Antifungal Efficacy of Chemical Based Shampoos and Natural Plant Extracts Against Dandruff Causing Fungi

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How to Cite this Article:

Kumar, D., Pandey, V. & Chaudhary, S. (2026). Antifungal Efficacy of Chemical Based Shampoos and Natural Plant Extracts Against Dandruff Causing Fungi. International Journal of Creative and Open Research in Engineering and Management, 02(6). https://doi.org/10.55041/ijcope.v2i6.003

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ABSTRACT

Dandruff is a scalp disorder usually linked to overgrowth of Malassezia fungi. It is influenced by excess sebum, dry skin, poor scalp hygiene, weather shifts, stress, and humidity. It appears as white or yellow flakes and causes scalp redness, greasiness, itching, and irritation. Literature highlights basic prevention of dandruff through scalp hygiene, oil control, and hair care that does not promote fungal growth. Chemical-based shampoos provide relief but many users now look for natural formulations with fewer side effects. The present investigation examines whether plant-based extracts can offer comparable antifungal action. Samples were collected from individuals affected with dandruff and efforts were made to isolate causative fungi. Identification was confirmed through cultural and microscopic characteristics. Antifungal efficacy will be assessed through standard assays. Two samples will be tested for present study. The first being commercial chemical-based shampoos commonly marketed as a cure for dandruff. The second sample would be plant extract(s) with reported antimicrobial potential. A measure of zone of inhibition at different concentrations of each sample would present a comparative performance to show which treatment exhibit stronger and promising antifungal activity. The study aims to determine if plant derived extracts can match or support synthetic formulations and offer an effective and safer choice for managing dandruff.

Keywords: Dandruff, Malassezia, Chemical Shampoo, Plants Extract, Antifungal Efficacy.

1. INTRODUCTION

Dandruff is one of the most common scalp disorders affecting humans worldwide and is considered a chronic relapsing condition characterized by excessive desquamation of the scalp epidermis. The disorder affects approximately 50% of the global post-pubertal population irrespective of gender or ethnicity and represents a significant dermatological and cosmetic concern (Ranganathan and Mukhopadhyay., 2010; Borda and Wikramanayake., 2015). Although dandruff is not a life-threatening disease, its visible manifestations often result in psychological distress, reduced self-confidence, social embarrassment, and diminished quality of life (Turner *et al.,* 2012; Hay *et al.,* 2017).

Lipophilic yeasts belonging to the genus *Malassezia* have been identified as the principal etiological agents associated with dandruff and seborrheic dermatitis (Guého *et al.,* 1996; Ashbee and Evans, 2002; Gaitanis *et al.,* 2012). These fungi normally exist as commensal organisms on healthy skin but become pathogenic under favorable environmental and physiological conditions. Studies predominance of *Malassezia globosa* and *Malassezia restricta*, *Malassezia furfur* on dandruff-affected scalps (Sugita *et al.,* 2002; Cafarchia *et al.,* 2024).

Malassezia species possess lipase enzymes that hydrolyze triglycerides present in scalp sebum into free fatty acids, including oleic acid and arachidonic acid (DeAngelis *et al.,* 2005). These fatty acids penetrate the stratum corneum and disrupt epidermal barrier integrity, leading to inflammation, irritation, and accelerated keratinocyte turnover (Warner *et al.,* 2001; Borda and Wikramanayake, 2015).

Several intrinsic and extrinsic factors contribute to dandruff development. Excessive sebum production, hormonal fluctuations, genetic predisposition, stress, nutritional deficiencies, climatic variations, and immune dysfunction are recognized as important predisposing factors (Piérard-Franchimont *et al.,* 2006; Kim *et al.,* 2024). The condition is particularly prevalent after puberty due to increased sebaceous gland activity stimulated by androgenic hormones, which provides an abundant lipid source required for *Malassezia* growth (Borda *et al.,* 2015). Recent investigations have further emphasized the role of scalp microbiome dysbiosis in dandruff pathogenesis, indicating that disruption of normal microbial balance facilitates fungal overgrowth and inflammation (Ranganathan *et al.,* 2024; Kim *et al.,* 2024).

For decades, chemical-based anti-dandruff shampoos have served as the primary therapeutic approach for dandruff management. These formulations typically contain active antifungal ingredients such as ketoconazole, zinc pyrithione, selenium sulfide, ciclopirox olamine, piroctone olamine, salicylic acid, and coal tar (Schwartz *et al.,* 2013; Gupta *et al.,* 2014). Zinc pyrithione exhibits antifungal and antibacterial activity by interfering with membrane transport systems and disrupting cellular metabolism (Warner *et al.,* 2001).

Despite their effectiveness, long-term application of synthetic anti-dandruff agents has raised concerns regarding adverse effects and environmental safety. Frequent use of medicated shampoos may result in scalp irritation, dryness, erythema, allergic contact dermatitis, hair texture alterations, and occasional follicular damage (Hay *et al.,* 2017). Additionally, the repeated exposure of fungal populations to chemical antifungal agents may contribute to the emergence of resistant strains, reducing therapeutic effectiveness over time (Gupta *et al.,* 2024).

Growing consumer awareness regarding natural healthcare products has stimulated interest in herbal alternatives for scalp disorders. Medicinal plants contain diverse bioactive secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, saponins, and essential oils that exhibit antimicrobial, antioxidant, and anti-inflammatory properties (Sharma and Sharma, 2019; Patel *et al.,* 2025). Unlike synthetic antifungal compounds that typically act through a single molecular target, phytochemicals often exert their effects through multiple mechanisms simultaneously, thereby minimizing the likelihood of microbial resistance development (Lopes *et al.,* 2024).

Among medicinal plants, neem (*Azadirachta indica*) has attracted considerable scientific attention due to its broad-spectrum antimicrobial activity. Neem contains biologically active compounds such as azadirachtin, nimbidin, gedunin, salannin, flavonoids, and tannins that inhibit fungal growth through disruption of membrane integrity and interference with metabolic pathways. Previous studies have demonstrated significant inhibitory effects of neem extracts against dermatophytic and dandruff-associated fungi (Madhavi *et al.,* 1998; Kaur *et al.,* 2024; Hashem *et al.,* 2024).

Rosemary (*Rosmarinus officinalis*) is another medicinal plant widely investigated for its therapeutic properties. Rosemary essential oil contains 1,8-cineole, camphor, α-pinene, borneol, and rosmarinic acid, compounds known for their antimicrobial and anti-inflammatory activities Experimental studies have suggested that rosemary oil may reduce microbial colonization, improve scalp circulation, and alleviate inflammatory symptoms associated with scalp disorders (Ahmed *et al 2011;* Moghtader *et al* 2011; Ravichandran *et al.,* 2015; Murtiastutik *et al* 2022; Li *et al.,* 2024).

Recent investigations have increasingly focused on comparing herbal formulations with conventional chemical treatments. (Satchell *et al.,* 2002) reported significant reductions in dandruff severity following treatment with tea tree oil shampoo. Similarly, studies evaluating neem, aloe vera, eucalyptus, rosemary, and tulsi extracts have demonstrated varying degrees of antifungal activity against *Malassezia* species (Sharma and Sharma, 2019; Hashem *et al.,* 2024). Although chemical shampoos generally exhibit stronger and faster antifungal effects, herbal formulations are often associated with lower toxicity, improved scalp tolerance, and enhanced consumer acceptance (Lopes *et al.,* 2024).

Considering the increasing prevalence of dandruff and the growing demand for safer therapeutic alternatives, the present study was undertaken to isolate and identify dandruff-causing fungal species and evaluate the comparative antifungal efficacy of selected chemical shampoos and natural plant extracts against *Malassezia*. The findings may contribute to the development of effective, affordable, and environmentally sustainable anti-dandruff formulations for long-term scalp health management.

2. MATERIALS AND METHODS

2.1 Study Design

The present study was conducted to evaluate and compare the antifungal efficacy of selected chemical-based shampoos and natural plant extracts against dandruff-causing fungi. The investigation involved isolation and identification of *Malassezia* species from dandruff samples followed by determination of the Minimum Inhibitory Concentration (MIC) of commercial anti-dandruff shampoos and plant-derived extracted essential oils.

2.2 Collection of Dandruff Samples

Dandruff samples were collected aseptically from individuals exhibiting visible symptoms of dandruff, including scalp flaking and itching. Sterile scalp scrapers and sterile cotton swabs were used to obtain scalp scrapings from affected regions (Gupta *et al.,* 2004).

Figure 1 Sample Collection

2.3 Isolation of Dandruff-Causing Fungi

Isolation of fungal organisms was performed using supplemented Sabouraud Dextrose Agar (Guillot *et al.,* 1996; Gaitanis *et al.,* 2012).

Dandruff sample was inoculated onto the prepared SDA plates using sterile inoculating loops. The inoculated plates were incubated at 32–35°C for 3–5 days under aerobic conditions (Gaitanis *et al.,* 2012).

Figure 2: Isolation of Dandruff causing Fungi on SDA supplemented with sterile Olive oil and Tween-80

2.4 Identification of Fungal Isolates

Identification of fungal isolates was carried out using colony morphology, microscopic examination and biochemical tests.

2.4.1 Colony Morphology

Colony characteristics such as color, texture, margin, elevation, and consistency were observed after incubation. Typical *Malassezia* colonies appeared creamy white to pale yellow, smooth, glossy, circular, and slightly raised (Guillot *et al.,* 1996; Gupta *et al.,* 2004; Gaitanis *et al.,* 2012).

2.4.2 Microscopic Examination

Microscopic identification was performed using Lactophenol Cotton Blue (LPCB) staining and Gram staining techniques for *Malassezia* species (Guillot *et al.,* 1996; Kindo *et al.,* 2004; Inamdar *et al.,* 2003).

2.4.3 Catalase Test

Catalase activity was determined by placing a small amount of fungal growth on a clean glass slide and adding one drop of 3% hydrogen peroxide (Nouripour-Sisakht *et al.,* 2014; Shah *et al* 2013).

2.4.4 Urease Test

The urease test was performed using Christensen's Urea broth. Test cultures were inoculated onto the medium and incubated at 32–35°C (Guillot *et al.,* 1996; Kindo *et al.,* 2004; Shah *et al* 2013; Nouripour-Sisakht *et al.,* 2014).

2.5 Preparation of Fungal Inoculum

Fresh colonies obtained from SDA plates were suspended in sterile normal saline solution. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard using a spectrophotometer. This standard corresponds approximately to 1×10^6 to 5×10^6 CFU/mL and ensures uniform inoculum density among all experimental tubes (Peano *et al.,* 2017).

Figure 3: Absorbance value of Spectrophotometer in correspondence to the 0.5 McFarland Standard

2.6 Preparation of Culture Medium

Sabouraud Dextrose Broth (SDB) was prepared and supplemented with 100 µL sterile olive oil and 50–100 µL Tween-80 to every 10 mL of sterile broth.

The supplemented broth served as the growth medium for MIC determination since *Malassezia* requires external lipid sources for growth (Guillot *et al.,* 1996; Ashbee & Evans, 2002; Kindo *et al.,* 2004; Gaitanis *et al.,* 2012).

2.7 Plant Material and Extraction of Essential Oils

Fresh plant materials used for oil extraction included neem (*Azadirachta indica*) leaves and rosemary (*Rosmarinus officinalis*) leaves. Essential oils were extracted using a Clevenger-type apparatus by hydro-distillation. (Burt *et al.,* 2004)

Figure 4: Extraction of plant oil using Clavanger

2.8 Test Samples

The following antifungal agents were evaluated:

Chemical-Based Formulations

1. Ketoconazole based Shampoo (2%)
2. Zinc Pyrithione based Shampoo (3.5%)

Natural Plant Extracts

1. Neem Oil (*Azadirachta indica*)
2. Rosemary Oil (*Rosmarinus officinalis*)

2.9 Preparation of Serial Dilutions

Serial two-fold dilutions of each test sample were prepared using supplemented Sabouraud Dextrose Broth.

The concentrations evaluated were:

- 50%
- 25%
- 12.5%
- 6.25%
- 3.125%

Each dilution was prepared aseptically and mixed thoroughly and then inoculated.

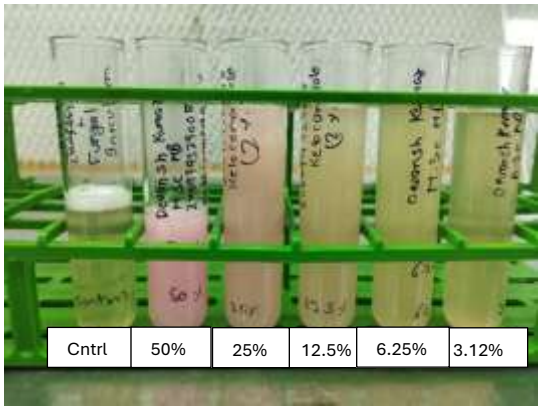


Figure 5: Two-Fold Serial Dilution of Ketoconazole shampoo

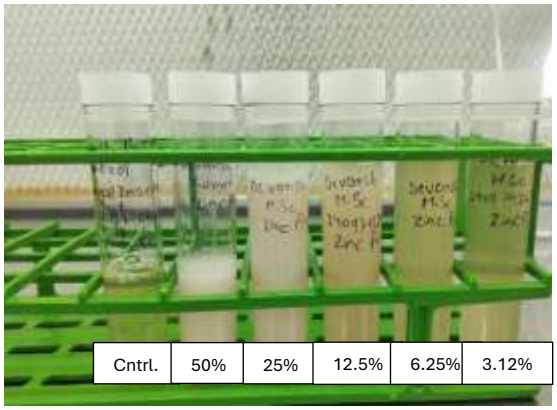


Figure 6: Two-Fold Serial Dilution of Zinc Pyrithione

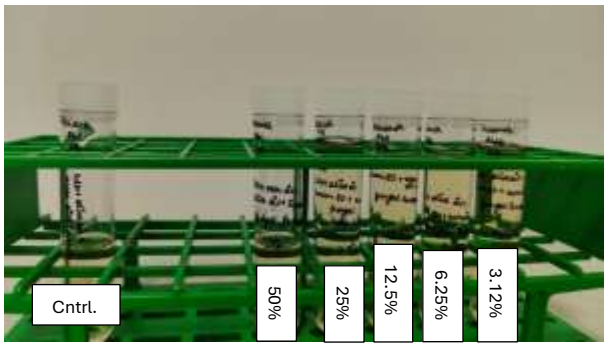


Figure 7: Two-Fold Serial Dilution of Neem Oil

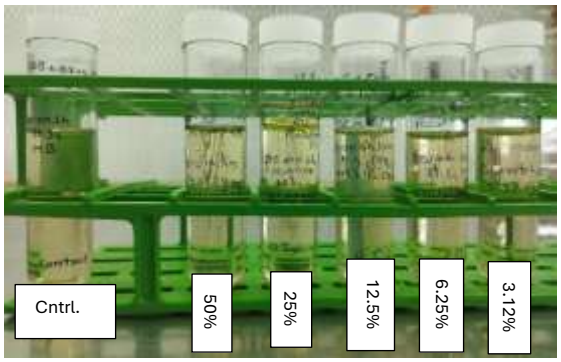


Figure 8: Two-Fold Serial Dilution of Rosemary Oil

2.10 Determination of Minimum Inhibitory Concentration by Subculturing

To confirm MIC results, 100 µL aliquots from each broth dilution tube were aseptically spread onto SDA plates supplemented with olive oil and Tween-80. The inoculated plates were incubated at 32–35°C for 3–5 days. Following incubation: Presence of colonies indicated fungal survival and growth and absence of colonies indicated complete fungal inhibition. The lowest concentration showing no visible colony formation after subculturing was recorded as the confirmed MIC value.

2.11 Controls Used in the Study

To ensure reliability of results, the following controls were maintained:

Positive Control: Supplemented SDB containing fungal inoculum without antifungal agents.

Figure 9: SDB + Olive Oil + Tween 80 + Fungal Inoculum

Figure 10: Growth on Positive Control

Negative Control: Supplemented SDB containing culture medium only without fungal inoculum.

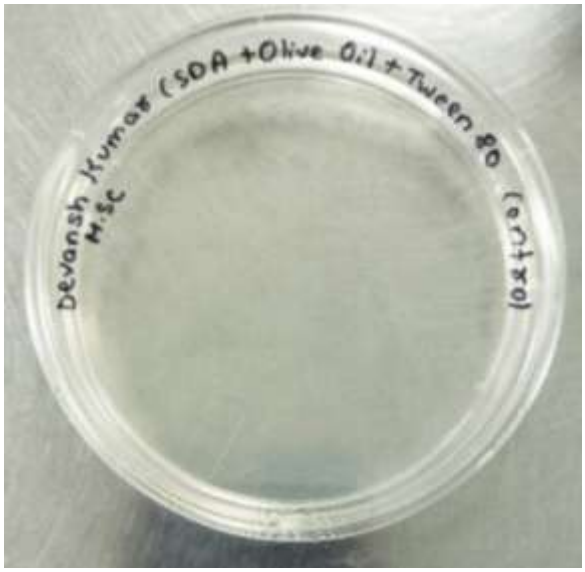


Figure 11: Negative control showing absence of fungal growth on SDA

Solvent Control: Medium containing Tween-80 and olive oil and fungal inoculum without antifungal agents to evaluate any inhibitory effect of solvents used in the experiment.

Figure 12: Solvent control showing fungal growth in presence of solvent only.

3. RESULT AND DISCUSSION

3.1 Isolation of Dandruff causing Fungi

The inoculated plates were incubated at 32–35°C for 3–5 days under aerobic condition and Fungal colonies were observed.

3.2 Identification of Fungal Isolates

3.2.1 Colony Morphology

The fungal isolate produced creamy, smooth, yeast-like colonies on SDA supplemented with olive oil.

Figure 13: Mallasezia Mother Culture

3.2.2 Microscopic Examination

Microscopic examination revealed Gram positive result and oval budding yeast cells and short hyphae, characteristic of *Malassezia* species.

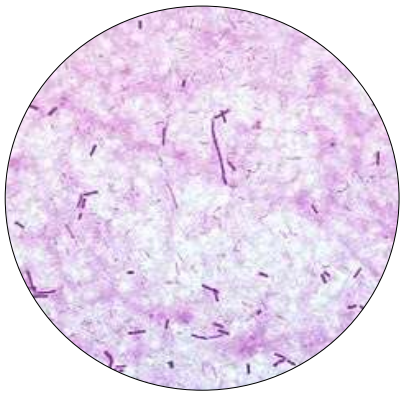


Figure 14: Gram Stain: Gram positive, Thick Walled

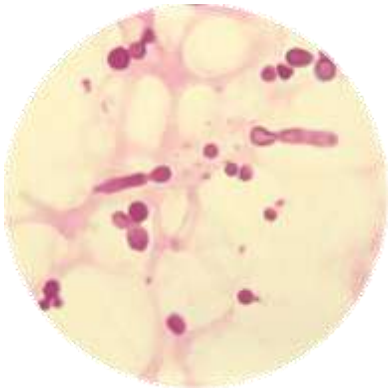


Figure 15: LPCB Staining of Fungi

3.2.3 Catalase Test and Urease Test

The isolate also showed positive catalase and urease reactions, confirming its identity.

Figure 9: Positive Catalase test (Active bubble Formation)

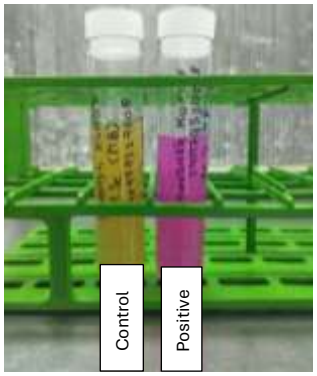


Figure 10: Positive Urease Test

3.3 Determination and Confirmation of MIC

3.3.1 Ketoconazole Shampoo (2%)

No fungal growth was observed at concentrations of 50%, 25%, 12.5%, and 6.25%, whereas visible growth was observed at 3.125%. Therefore, the MIC of Ketoconazole Shampoo was determined to be **6.25%**.

Figure 11: Fungal growth at 3.12%

Figure 12: No fungal growth at 6.25%

3.3.2 Zinc Pyrithione Shampoo (3.5%)

Zinc Pyrithione Shampoo (3.5%) demonstrated antifungal activity against *Malassezia* species. No fungal growth was observed at concentrations of 50%, 25%, and 12.5%, while growth was detected at lower concentrations. The MIC was determined to be 12.5%.

Figure 13: Growth of Fungi at 3.12% concentration of Zinc Pyrithone Shampoo

Figure 14: Growth of Fungi at 6.25% concentration of zinc Pyrithone Shampoo

Figure 15: No growth at 12.5% concentration of the Zinc Pyrithione Shampoo

3.3.3 Neem Oil

Neem oil exhibited significant antifungal activity against *Malassezia* species. Complete inhibition of fungal growth was observed up to a concentration of 12.5%, whereas fungal colonies were detected at lower concentrations. The MIC of neem oil was therefore recorded as 12.5%.

Figure 16: Growth of Fungi at 3.12% concentration of Neem Oil

Figure 17: Growth of Fungi at 6.25% concentration of Neem Oil

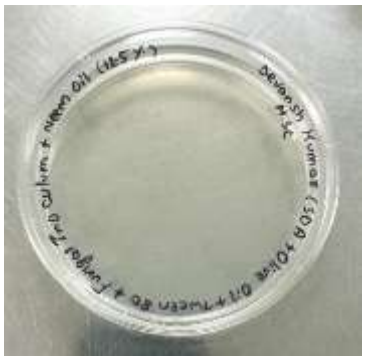


Figure 18: No growth at 12.5% concentration of Neem Oil

3.3.4 Rosemary Oil

Rosemary oil showed comparatively lower antifungal activity than the other test samples. Complete inhibition of fungal growth was observed only at concentrations of 50% and 25%, while fungal growth occurred at lower concentrations. The MIC was determined to be 25%.

Figure 19: Growth of Fungi at 3.12% concentration Of Rosemary oil

Figure 20: Growth of Fungi at 6.25% concentration Of Rosemary oil

Figure 21: Growth of Fungi at 12.5% concentration Of Rosemary oil

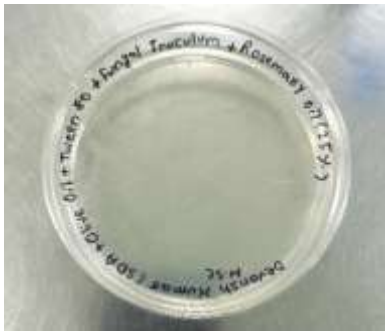
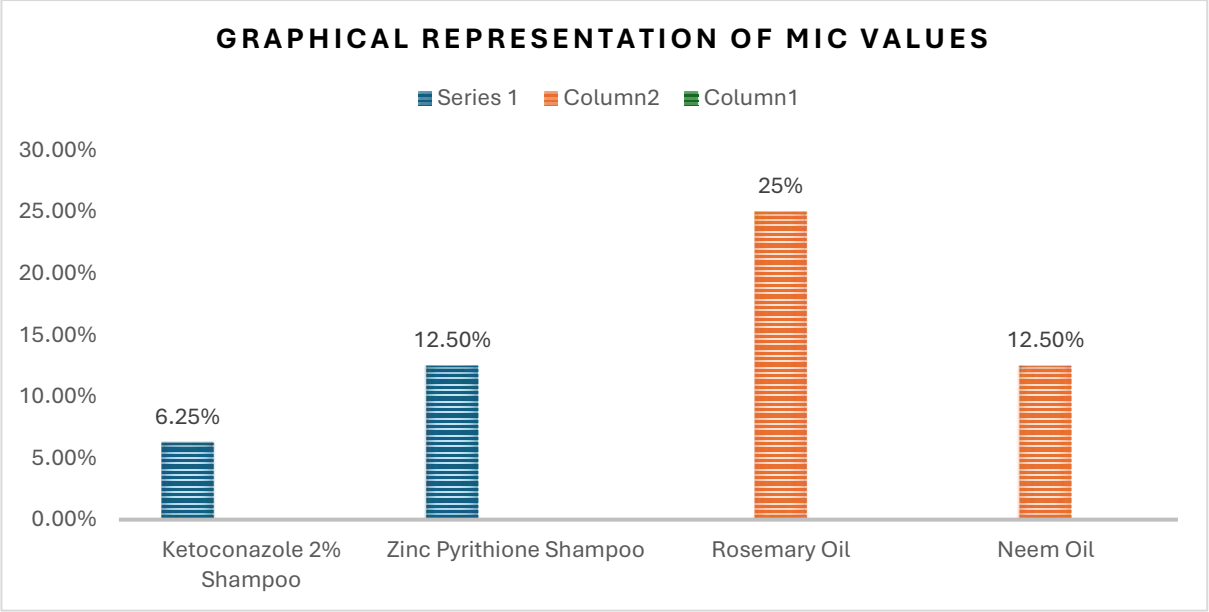


Figure 22: No growth of fungi at 25% of Rosemary Oil

Table: MIC Values of Tested Samples Against *Malassezia*

Test Sample	Active Ingredient	MIC (%)
Ketoconazole Shampoo	2% Ketoconazole	6.25%
Zinc Pyrithione Shampoo	3.5% Zinc Pyrithione	12.5%
Neem Oil	100% Neem Oil	12.5%
Rosemary Oil	100% Rosemary Oil	25%



3.4 DISCUSSION

The present study compared the antifungal efficacy of two commercially available anti-dandruff shampoos and two natural plant oils against *Malassezia* species. The results demonstrated that all tested samples exhibited antifungal activity, although their effectiveness varied considerably.

Among the tested formulations, ketoconazole shampoo showed the strongest antifungal effect with the lowest MIC value of 6.25%. This finding suggests that ketoconazole remains highly effective in controlling *Malassezia* growth and supports its widespread use in the treatment of dandruff and seborrheic dermatitis. Zinc pyrithione shampoo also showed good antifungal activity with an MIC value of 12.5%, indicating its effectiveness as an anti-dandruff agent.

Among the natural products, neem oil demonstrated significant antifungal activity with an MIC value comparable to zinc pyrithione shampoo. The inhibitory effect of neem oil may be attributed to the presence of bioactive compounds such as azadirachtin, nimbidin, and flavonoids, which are known to possess antimicrobial properties. These findings suggest that neem oil has potential as a natural alternative for managing dandruff.

Rosemary oil exhibited antifungal activity but required a higher concentration to inhibit fungal growth, resulting in an MIC value of 25%. Although its activity was lower than that of the other tested samples, the presence of antimicrobial phytochemicals may contribute to its inhibitory effect against *Malassezia*.

Overall, the study indicates that chemical-based shampoos were more effective than the tested plant oils in inhibiting *Malassezia* growth. However, the considerable antifungal activity observed in neem oil highlights its potential for inclusion in herbal anti-dandruff formulations. Further studies involving larger sample sizes and clinical evaluation are required to confirm the effectiveness of these natural products in routine dandruff management.

CONCLUSION

The present study demonstrated that both chemical-based shampoos and natural plant oils possess antifungal activity against *Malassezia* species, the major fungus associated with dandruff. Among the tested samples, ketoconazole shampoo showed the highest antifungal efficacy, followed by zinc pyrithione shampoo and neem oil. Rosemary oil exhibited comparatively lower activity.

The findings suggest that while chemical formulations remain more effective in controlling fungal growth, neem oil also shows promising antifungal potential and may serve as a natural alternative for dandruff management. Further studies are needed to evaluate the clinical effectiveness of herbal formulations and their potential use in the development of safer and eco-friendly anti-dandruff products.

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