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IN VITRO EVALUATION OF ANVAYA AYURVEDA'S ANTI-DANDRUFF HAIROIL AGAINST *M. FURFUR* AND *C. ALBICANS* BY MINIMUM INHIBITORY CONCENTRATION METHOD

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ABSTRACT

Dandruff is a common scalp disorder strongly associated with the overgrowth of lipophilic yeasts, particularly *Malassezia furfur*, and opportunistic fungi such as *Candida albicans*. Inhibition of these microorganisms remains a key strategy in the management of dandruff and related seborrheic scalp conditions. The present study aimed to evaluate the in-vitro anti-dandruff activity of Anvaya Ayurveda's *Anti-Dandruff Hair* Oil by determining its minimum inhibitory concentration (MIC) against *M. furfur* and *C. albicans*. *M. furfur* was cultured on RPMI-A medium and incubated at 25 °C for 72h, while *C. albicans* was grown on Sabouraud Dextrose Agar and incubated at 25 °C for 48h. The *anti-dandruff hair oil* was evaluated in its undiluted form and across serial dilutions, with ketoconazole (0.1% w/v) serving as the reference standard. Antifungal activity was assessed using a resazurin-based microdilution assay to determine MIC values. The formulation demonstrated antifungal activity against both test organisms over concentrations ranging from 100% to 0.0488%. Growth inhibition of *M. furfur* was observed at a minimum concentration of 25%, indicating retained activity upon dilution, whereas inhibition of *C. albicans* was observed at the highest tested concentration. These findings demonstrate that Anvaya Ayurveda's *Anti-Dandruff Hair Oil* exhibits *in-vitro* antifungal activity, particularly against *M. furfur*, the primary dandruff-associated pathogen. The *in-vitro* results were complemented by a human volunteer survey, in which individuals with dandruff-prone scalps reported reductions in dandruff severity, scalp flaking, and itching, along with improved scalp comfort following regular use. Collectively, these preliminary findings support the potential of the formulation as a safer and more sustainable approach to dandruff management.

KEYWORDS: *Anti-dandruff oil*; *anti-fungal*; *M.furfur; C.albicans*; ketoconazole; Anvaya Ayurveda.

INTRODUCTION

Dandruff is a highly prevalent, chronic, and benign scalp disorder characterized by excessive flaking of the *stratum corneum*, often accompanied by pruritus and mild inflammation. Although not medically serious, dandruff can significantly affect quality of life due to its visible nature, associated discomfort, and psychosocial impact. The condition demonstrates seasonal variability, with symptom severity frequently increasing during colder months due to low ambient humidity and scalp dryness, while improvement is commonly observed in warmer, more humid environments.

The pathogenesis of dandruff is multifactorial, involving complex interactions between scalp barrier function, sebaceous activity, individual susceptibility, and microbial colonization. Among these factors, the proliferation of *Malassezia* species, lipophilic commensal yeasts naturally present on the scalp, plays a pivotal role. These organisms metabolize sebum-derived lipids into free fatty acids, which can disrupt the epidermal barrier and elicit inflammatory responses, leading to scaling, itching, hair fall and erythema.

A closely related condition, seborrheic dermatitis, represents a more inflammatory spectrum of the same disorder and may present with erythematous plaques and greasy scales extending beyond the scalp. In infants, seborrheic dermatitis manifests as cradle cap, characterized by yellow or white scaling with mild crusting of the scalp during early infancy. This condition is benign and self-limiting, typically resolving spontaneously within the first year of life.

Clinical presentation and disease severity may vary across populations. Individuals with darker skin tones may develop a distinct presentation known as petaloid seborrheic dermatitis, marked by annular or ring-shaped lesions along the scalp margins and a higher likelihood of post-inflammatory pigmentary changes. Understanding these variations is essential for accurate diagnosis and effective management. Despite its high prevalence, dandruff remains an area of active research, particularly with respect to improved therapeutic strategies targeting microbial balance, inflammation, and scalp barrier integrity. Even today ketoconazole remains as one of the main anti-fungal agents among others in treating dandruff.[1]

A variety of synthetic, over-the-counter products with ketoconazole, salicylic acid, zinc pyrithione, selenium sulphide, and lipase inhibitors as prime ingredients, are widely used for dandruff management. Despite their established antifungal activity, these agents often come with limitations such as reduced long-term effectiveness, potential safety concerns, and limited ability to prevent recurrence, thereby necessitating the exploration of safer and more sustainable alternatives. In contrast, herbal and naturally derived formulations offer a safer and multi-targeted approach by targeting multiple pathological mechanisms involved in dandruff, including fungal proliferation, inflammation, and scalp barrier dysfunction. In this context, the present study focuses on the development and evaluation of Anvaya Ayurveda's *Anti-Dandruff Hair Oil* enriched with bioactive constituents, aimed at providing effective antifungal activity against *M. furfur* and *C. albicans* while improving scalp health, promoting hair growth and reducing recurrence.

MATERIALS AND METHODS[2,3,4,5]

M. furfur was cultured on RPMI-A medium and incubated at 25°C for 72h, while *C. albicans* was grown on Sabouraud Dextrose Agar at 25 °C for 48 h. Following incubation, cell suspensions were standardized to 1×10^6 CFU/mL using a digital colorimeter at 620 nm. The anti-dandruff hair oil was evaluated in its undiluted (100%) form. Ketoconazole

(0.1% w/v) prepared in sterile distilled water served as the reference standard. A resazurin working solution was prepared from a stock solution of 2.7mg in 5mL sterile saline and used as a growth indicator.

The minimum inhibitory concentration (MIC) was determined by a broth microdilution method performed in triplicate under aseptic conditions. RPMI broth (for *M. furfur*) or Sabouraud dextrose broth (for *C. albicans*) was dispensed into 96-well microtiter plates. Undiluted test item (200µL) was added to the initial wells and serially two-fold diluted across the plate to obtain the required concentration range.

Ketoconazole (200µL) was added to designated wells and serially two-fold diluted to obtain the required concentration range. Subsequently, 10µL of standardized fungal inoculum (1×10^6 CFU/mL) was added to all wells containing the test item and standard. Positive control wells contained microbial inoculum with broth, while negative control wells contained broth alone.

The microtiter plates were incubated under organism-specific conditions. Following incubation, 20µL of resazurin working solution was added to each well, and plates were further incubated for 1h in the dark. Antifungal activity was assessed visually based on color change and turbidity. A change from purple to pink or colorless indicated fungal growth, whereas retention of purple color indicated growth inhibition. The MIC was defined as the lowest concentration at which no visible growth or color change was observed.

RESULTS

Table 1: *Anti-dandruff* activity of *Anti-dandruff hair oil* against *M. furfur* and *C. albicans.*

Test Item	RR No.	*M. furfur* MIC %	*C. albicans* MIC %	Concentration tested
Anvaya Ayurveda's *Anti-Dandruff Hair Oil*	RR250654	25%	100%	100% to 0.0488%
Ketoconazole (Standard)		0.00019%	0.000048%	0.1% to 0.000048 %

The Anvaya Ayurveda's *Anti-Dandruff Hair Oil* was evaluated for anti-fungal activity against *M. furfur* and *C. albicans* by MIC at different concentrations ranging from 100% to 0.0488%. The oil was able to inhibit the growth of *M. furfur* at 25% concentration, this suggest that even at diluted concentration (25%), the oil effectively worked against *M. furfur*.

Anvaya Ayurveda's *Anti-Dandruff Hair Oil* tested against *C. albicans* showed inhibition at highest concentration (100%).

VOLUNTEER SURVEY TO ESTABLISH THE EFFICACY OF ANVYAYA AYURVEDA'S ANTI-DANDRUFF HAIR OIL

A volunteer-based survey was conducted to assess the real-world efficacy of the Anvaya Ayurveda's *Anti-Dandruff Hair Oil*. In this survey, the participants with self-reported dandruff symptoms were instructed to apply the Anvaya Ayurveda's *Anti-Dandruff Hair Oil* minimum of 1 hour prior to hair wash twice weekly for a duration of 1 month. A follow-up assessment was done on 15th day to monitor its efficacy and compliance of use. The results were recorded, and a 2nd follow-up was done on the 30th day.

Demographic data

A total of 11 eligible subjects were enrolled in the clinical study of *anti-dandruff oil*, comprising nine females and four males in the age group between 18-40 years. Of these, two participants were unavailable for follow-up and were therefore withdrawn, while one participant was considered drop-out due to irregular use of the oil.

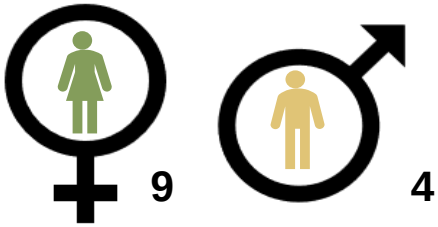
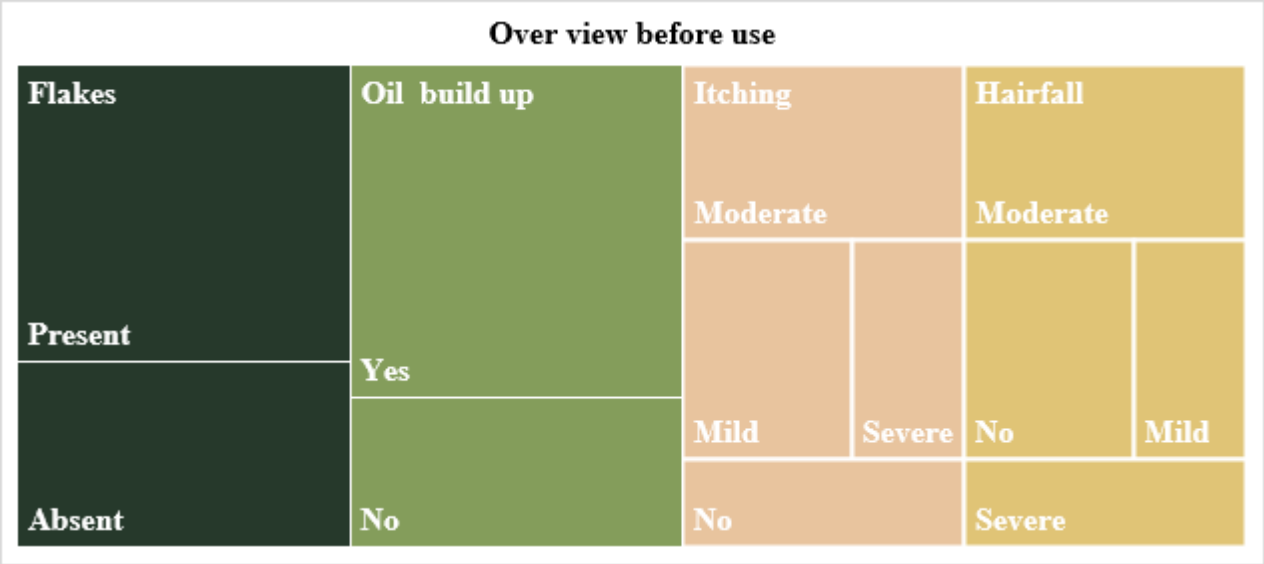


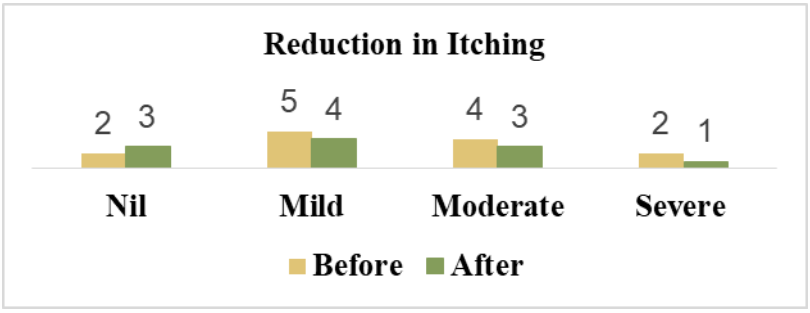
Fig. 1: Demographic data.

At baseline, the primary complaints reported by the subjects included scalp itching, hair fall, visible flakes, and oil build-up after hair wash. Among them, two reported no itching, three had mild itching, four had moderate itching, and two experienced severe itching. With respect to hair fall, three had no complaints, two reported mild, four moderate, and two severe hair falls. Regarding flakes, five subjects reported none, while eight presented with scalp flakes. Additionally, nine participants complained of post-wash oil build-up, whereas four reported no such concern.



Effect on itching after use of the trial oil

The number of participants reporting increased from two to three after intervention, while those with mild itching decreased from five to four, moderate itching from four to three and severe itching from two to one following intervention.

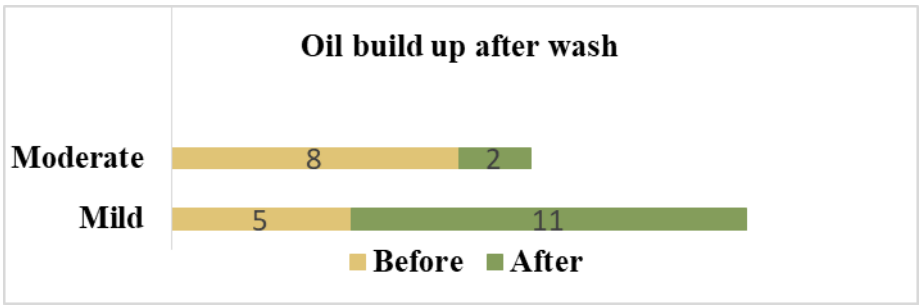


Impact on hair fall after intervention

At baseline, four subjects reported no hair fall despite having dandruff; this number increased to five after the intervention. Two participants with mild hair fall showed no improvement. The number of subjects with moderate hair fall increased from three to four, while the three participants with severe hair fall did not experience any improvement.

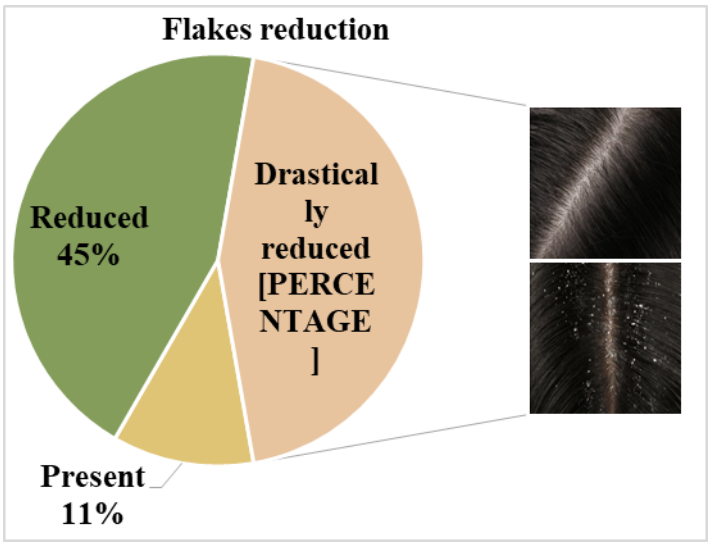
Effect on oil-build up post hair wash

Five subjects reported mild oil build-up before the intervention, which increased to 11 after the intervention. In contrast, eight subjects initially reported moderate oil build-up, and this number decreased to two following the intervention.



Effect on reduction of flakes

Among the eight responses received, 45% reported a reduction in flake formation, 44% experienced a drastic reduction, while 11% reported no reduction after the intervention.



DISCUSSION

Dandruff is a common scalp disorder which affects almost half of the young population of both genders. The pathogenesis involves hyperproliferation of dandruff which is resulting in deregulation of keratinization.

Ayurveda perspective on treatment of Dandruff

Dandruff can be correlated to *Darunaka* in Ayurveda. According to *Acharya Vagbhatta* and *Acharya Sarangadhara*. *Darunaka* is a *kapalagata roga* (scalp disease) but *Acharya Susruta* and other *Acharyas* mentioned it under *kshudra roga*. Dry dandruff is caused because of *Vata Pradhana Kapha dosha* and wet dandruff due to *Kapha Pradhana Vata dosha*. The *Pitta dosha* also play an important role in genesis of dandruff. The increase of *Vata and Pitta* in the body leads to destruction of *Rakta dhatu*, thus causing vitiation of blood. So, the line of treatment should be based on *Tridosha dushti* incorporating *Rakta* with it. The symptoms of Dandruff according to Ayurveda are: 1) *Shirah kandu* [itching on scalp] 2) *Twak sputana* [cracks on the scalp] 3) *Sookshma pidaka* [minute pustules] 4) *Shirah daha* [burning sensation on head] 5) *Kesha chyuti* [hair fall].

The causative factors for *Darunaka* are non-application of hair oil, poor scalp hygiene, sleeping during day time, night vigil, exposure to dust, heat etc., So, treatment protocol used here is *Hetu Viparita Chikitsa*, i.e identification of cause - identify the *dosha prakopa* due to cause -identification of which quality increased in that *dosha prakopa* due to that specific cause - to stop the usage of that specific cause. Treatment is given to to reverse the pathology of *dosha* caused by this specific cause.[6,7,8]

Rising Demand for Natural and Herbal Hair Care Products

One of the primary drivers of the global hair oil market is the increasing consumer preference for natural and herbal formulations. Consumers today are more conscious about the ingredients used in personal care products and actively avoid those containing harsh chemicals, sulfates, and parabens. This has resulted in a noticeable shift toward hair oils enriched with natural ingredients such as coconut, argan, amla, jojoba, castor, and almond oils. Herbal infusions and Ayurvedic solutions are also gaining popularity, particularly in regions like Asia-Pacific, where traditional remedies are deeply rooted in cultural practices.

The clean beauty movement has further amplified the demand for organic and plant-based hair oils that promote scalp nourishment, hair growth, and overall health without harmful side effects. Brands are responding by offering certified organic products and highlighting sustainable sourcing methods, appealing to eco-conscious consumers. For instance, cold-pressed oils that retain nutrients and vitamins are highly sought after. This rising focus on natural and herbal solutions has become a major growth catalyst, with both established and emerging brands positioning themselves around authenticity and transparency in ingredient use.[9]

Conventional treatments for dandruff and hair fall (topical/systemic) are extensively employed, these include keratolytic agents, anti-inflammatory, and antimicrobial agents. The need for novel and reliable methods for dandruff and hair fall management is crucial. Medicinal plants offer promising alternatives, as they are rich sources of biologically active metabolites. Plant extracts and essential oils exhibit potent antioxidant, antimicrobial, and anti-inflammatory activities, often with fewer adverse effects, making them attractive candidates for effective and sustainable scalp and hair care management.

The Anvaya Ayurveda's *Anti-Dandruff Hair Oil* is specially curated with the richness of *Cajuput oil, Rosemary oil, Ginger, Coconut oil*. The blend of the ingredients works efficiently in mitigating the fungal growth, relieving of symptoms and promoting hair growth.

Melaleuca cajuputi. cajuput tree is widely used in the traditional medicine of South Eastern Asia. The antimicrobial constituents of cajuput oil are identified as *1,8-cineol, linalool, terpinen-4-ol, α-terpineol, and 3,5-dimethyl-4,6-di-O methylphloroacetophenone*, among which 1,8-*cineole and α-terpineol* have shown significant anti-fungal activity against *C.albicans*.[10,11,12]

Rosemary oil is a highly volatile oil extracted from fresh flowering tops of Rosemary plant (*Salvia Rosmarinus*). It is recognized for its valuable phytoconstituents, *carnosic* and *rosmarinic acid, volatile oil*, and *phenolics* along with their derivatives which have antioxidant, anti-microbial, anti-inflammatory and neuroprotective diabetic activity. They also aid in hair growth, nourishes scalp and relieves dryness.[13,14]

Ginger *(Zingiber officinale)* rhizomes contain a diverse array of essential oils, minerals, and pharmacologically active constituents, particularly *gingerols* and *shogaols*, which contribute to their conditioning and protective effects on hair and scalp. These bioactive compounds exhibit significant anti-inflammatory and antiseptic activities, rendering ginger especially beneficial in the management of prevalent scalp conditions. By mitigating inflammatory responses, ginger helps soothe scalp irritation, pruritus, and dryness, thereby improving overall scalp comfort.[15]

Coconut oil is an edible oil extracted from the kernel of mature coconuts of the coconut palm. For thousands of years tropical countries have used coconut from the tree *Cocos nucifera*, as an integral part of their diet and livelihood. Known as "kalpa vriksha", in Sanskrit, this interprets as the palm which supplies all the necessities of life. In recent years this oil has attained superstardom in the health food world. Phenolic compounds of coconut consist *of tocotrienols, polyphenols and tocopherols. Lauric acid* is the most abundant fatty acid. It is an effective anti-inflammatory and anti-microbial agent, helps relieve dandruff, itchy scalp, dryness and promotes hair growth.[16]

The findings from this *in-vitro* study provide a scientific rationale for further evaluation of the anti-dandruff oil formulation as a natural approach for managing dandruff and related scalp conditions. Future *in-vivo* and clinical studies are warranted to confirm anti-fungal efficacy against *M. furfur* and *C. albicans* under physiological conditions and to elucidate underlying mechanisms. Collectively, these would enhance the translational relevance of the formulation and support its development as a safe, effective, and evidence-based natural anti-dandruff product.

CONCLUSION

The present study demonstrates that the Anvaya Ayurveda's *Anti-Dandruff Hair Oil* possesses significant in-vitro antifungal activity, particularly against *M. furfur*, the primary etiological agent implicated in dandruff. The ability of the formulation to inhibit fungal growth even at minimum concentration of 25% highlights its intrinsic antifungal potential. These findings were further supported by outcomes from a volunteer-based survey, which indicated improvements in dandruff severity, scalp flaking, itching, and overall scalp comfort following regular use. Together, the concordance between in-vitro efficacy and real-world user-reported outcomes suggests that the formulation may serve as an effective, safer, and sustainable alternative to conventional synthetic antifungal agents for dandruff management.

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