ISOLATION OF DANDRUFF CAUSING FUNGUS AND COMPARISON OF ANTI-DANDRUFF ACTIVITY OF COMMERCIAL AND MEDICATED SHAMPOOS

Project work by

Sreelakshmi Santhosh
AB21ZOO014

Under the guidance of

Dr. Soja Louis

Associate Professor

Department of Zoology, St. Teresa's College (Autonomous), Ernakulam

Kochi 682011

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CERTIFICATE

This is to certify that the project report entitled "**ISOLATION OF DANDRUFF CAUSING FUNGUS AND COMPARISON OF ANTI-DANDRUFF ACTIVITY OF COMMERCIAL AND MEDICATED SHAMPOOS**" submitted by Ms. Sreelakshmi Santhosh, Reg No: AB21ZOO014 in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.

Dr. Soja Louis

Head of the Department & Associate Professor

Department of Zoology

St. Teresa's College (Autonomous)
Ernakulam

EXAMINERS:

1.

2.

DECLARATION

I, hereby declare that this project work entitled "**ISOLATION OF DANDRUFF CAUSING FUNGUS AND COMPARISON OF ANTI-DANDRUFF ACTIVITY OF COMMERCIAL AND MEDICATED SHAMPOOS**" is submitted to St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirement of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

Name: Sreelakshmi Santhosh Signature:
Register no.: AB21ZOO014

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ABSTRACT

Dandruff, a common perennial scalp condition affecting millions worldwide, is primarily attributed to the overgrowth of the lipophilic yeast *Malassezia* spp. Despite its widespread occurrence, effective treatment options remain limited. In this project **titled "ISOLATION OF DANDRUFF CAUSING FUNGUS AND COMPARISON OF ANTI-DANDRUFF ACTIVITY OF COMMERCIAL AND MEDICATED SHAMPOOS**", we aimed to isolate a potent dandruff-causing isolate using SDA medium with Olive oil and evaluate its anti-dandruff activity against both commercial and medicated shampoos.

The isolate was identified through microscopic and biochemical examination. Following isolation, anti-dandruff activity was assessed using in vitro assays measuring yeast growth inhibition and scalp flaking reduction. Our findings reveal the isolation of a robust dandruff-causing isolate and demonstrate its significant anti-dandruff activity compared to conventional and medicated shampoos. On comparison, it was observed that medicated shampoos showed a higher zone of inhibition than the commercial ones. This research contributes to understanding dandruff pathogenesis and offers potential insights for the development of improved anti-dandruff treatments. The identification of a potent dandruff causing isolate, along with its demonstrated efficiency against conventional and medicated shampoos, paves way for further research in this area to improve dandruff management strategies and enhance patient outcomes.

INTRODUCTION

Dandruff, an inflammatory scalp condition affecting a significant portion of the global population, has been a subject of persistent curiosity and scrutiny. While it is generally considered a benign condition, dandruff can significantly impact an individual's quality of life, leading to social embarrassment and discomfort. The exact etiology of dandruff remains elusive, but numerous factors, including genetic predisposition, hormonal fluctuations, and microbial colonization, are thought to contribute to its development. Dandruff is a common scalp condition characterized by the excessive shedding of skin cells from the scalp affecting individuals of all ages and backgrounds. It often presents as white or grayish flakes that may be visible on the scalp, hair, eyebrows or clothing. While shedding of skin cells is a natural part of the skin renewal process, individuals with dandruff experience an accelerated shedding cycle.

Malassezia, a type of lipophilic yeast found on the skin's surface, has been closely associated with dandruff. Its overgrowth or alteration in scalp lipid metabolism, particularly an elevated response to sebum production, is believed to trigger an inflammatory reaction in some individuals, leading to increased skin cell turnover and the manifestation of visible dandruff flakes. They naturally inhabit the skin of humans and other animals. *Malassezia's* lipophilic nature allows it to thrive in oily environments like the scalp, where it metabolizes skin lipids as a source of energy. This proliferation or imbalance in *Malassezia* populations can contribute to the intensification of dandruff symptoms.

Anti-dandruff treatments often incorporate active agents targeting *Malassezia* to regulate its population and alleviate associated scalp conditions. While *Malassezia* is a natural skin resident, its presence can influence scalp health, underscoring the significance of specific interventions to manage its impact on our daily life.

Among the various treatment modalities available, anti-dandruff shampoos represent a cornerstone in the management of these scalp conditions. Commercially available shampoos formulated with antifungal agents offer a convenient and accessible option for individuals seeking relief from fungal scalp infections. These shampoos typically contain active ingredients such as zinc pyrithrone, ciclopirox olamine, known for their antifungal properties. By targeting the underlying fungal overgrowth, these shampoos aim to alleviate symptoms such as scalp itching, flaking, and inflammation associated with dandruff and seborrheic dermatitis.

Commercial shampoos selected for this study are:

- **Head and Shoulders**: is an American brand of anti-dandruff and non-dandruff shampoo produced by the company Procter & Gamble. It was introduced in the United States and used the slogan THE AMERICA AND WORLD's NO. 1 SHAMPOO. The active anti-fungal ingredient in Head & Shoulders is piroctone olamine, with some "clinical strength" varieties also containing selenium disulfide.
- **Clear**: Clear is a global brand of anti-dandruff shampoo, the manufacturer is owned by the British-Dutch company Unilever. Clear shampoo is originated from Italy. It is sold under the Clear name in most certain global countries, and also known as Ultrex in Greece, Linic in Portugal and Pure Derm in India. Until 2010, the product was sold in Thailand under the name Clinic Clear, and Clinic before the early 2000s.
- **St. Botanica**: All the products are designed and manufactured in India with the best of ethically sourced ingredients that are natural, safe, and skin-friendly. Has cleared all the quality tests by CTFA, the Cosmetics, Toiletries and Fragrances Associations of the United States. Products are devoid of harmful chemicals and dermatologically tested.
- **Sunsilk**: Sunsilk is a British hair care brand produced by the company Unilever. The brand was introduced in the UK and available in most countries globally. It has biotin and aloe vera that work together to deeply

nourish your hair. It helps your hair grow stronger so you can experiment with any hairstyle.

In addition to commercial formulations, medicated shampoos prescribed by healthcare professionals provide targeted therapy for more severe or refractory cases of fungal scalp infections. These medicated shampoos may contain higher concentrations of antifungal agents or additional therapeutic ingredients to enhance efficacy and mitigate potential side effects. Commonly prescribed medicated shampoos include ketoconazole shampoo, ciclopirox shampoo, and prescription-strength formulations of over-the-counter antifungal agents. The following medicated shampoos were taken for this study.

- Selsun Blue
- Ketafung lotion (Ketoconazole)
- Scalp + Anti-dandruff shampoo
- Keraglo- AD

Despite their widespread use, the antifungal effects of commercial and medicated shampoos vary depending on factors such as formulation, concentration of active ingredients, and individual response to treatment. While some individuals experience significant improvement in symptoms with regular use of antifungal shampoos, others may exhibit partial or inadequate response, necessitating alternative treatment approaches.

In this context, understanding the antifungal effects of commercial and medicated shampoos is essential for optimizing treatment outcomes and guiding clinical decision-making. This study aims to evaluate the efficacy of various commercial and medicated shampoos against fungal scalp infections, including dandruff and seborrheic dermatitis. By elucidating the antifungal properties of these shampoos and their impact on clinical outcomes, we can enhance our understanding of scalp fungal infections and inform evidence-based approaches to their management.

REVIEW OF LITERATURE

Fungi comprise a vast and diverse group of microorganisms inhabiting a wide range of environments, from terrestrial ecosystems to the human body. With an estimated 1.5 to 5 million species, fungi exhibit remarkable variability in morphology, physiology, and ecological roles, contributing significantly to ecosystem functioning and biodiversity. Among the vast diversity of fungi, certain species are known to inhabit the human body as part of the normal microbiota or as opportunistic pathogens capable of causing infectious diseases. Fungal infections, or mycoses, can affect different anatomical sites, including the skin, nails, respiratory tract, and mucous membranes, with varying degrees of severity ranging from mild superficial infections to life-threatening systemic diseases.

One group of fungi commonly associated with human skin and scalp conditions is the genus *Malassezia*. These lipophilic yeasts are natural inhabitants of the skin microbiota, particularly in sebum-rich areas such as the scalp, face, and upper trunk. While *Malassezia* spp. typically maintain a symbiotic relationship with the host, their overgrowth or dysregulation has been implicated in the pathogenesis of various dermatological disorders, including dandruff, seborrheic dermatitis, and folliculitis.

Understanding the role of fungi, particularly *Malassezia* spp., in the pathogenesis of dandruff is essential for developing effective therapeutic strategies for its management. Research efforts focused on understanding the mechanisms of fungal colonization, host-fungus interactions, and immune responses can provide valuable insights into dandruff pathogenesis and guide the development of targeted antifungal therapies and anti-dandruff treatments.

Naga Padma *et al.,* (2015) conducted a study on "Comparison of potency of antifungal action of dandruff shampoos and different plant extracts". In which, commercially available shampoos were assessed for antifungal activity against a human dandruff isolate of *M. furfur*. The shampoos were Head & Shoulders, Clinic All Clear, and Pantene etc. The results demonstrated that all six of the assayed hair shampoos have some antifungal effect on growth of *M. furfur*. These products have poor efficacies, more side effects and give scope for recurrence of symptoms.

A comparative study of the effect of commercial antidandruff shampoos and natural plant products to evaluate their antifungal efficacy leads to the conclusion that the activity of some of the natural extracts was equivalent to that of the commercially available branded shampoos. As crude herbal drugs have been included in traditional medicine and household remedies for a long time, regular usage of these tested plant extracts can reduce the incidence of dandruff (Kutcharlapati, 2019).

A study on the Anti-dandruff activity of synthetic and herbal shampoos on dandruff causing isolate: *Malassezia* by Mistry Zoya et al., (2018) explored the comparison between antifungal effects of synthetic shampoos and herbal shampoos against the dandruff causing isolate *Malassezia* using SDA agar. All the selected shampoos were proved to be effective as they all showed the inhibition against *Malassezia*. Synthetic shampoos were proved to be more effective compared to herbal shampoos. The highest zone of inhibition was obtained by Cipla-8X while minimum inhibition was observed by Nature's Essence.

Kohinur Begum et al., (2019) focused on the isolation and identification of dandruff isolates by molecular biology techniques and testing its sensitivity towards antifungal agents. This study was conducted to isolate and identify the *Malassezia* spp. from dandruff samples and to determine their responsiveness towards antifungal agents such as fluconazole, ketoconazole, miconazole and nystatin. *Malassezia spp*. was isolated using selective media and identified by

biochemical tests and microscopic examination. Antifungal activities were assessed by the Kirby Bauer method using well diffusion technique.

Antifungal activities of azole agents against the *Malassezia* species by Karla Carvalho Miranda et al., (2007), revealed 95 *Malassezia* isolates, which were identified by various biochemical and morphological criteria and its sensitivity towards various azole agents were studied. The modified Leeming-Notman medium used for susceptibility testing allowed good growth of *Malassezia* spp.

A study by Kindo et al., (2003), focused on the isolation and identification of dandruff causing *Malassezia* fungus by various biochemical tests and microscopic analysis like catalase test, tween assimilation test. KOH microscopy etc. This study was aimed at using a simple practical approach to speciate *Malassezia* yeasts from clinical material. Seventy skin scrapings from patients with pityriasis versicolor infection, positive in 10% potassium hydroxide (KOH), were cultured onto modified Dixon's agar (mDixon's agar) and Sabouraud dextrose agar (SDA) and incubated at 32ºC. Speciation was done on the basis of Gram stain morphology, catalase test, and utilization of Tweens. Out of 70 scrapings 48 (68.75%) showed growth on mDixon's agar. The commonest isolate was M. sympodialis (28, 58%) followed by M. globosa (19, 40%) and one isolate was (2%) of M. restricta. M. sympodialis was the commonest species affecting our population and there was no isolation of M. obtusa, M. slooffiae, M. pachydermatis and M. furfur.

METHODOLOGY

Sample Collection:

1. **Dandruff Sample Collection**: Dandruff samples are obtained from individuals experiencing this condition with moderate to severe dandruff symptoms. Samples are collected using sterile swabs or scraping affected areas of the scalp using a sterilized comb.

Processing Dandruff Samples:

2. **Preparation of Samples:** Transfer the collected scalp samples into sterile containers containing the SDA medium to maintain fungal viability during transportation to the laboratory.

Isolation and Culture:

3. **Plating on Selective Media**: Selective agar plates such as Sabouraud Dextrose Agar (SDA) incorporated with 0.01 g of ampicillin per 100ml of medium with an overlay of olive oil is used and plates containing medium without olive oil is kept as control, which is optimized for the growth of *Malassezia* species. Spread or streak the dandruff samples onto these plates.

4. **Incubation**: Incubate the plates at the optimal temperature (usually around 32-37°C) for *Malassezia* growth for a specified period (usually3-4 days). Monitor the plates regularly for fungal growth.

Identification and Characterization:

5. **Microscopic Examination**: Examine the colonies of typical *Malassezia* for characteristic morphology including color, texture and growth pattern using microscopy.

i. Direct microscopy:
   A drop of 10% KOH was added onto a clean slide containing the smear of sample and the smear was covered with a cover slip. The slides were viewed under 40x objective lens.

ii. Staining with Lactophenol Cotton Blue:
   A drop of lactophenol cotton blue was introduced at the center of a clean slide. Fragments of 2-3mm from the fungus colony was collected using wire loop and the fragment was dropped on the stain and teased gently, a cover slip was applied. The preparation was examined under low and high magnifications.

Malassezia typically exhibit yeast-like or mycelial forms (spaghetti and meatball forms) under microscopic examination by gram staining, lactophenol cotton blue staining.

6. **Subculture and Purification**: Subculture representative colonies onto fresh SDA agar plates to obtain pure cultures. This step ensures the isolation of individual *Malassezia* species for further analysis.

7. **Biochemical Tests**: Conduct biochemical tests for accurate identification of *Malassezia* species by Catalase test or urease test.

i. Catalase test: Catalase test was carried out to ascertain the presence of *Malassezia* species as it is catalase positive. 3 mL of 3% hydrogen peroxide (H202) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using a

sterile glass rod. The gas bubbles observed is the breakdown of hydrogen peroxide to oxygen and water by enzyme catalase.

8. **Antifungal Susceptibility Testing**:

Evaluation of the susceptibility of isolated species to antifungal agents commonly used in dandruff treatment like commercial and medicated shampoos and the zone of inhibition was measured. These were done through the following steps:

i. **Preparation of Agar Plates**: Prepare Sabouraud dextrose agar plates supplemented with appropriate antifungal concentrations to achieve desired inhibitory effects. Pour the agar medium into sterile petri dishes and allow it to solidify.

ii. **Inoculation of Agar Plates**: Using a sterile swab or inoculation loop, evenly spread the standardized fungal inoculum onto the surface of the agar plates. Allow the inoculum to dry before proceeding to the next step.

iii. **Antifungal assay**: Anti-fungal (anti-dandruff) effects of the shampoo against *Malasezzia* species by Agar well diffusion or Agar cup plate method.

AGAR WELL DIFFUSION METHOD:

Wells were punched aseptically with corkborer round the margin of the plates equidistantly (3cm apart). In to each of these wells 50microlitres of extracted solutions were placed carefully.

iv. **Incubation**: Incubate the agar plates at appropriate temperature and humidity conditions conducive to fungal growth (typically 25-30°C) for a predetermined incubation period (e.g., 24-48 hours) to allow fungal growth and antifungal diffusion.

v. **Measurement of Zones of Inhibition**: After the incubation period, visually inspect the agar plates for the presence of clear zones of inhibition around the antifungal discs, indicating susceptibility of the dandruff-causing fungus to the respective antifungal agent. Measure the diameter of the inhibition zones using a calibrated ruler.

Documentation and Reporting:

9. **Interpretation of Results**: Compare the diameter of the inhibition zones to established formulated criteria for antifungal susceptibility testing. Zones of inhibition larger than the defined breakpoints indicate susceptibility, while smaller zones or absence of inhibition suggest resistance or reduced susceptibility.

10. **Data Analysis**: Record the results of antifungal susceptibility testing, including the diameter of inhibition zones for each shampoo tested. Analyze the data to determine the susceptibility profile of the dandruff-causing fungus and identify the most effective shampoo for treatment

RESULT

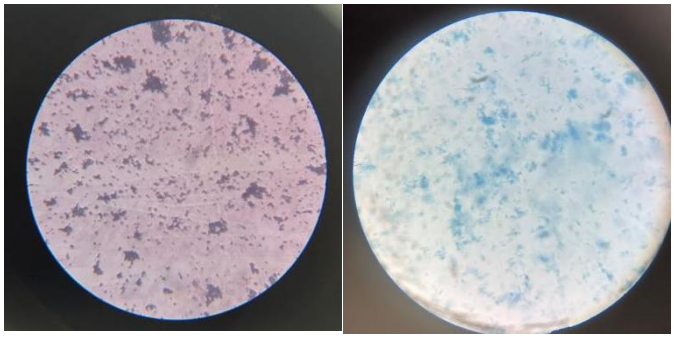
Isolation of Dandruff

The collected samples of dandruff were inoculated over SDA medium for a period of two days and according to the morphological characteristics, *Malassezia* was identified and further inoculated in SDA medium. Its sub culturing and isolation by pure culture resulted in the appearance of phenotypically similar creamy white dotted colonies of fungus along the streaked lines. The inoculum was then subjected to various tests to confirm the presence the dandruff causing fungus *Malassezia*.

The inoculum was analysed based on direct microscopy, which showed the hyphae and conidiospores giving the characteristic "spaghetti and meatball" appearance in KOH preparation. Gram staining showed ovoid shaped yeast cells under the microscope and gave a positive test.

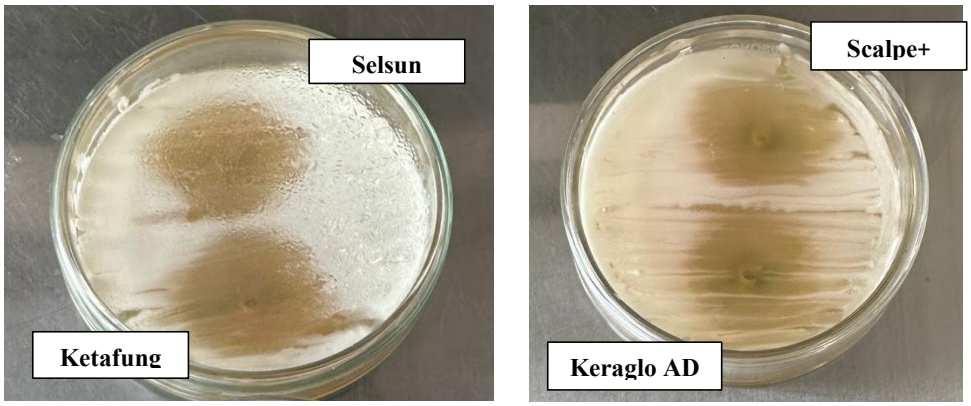
The catalase test was performed which showed active bubbling when the isolated colony of fungus was inoculated in a test tube containing 3ml of H_2O_2. Microscopic observation using lactophenol cotton blue also showed the "spaghetti and meatball" appearance.

(a) (b) (c)

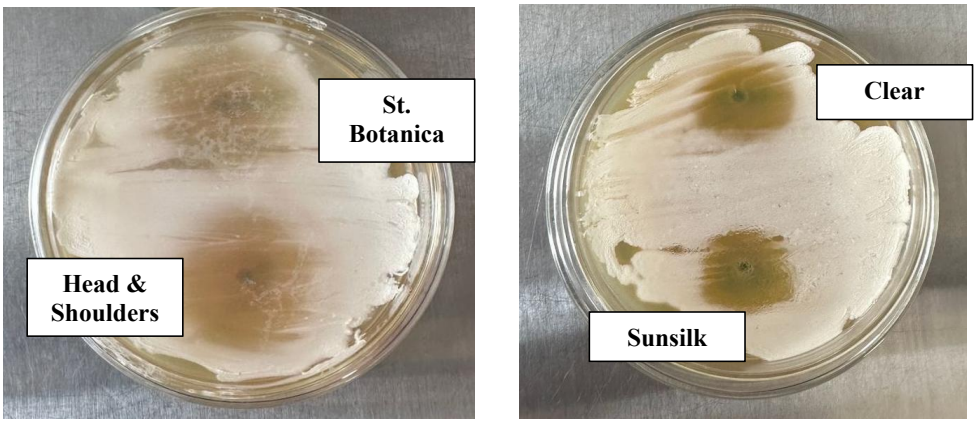


(d) (e)

(a) Initial dandruff culture (b) Streak culture of Malassezia (c) Catalase test (d) Gram's test (e) Lactophenol cotton blue test



Zones of Inhibition of Medicated Shampoos



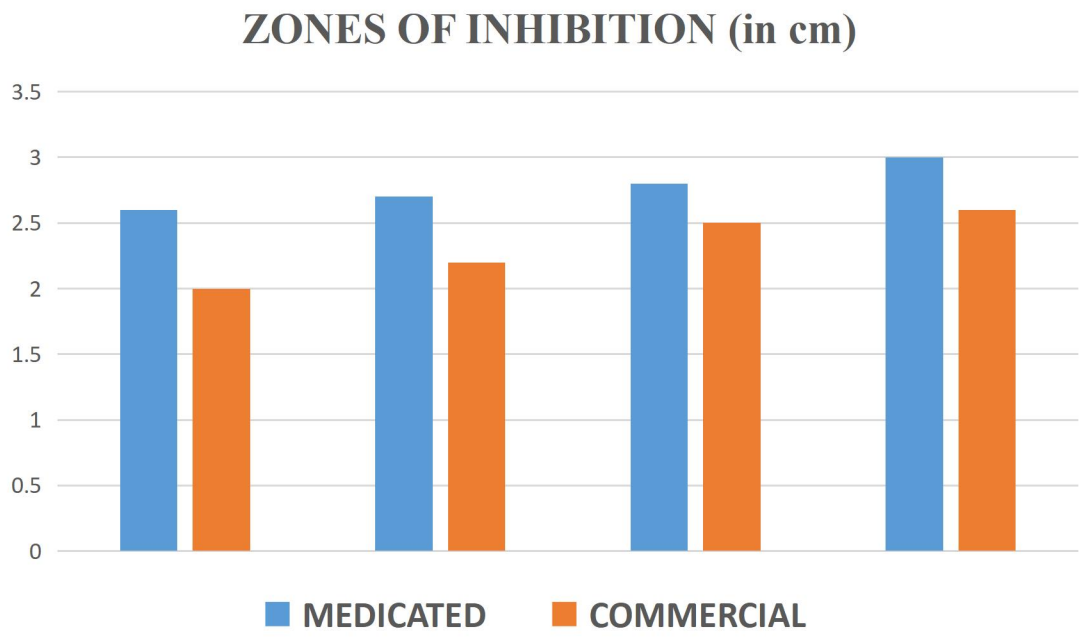
Zones of Inhibition of Commercial Shampoos

Antifungal Activity

Agar well diffusion: Agar well diffusion method was performed throughout the experiment for different samples of shampoos to check the inhibition levels, and the zone of inhibition was noted.

Name of Shampoo	Zone of Inhibition (in mm)
Clear	22
Sunsilk	20
St. Botanica	25
Head and Shoulders	26

Name of Shampoo	Zone of Inhibition (in cm)
Selsun	27
Ketafung	28
Scalpe plus	30
Keraglo AD	26



Graph showing the comparison of Medicated and Commercial shampoos. It is observed that the average zone of inhibition of medicated shampoos is higher than commercial shampoo

DISCUSSION

In this day and age, understanding the cause of dandruff formation and the anti-dandruff activity of various commercial shampoos and medicated shampoos is of importance as it gives an enhanced possibility of eradicating the condition.

The present study showed that the dandruff causing organism belongs to genus *Malassezia*, which is known for its lipolytic activity of converting human sebum to free fatty acids. The present isolate being lipolytic grew well on the SDA medium, in accordance with other researches. The presence of *Malassezia* was confirmed by catalase test and observation by direct microscopy with KOH, Lactophenol cotton blue test and Gram's test all of which showed positive results.

In this research, the anti-dandruff activity of various commercial and medicated shampoos were checked along with its comparative study. The zones of inhibition (ZOI) of the commercial shampoos were as follows: Clear (22mm), Sunsilk (20mm), Head and Shoulders (26mm) and St. Botanica (25mm), whereas the medicated shampoos showed a greater zones of inhibition, they were - Selsun (27mm), Ketafung (28mm), Keraglo AD (26mm), Scalpe plus (30mm).

Among the commercial shampoos, it was observed that Head and Shoulders have a higher zone of inhibition with 26mm and in medicated shampoos, Scaple+ has a zone of inhibition of 30mm.

Comparing the average zones of inhibition it can be noted that, the average zone of inhibition of commercial shampoos is 23.25mm and that of medicated shampoos is 27.75mm.

The reason for the enhanced activity of medicated shampoo is due to the active ingredients present in it which is - Ketaconazole, a drug, which belongs to an imidazole class of antifungal agents, effective in treatment of dandruff and Selenium sulphide, which is a chemical compound and medication used to treat

dandruff, and seborrheic dermatitis, along with ingredients like Zinc pyrithrone and other commercial ingredients which is present in the commercial shampoos. These mentioned ingredients work by destroying the cell membrane of the dandruff and acting as a cytostatic causing fungus preventing its growth.

Based on our results, it can be interpreted that medicated shampoos showed greater antifungal and antidandruff activity compared to commercial shampoos and is a better choice for eradicating this condition.

This study therefore indicates the significance of selecting an appropriate shampoo for treating dandruff based on the ingredients present in it that will help eradicate this condition, which if left untreated may result in hair loss and scalp itching.

CONCLUSION

Dandruff is a condition faced by 50% of the general population worldwide according to the statistics published by the National Institute of Health (*Seborrheic Dermatitis and Dandruff: A Comprehensive Review, 2015. Journal of Clinical and Investigative Dermatology; 3(2): 10.13188/2373-1044.1000019.*). There are several surveys citing that almost 70% of Indian population is faced with this condition in various forms.

This study has confirmed the presence of a lipolytic fungus *Malassezia* as the root cause of dandruff and the comparative study has analysed the antidandruff activity of various commercial and medicated shampoos that are commonly used by the general public and available in markets.

The results establish that medicated shampoos have enhanced efficacy in antidandruff properties compared to commercially available shampoos in treating dandruff. This may be due to the presence of active ingredients present in medicated shampoos that are targeted at dandruff causing fungus. It may also be noted that, commercial shampoos are more preferred by the general population as it might provide alternate benefits like reduction in frizziness of hair and so on.

These findings helps in informed decision making when choosing antidandruff shampoos that contain active ingredients against *Malassezia* and help in managing this scalp condition and associated problems.

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