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# Formulation, Evaluation and Analysis of Antidandruff Activity of Polyherbal Shampoo

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**Abstract**

Dandruff is a chronic, relapsing scalp disorder characterized by excessive scaling, pruritus, and mild inflammation, predominantly associated with the proliferation of Malassezia species. Conventional anti-dandruff treatments rely on synthetic antifungal agents such as Ketoconazole and Zinc pyrithione, which may produce adverse effects upon long-term use. The present investigation aimed to formulate and evaluate a polyherbal anti-dandruff shampoo using extracts of Ocimum tenuiflorum L., Hibiscus rosa-sinensis, and Aloe vera. Ethanolic extracts were prepared via maceration and incorporated into a shampoo base. The formulations were evaluated for physicochemical properties including pH, viscosity, surface tension, solid content, and foaming ability. Antifungal activity was assessed against Candida albicans using the agar well diffusion method. Among the tested formulations (1:1:1, 2:1:1, 3:1:1), the 3:1:1 ratio demonstrated the highest antifungal activity with a maximum zone of inhibition of 23 mm. The results suggest that the optimized polyherbal shampoo possesses promising antifungal potential and acceptable cosmetic properties, supporting its use as a safer alternative to synthetic formulations.

**Keywords:** Polyherbal shampoo; Anti-dandruff; Pityriasis capitis; Malassezia; Aloe vera; Hibiscus rosa-sinensis; Ocimum tenuiflorum L; Antifungal activity; Herbal formulation.

## 1. INTRODUCTION

Dandruff is one of the most prevalent dermatological scalp disorders worldwide, affecting nearly half of the global population at some stage of life. It manifests as visible flakes of dead epidermal cells accompanied by itching, dryness, and occasional inflammation. Though not life-threatening, dandruff significantly impacts psychological well-being and social confidence.

The condition is primarily associated with the overgrowth of lipophilic yeasts belonging to the genus Malassezia. These fungi metabolize sebum triglycerides into free fatty acids, leading to irritation of the scalp and accelerated turnover of keratinocytes. Environmental factors such as humidity, stress, hormonal imbalance, and poor hygiene further contribute to disease progression.

Conventional management includes antifungal and keratolytic agents such as Clotrimazole, Selenium sulfide, and coal tar preparations. While effective, these treatments may cause adverse reactions including irritation, erythema, dryness, and rebound recurrence upon discontinuation.

The increasing awareness of chemical-associated side effects has led to renewed interest in herbal therapeutics. Polyherbal formulations combine multiple plant extracts to achieve synergistic effects, enhancing therapeutic efficacy while minimizing toxicity. Traditional systems of medicine, including Ayurveda, have long utilized medicinal plants for scalp health and dandruff control.

## 2. LITERATURE REVIEW

- Dandruff is a common scalp condition linked to flaking, itching, and fungal growth (especially Malassezia species). Herbal remedies are considered safer alternatives to synthetic treatments. A study by Baig et al. (2021) showed that Hibiscus extract (1.5 mg) and Aloe vera (400 µL) had antifungal activity against *Candida albicans,* supporting their use in dandruff control. Aloe vera soothes and moisturizes the scalp, while Hibiscus nourishes it. The formulation showed suitable pH, good stability, and no skin irritation, indicating safety and effectiveness.
- Sivareddy et al. evaluated the antifungal activity of Tulsi (Ocimum sanctum) against *Candida albicans* using the disc diffusion method. The ethanolic extract showed dose-dependent activity, producing a 13 mm zone of inhibition at 1 mg/mL. This effect is attributed to phytochemicals like eugenol and linalool, suggesting Tulsi as a useful natural antifungal ingredient in polyherbal anti-dandruff formulations.

## 3. DANDRUFF

### 3.1. Definition

Dandruff, also known as pityriasis capitis, is a common chronic scalp disorder characterized by excessive shedding of dead skin cells from the scalp. It presents clinically as white or yellowish flakes accompanied by itching and mild irritation. Although not a serious medical condition, dandruff is recurrent and often requires long-term management.

### 3.2. Epidemiology

Dandruff affects nearly 40–50% of the adult population worldwide. It commonly appears after puberty due to increased sebaceous gland activity and is more prevalent in individuals aged

15–50 years. Males are slightly more affected than females, possibly due to higher androgen- induced sebum production. Environmental factors such as humidity, stress, and pollution can aggravate the condition.

### 3.3. Etiology and Causative Organisms

The primary cause of dandruff is the overgrowth of lipophilic yeasts belonging to the genus Malassezia, particularly:

- Malassezia globosa
- Malassezia restricta
- Malassezia furfur

These fungi are part of the normal scalp flora but proliferate excessively under favourable conditions such as increased sebum production, hormonal imbalance, or poor scalp hygiene.

### 3.4. Pathophysiology

Dandruff develops due to the interaction of three main factors:

- Excess sebum production
- Overgrowth of Malassezia species
- Individual susceptibility

The fungi release lipases that break down sebum triglycerides into free fatty acids, causing irritation and inflammation. This leads to accelerated turnover of epidermal cells, resulting in visible flakes and itching.

### 3.5. Classification

Dandruff is classified into:

**A. Dry Dandruff (Pityriasis Simplex)**

- Fine, dry white flakes
- Minimal inflammation

- Associated with dry scalp

**B. Oily Dandruff (Pityriasis Steatoides)**

- Greasy, yellowish flakes
- Associated with excess sebum
- May show mild redness

### 3.6. Clinical Features

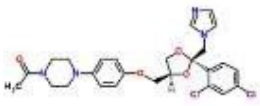
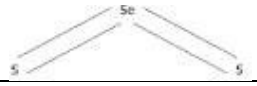
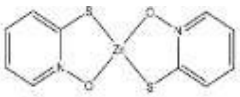
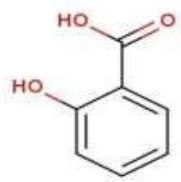
Common symptoms include:

- White or yellow flakes on scalp
- Itching
- Dry or oily scalp
- Mild redness
- Temporary hair fall in severe cases

### 3.7. Management Overview

Treatment focuses on reducing fungal growth and controlling scalp oiliness. Synthetic antifungal agents such as Ketoconazole and Selenium sulfide are commonly used, but long- term use may cause irritation and dryness. This has encouraged the development of safer herbal alternatives.

## 4. ALLOPATHIC SYSTEM OF MEDICINE

| Active Ingredient | Structure | Drug Class | Mechanism of Action | Common Adverse Effects |
|---|---|---|---|---|
| toconazole |  | Imidazole antifungal | Inhibits fungal ergosterol synthesis ,disrupting cell membrane integrity of Malassezi species | Scalp irritation ,dryness, hair texture changes |
| Selenium sulfide |  | Cytostatic & antifungal agent | Reduces epithelial cell turnover and suppresses fungal growth | Oily scalp, discoloration of hair, irritation |
| Zinc pyrithione |  | Antifungal & antibacterial agent | Inhibits fungal proliferation and reduces microbial load on scalp | Mild burning sensation,dryness |
| Salicylic acid |  | Keratolytic agent | Promotes shedding of dead skin cells and removes scales | Excessive dryness, scalp peeling |

## 5. HERBS USED FOR THE TREATMENT OF DANDRUFF

- Tulsi (*Ocimum tenuiflorum*) – Antifungal and anti-inflammatory activity
- Hibiscus Flower (*Hibiscus rosa-sinensis*) – Nourishes scalp and reduces flaking
- Aloe *(Aloe vera*) – Moisturizing and soothing effect
- Neem (*Azadirachta indica*) – Strong antifungal activity
- Tea Tree (*Melaleuca alternifolia*) – Controls dandruff-causing fungi
- Rosemary (*Rosmarinus officinalis*) – Improves scalp circulation

- Thyme (*Thymus vulgaris*) – Antimicrobial activity
- Sage (*Salvia officinalis*) – Reduces scalp irritation
- Garlic *(Allium sativum*) – Natural antifungal properties
- Bhringraj (*Eclipta alba*) – Supports scalp health
- Hibiscus leaves (*Hibiscus rosa-sinensis*) – Helps reduce dryness
- Guava (*Psidium guajava*) – Antioxidant and antimicrobial
- Henna (*Lawsonia inermis*) – Cooling and antimicrobial
- Pomegranate *(Punica granatum*) – Antioxidant activity
- Senna (*Cassia fistula*)– Traditional scalp treatment
- Kusum (*Schleichera oleosa*) – Helps maintain healthy scalp

**5.1. Plant Profile**

**A. Hibiscus**

- Synonyms: Jaswand, Shoe flower, Jaswandi
- Biological source: Flowers of Hibiscus rosa-sinensis
- Family: Malvaceae
- Chemical constituents: Taraxeryl acetate, beta-sitosterol, stigmasterol, cholesterol, flavonoids, vitamins, and niacin.
- Uses: Hibiscus has antidandruff properties and helps maintain scalp health. It nourishes the scalp, reduces flaking, and supports healthy hair.

**B. Aloe**

- Synonym: Acemannan
- Biological source: Pulp obtained from the leaves of Aloe vera
- Family: Asphodelaceae
- Chemical constituents: Aloins (barbaloin, beta-barbaloin, isobarbaloin),
- emodin, aloe-emodin, and resins.
- Uses: Aloe helps reduce dandruff by moisturizing and soothing the scalp. It
- Relieves itching, reduces flaking, and promotes a healthy scalp.

- Synonym: Holy Basil
- Biological source: Fresh and dried leaves of *Ocimum tenuiflorum*

- Family: *Lamiaceae*
- Chemical constituents: Eugenol, methyl eugenol, carvacrol, caryophyllene, and phenolic compounds such as apigenin and rosmarinic acid.
- Uses: Tulsi has antifungal and antimicrobial properties that help control dandruff. It reduces scalp irritation, fights scalp infections, and promotes a healthy scalp.

## 6. MATERIALS AND METHODS

### 6.1. Authentication and collection of Plant Materials

*6.1.1. Authentication*

Fresh leaves of Tulsi, Aloe and Hibiscus were collected locally and authenticated by a qualified taxonomist.

*6.1.2. Collection*

- Tulsi and Hibiscus

The leaf of Tulsi and hibiscus were collected from the local area of Madurai.

- Aloe Leaf

A standard sample of aloe vera gel was extracted by simple drain procedure, where 2-4 leaves of aloe were cut at about ½ inch from the base so as to drain out all the yellow sap material.

### 6.2. Preparation of Extracts

*6.2.1. Maceration Method (Tulsi and Hibiscus)*

Plant leaves were washed, shade dried, and coarsely powdered. The powdered Tulsi and Hibiscus leaves were extracted with 95% ethanol for three days with occasional shaking. The extracts were then vacuum filtered and concentrated under reduced pressure using a rotary evaporator.

*6.2.2. Maceration Method (Aloe)*

Fresh Aloe vera leaves were washed with distilled water, and the yellow latex (containing aloin) was drained by cutting the base to prevent irritation. The outer peel was removed, and the clear inner gel was collected by gentle pressing. The gel was macerated with 95% ethanol for three days with occasional shaking for proper extraction. After filtration using Whatman No. 1 filter paper, the extract was concentrated using a rotary evaporator and the dried extract was collected and stored.

**Fig 1.** *Macerated Hibiscus, Aloe, Tulsi extract*

### 6.3. Phytochemical Screening

Preliminary phytochemical analysis confirmed:

- Flavonoids in Hibiscus
- Anthraquinone glycosides in Aloe vera
- Phenolic compounds in Tulsi

These bioactive constituents are known to contribute to antifungal and antioxidant activity.

### 6.4. Formulation of Polyherbal Shampoo Ingredients

| Ingredient | Quantity | Function |
|---|---|---|
| Tulsi extract | 10 mL | Antifungal |
| Hibiscus extract | 10 mL | Conditioning |
| Aloe vera extract | 10 mL | Moisturizing |
| Sodium lauryl sulfate | 10 g | Surfactant |
| Methyl cellulose | 2 g | Thickener |
| Sodium benzoate | 0.02 g | Preservative |
| Distilled Water | q.s | Vehicle |

**Fig 2.** *Prepared Herbal Shampoo Product*

## 7. METHOD

- Measured 10 mL each of Tulsi extract, Hibiscus extract, and Aloe vera extract. (3:1:1)
- The extracts were mixed thoroughly to obtain a uniform polyherbal base.
- Sodium lauryl sulfate (10 g) was gradually added to the herbal mixture.
- The mixture was triturated continuously to ensure proper blending and formation of foam base.
- Methyl cellulose (2 g) was added slowly while triturating to achieve the desired viscosity and consistency.
- Sodium benzoate (0.02 g) was added as a preservative and mixed uniformly.
- The final mixture was triturated until a homogeneous, smooth shampoo was obtained.
- The prepared polyherbal shampoo was transferred into a clean, airtight container and stored at room temperature for further evaluation

### 7.1. Evaluation of Formulation

*7.1.1. Organoleptic Properties*

Herbal shampoo formulation was evaluated for its:

- Color
- Physical state
- Odor
- Solubility and the results were reported in table.

**Fig 3.** *Organoleptic Evaluation*

### 7.2. pH Determination

10% v/v shampoo solution is prepared in distilled water and pH of this solution was measured with digital pH meter at room temperature 30±2°C and the results were reported in table.


**Fig 4.** *pH tested on pH paper and Electronic pH meter*

### 7.3. Solid Content

A clean dry dish was weighed and added with 4 grams of shampoo. The dish with shampoo was weighed. The exact weight of the shampoo was calculated. The dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight after drying was calculated and the results were reported in table.

**Fig 5.** *Solid content analysis*

### 7.4. Surface Tension

Dilute the shampoo using distilled water to fix 10% as concentration. Measurements were carried out using stalagmometer.

$\gamma_2 = \gamma_1 (\rho_2 n_1)/ (\rho_1 n_2)$

γ is surface tension, ρ is density, n is the number of drops, and subscripts 1 and 2 refer to a standard liquid (water) and the test liquid, respectively and the results were reported in table.

**Fig 6.** *Surface Tension Evaluation*

### 7.5. Viscosity

The viscosity of the shampoo was determined by using Brookfield Viscometer (Model DV-l Plus, LV, USA) set at different spindle speeds from 0.3 to 10 rpm3. The viscosity of the shampoos was measured by using spindle T95. The temperature and sample container's size was kept constants during the study and the results were reported in table.

Viscosity (cP)= Dial Reading X Factor

Where:

Dial Reading = Value shown on the Brookfield viscometer scale

Factor = Constant value depending on spindle number and RPM (given in the Brookfield factor chart)

cP = Centipoise (unit of viscosity)

### 7.6. Foam Stability

Cylinder shake method was used. 15ml of 1% solution of shampoo is taken in graduated cylinder (1.5ml in 15ml water), shake for ten minutes and record the foam produced after 1 minute. Record the stability of foam after 4-5 minutes and the results were reported in table.

**Fig 7.** *Foam Formation and Stability*

## 8. RESULTS AND DISCUSSION

| Parameters | Observation |
|---|---|
| 1. Colour | Green |
| 2. Odour | Pleasant |

| | |
|---|---|
| 3. Clarity | Milky |
| 4. Physical State | Semi-solid |
| 5.Solubility | Water soluble |
| 6. p H | 5.91 |
| 7. % Solid content | 25 % |
| 8.Viscosity | |
| 9. Surface tension | 32.4 dynes/cm |
| 10. Foam formation & Foam stability | 15cm & 5 Mins |

### 8.1. Antifungal Activity

*8.1.1. Aim*

To evaluate the antifungal activity of the formulated polyherbal shampoo against *Candida albicans* using the agar well diffusion method.\

*8.1.2. Test Organism*

*Candida albicans*

Since *Malassezia furfur* is difficult to culture and maintain under laboratory conditions, *Candida albicans* is commonly used as a test organism to evaluate antifungal activity in vitro. Therefore, in this study, antifungal activity was assessed against *Candida albicans* as a representative fungal organism to indicate the potential anti-dandruff activity of the formulation.

*8.1.3. Composition of Test Formulations*

Three different formulations were prepared by varying the concentration of Ocimum tenuiflorum(Tulsi), while the amounts of Hibiscus rosa-sinensis and Aloe were kept constant.

| Formulation | Tulsi | Hibiscus | Aloe |
|---|---|---|---|
| 1:1:1 | 1 mg | 1.5 mg | 400µL |
| 2:1:1 | 2 mg | 1.5 mg | 400µL |
| 3:1:1 | 3mg | 1.5 mg | 400µL |

## 9. METHODOLOGY

- The antifungal activity was determined using the agar well diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines.
- Mueller Hinton Agar plates were prepared and sterilized.
- The plates were inoculated with *Candida albicans* under aseptic conditions.
- The inoculum was standardized using 0.5 McFarland standard.
- Wells were made using a sterile cork borer. Different volumes of the test sample (25 µL, 50 µL, 75 µL, and 100 µL) were added into the wells.
- A ketoconazole disc was placed as the positive control.
- Distilled water was used as the negative control.
- Plates were incubated at 37°C for 24 hours.
- After incubation, the zone of inhibition was measured in millimetres.


**Fig 8.** *Zone of Inhibition of (1:1:1) ratio*


**Fig 9.** *Zone of Inhibition of (2:1:1) ratio*


**Fig 10.** *Zone of Inhibition of (3:1:1) ratio*

## 10. RESULTS

| Formulation | Positive Control (Ketoconazole) | Negative Control (Distilled Water) | 25µl | 50µl | 75µl | 100µl |
|---|---|---|---|---|---|---|
| 1:1:1 | 33mm | No zone | 16mm | 17mm | 19mm | 20mm |
| 2:1:1 | 33mm | No zone | 12mm | 17mm | 19mm | 20mm |
| 3:1:1 | 33mm | No zone | 16mm | 17mm | 19mm | 23mm |

## 11. DISCUSSION

The antifungal activity of the polyherbal shampoo formulations was evaluated against *Candida albicans*, a common fungal pathogen responsible for scalp infections and dandruff. All three formulations showed zones of inhibition, indicating the presence of antifungal activity. The results demonstrated a concentration-dependent increase in antifungal activity, as the zones of inhibition increased with higher sample volumes. Among the tested formulations, the 3:1:1 ratio exhibited the highest antifungal activity, with a maximum zone of inhibition of 23 mm at 100 µL. This suggests that increasing the concentration of Ocimum tenuiflorum improves the antifungal effect of the formulation. Tulsi is known for its strong antimicrobial properties due to the presence of bioactive compounds such as eugenol, ursolic acid, and essential oils. These compounds can disrupt fungal cell membranes and inhibit fungal growth. Hibiscus contributes antifungal activity through flavonoids, tannins, and organic acids, while also providing conditioning and scalp-soothing effects. Aloe enhances the formulation by providing moisturizing, anti-inflammatory, and mild antimicrobial properties. The consistent inhibition observed in all formulations suggests a synergistic effect among the herbal ingredients. Although the herbal formulations showed lower activity compared to the standard drug ketoconazole, they still demonstrated significant antifungal potential with the added advantage of being natural and safer for regular use. The absence of inhibition in the negative control confirms that the antifungal activity was due to the herbal ingredients and not the solvent.

## 12. CONCLUSION

The antifungal evaluation confirmed that all three polyherbal shampoo formulations exhibited inhibitory activity against *Candida albicans*. The activity increased with concentration. The 3:1:1 formulation

showed the highest antifungal effect. Maximum zone of inhibition observed: 23 mm at 100 µL. Therefore, the 3:1:1 formulation can be considered the optimized formulation for antifungal activity. The developed polyherbal shampoo shows promising potential as a natural alternative for the management of fungal scalp conditions.

## 13. FUTURE SCOPE

### 13.1. Advanced Extraction Techniques

Improved extraction methods can increase the yield of active compounds and enhance anti-dandruff activity. For example, cold extraction of Tulsi helps preserve antifungal components.

### 13.2. Nano-Herbal Formulations

Nano-formulations reduce herbal extracts into tiny particles that penetrate deeper into the scalp, improving absorption and antifungal effectiveness. They provide better penetration, controlled release, improved stability, and require smaller quantities. Examples include nano-encapsulated Aloe vera for long-lasting hydration and nano Tulsi extract for enhanced antifungal action.

### 13.3. Sulfate-Free Formulations

Future shampoos may replace harsh sulfates with mild plant-based cleansers to reduce scalp dryness and irritation.

### 13.4. Natural Preservatives

Natural preservatives such as neem extract or vitamin E can be used instead of synthetic preservatives to improve product safety.

### 13.5. Optimized Polyherbal Combinations

Research can help determine the best ratio of Tulsi, Hibiscus, and Aloe vera for maximum anti-dandruff effectiveness. For example, higher Tulsi content may improve antifungal activity, while more Aloe vera may benefit dry scalp.

### 13.6. Scalp-Specific Formulations

Different formulations may be developed for different scalp types, such as Tulsi- rich shampoos for oily scalp and Aloe vera-rich shampoos for dry scalp.

### 13.7. Clinical Validation

Future studies and clinical testing on volunteers can help confirm the safety and effectiveness of polyherbal anti-dandruff shampoos.

### 13.8. Eco-Friendly Formulations

Eco-friendly shampoos may use biodegradable ingredients, plant-based surfactants, and recyclable packaging to reduce environmental impact.

### 13.9. Herbal Encapsulation Technology

Encapsulation protects herbal ingredients and allows controlled release during washing. For example, encapsulated Aloe vera beads can release moisturizing agents gradually.

### 13.10. Natural Surfactants and Herbal Oils

Natural surfactants like soapnut (reetha) can improve foaming and cleansing, while herbal oils such as coconut or neem oil can enhance scalp nourishment and dandruff control.

### 13.11. Dermatological Testing

Dermatological testing and patch tests can ensure that herbal shampoos are safe and non-irritating for regular use.

### 13.12. Novel Drug Delivery Systems (NDDS)

NDDS improves the delivery of herbal ingredients like Tulsi, Hibiscus, and Aloe vera into the scalp for better absorption and longer action. It enhances stability, reduces loss of active ingredients, and increases effectiveness.

**13.13. Types of NDDS include:**

- Nanoparticles: Improve scalp penetration (e.g., nano Aloevera for moisturization).
- Liposomes: Provide slow release of herbal extracts (e.g., Tulsi for antifungal action).
- Nanoemulsions: Improve spreading and absorption on the scalp.
- Microcapsules: Release herbal extracts during shampooing (e.g., Hibiscus for conditioning).

Controlled Release Systems: Provide long-lasting hydration and anti- dandruff effects.

## 14. CONCLUSION

The present study successfully developed and evaluated a polyherbal anti-dandruff shampoo containing extracts of *Ocimum tenuiflorum, Hibiscus rosa-sinensis*, and *Aloe vera*. The optimized 3:1:1 formulation demonstrated significant antifungal activity against *Candida albicans* along with acceptable physicochemical characteristics. The findings support the potential of polyherbal shampoos as safe, effective, and sustainable alternatives to conventional synthetic anti-dandruff products.