

## Original Research

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## Comparative Evaluation of Herbal and Commercial Anti-Dandruff Shampoos

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### KEYWORDS:

Anti-dandruff shampoo; Herbal shampoo; Azadirachta indica; Ocimum tenuiflorum; Aloe vera; Hibiscus; Tea tree oil; Malassezia; Physicochemical evaluation; Performance evaluation; Comparative study

**ABSTRACT:** Dandruff is one of the most common scalp conditions, caused by overgrowth of *Malassezia* genus, which causes flaking, itching and irritation of the scalp. Synthetic antifungal shampoos are readily available and help control dandruff, but may cause problems like dryness, irritation and allergies when used for a long time. So the herbal anti-dandruff shampoos made using extracts of medicinal plants have attracted a great deal of interest as safer and environmentally-friendly alternatives. The purpose of the present study was to compare the properties of the prepared herbal anti-dandruff shampoos and commercially available anti-dandruff shampoos with regard to their physical characteristics, physicochemical properties, performance attributes, and safety profile. Six formulations of herbal shampoo using neem and tulsi, aloe vera, hibiscus, tea tree oil were prepared using a standardized shampoo base and marketed anti-dandruff shampoo formulations (five) were compared. Standard evaluation procedures were employed for the evaluation of formulations with regard to organoleptic characteristics, pH, solid content (%), density, viscosity, surface tension, wetting time, dirt dispersion, foaming ability, foam stability, cleansing effect, conditioning effect, ease of rinsing, wet combability and dry combability, as well as literature-based safety assessment. All the prepared herbal formulations showed acceptable physical appearance, pH that is compatible to scalp, satisfactory viscosity and good foaming property with cleansing efficiency similar to the commercial shampoos. The prepared formulations were evaluated for their physicochemical properties and performance characteristics. Among them, the polyherbal formulation exhibited the most balanced overall properties due to the combination of multiple medicinal plant extracts. The herbal anti-dandruff shampoos demonstrated comparable quality and performance, indicating their potential as alternatives to commercially available anti-dandruff shampoos.

### 1.1 Hair Anatomy

The human hair is a keratinized structure that emerges from a hair follicle, which is a mini-organ that grows out of the epidermis into the dermis. It acts as a sun-screen, protects from mechanical damage and plays a role in thermoregulation and sensory perception. Between 80,000 and 150,000 hair follicles are on the scalp, depending on the individual's genetics and ethnicity. [1] There are several parts to the hair; the hair shaft, hair bulb, dermal papilla, hair matrix, inner root sheath, outer root sheath, sebaceous gland, and arrector pili muscle. The visible shaft of hair has three layers: cuticle, cortex and medulla. Of these, the bulk of the hair comes from the cortex, which determines its tensile strength, elasticity and pigmentation. [2] Cycles in hair growth include 4 phases, namely anagen (growth), catagen (regression), telogen (resting), and exogen (shedding). In physiological conditions, almost 85-90% of the scalp hair follicles are in anagen phase with the remaining ones being in the other phases. An imbalance of this cycle can lead to different disorders like alopecia and dandruff. [3]

### 1.2 Definition of Dandruff

Dandruff is a non-inflammatory, common, chronic condition of the scalp in which excessive shedding of fine white or gray flakes occur from the stratum corneum, or outer layer of the scalp, and is frequently accompanied by pruritus (itching). It is considered to be the least severe clinical form of seborrheic dermatitis and occurs mainly on the scalp without any demonstrable

erythema or excessive inflammation. Dandruff is not contagious or life-threatening, but it can impact a person's sense of self, their quality of life, and cosmetics. It is caused by a high epidermal cell turnover which causes abnormal desquamation of corneocytes that are seen as flakes on the scalp. <sup>[4, 5]</sup> Dandruff is thought to be a multifactorial condition, where the interaction of the factors of sebum production, *Malassezia* lipophilic yeast, epidermal barrier dysfunction and individual susceptibility are considered. Unlike scalp diseases like tinea capitis or psoriasis, dandruff is a disease that flakes off in a diffuse manner without causing the hair to fall out. Both environmental and host-related factors can also have an impact, as symptoms tend to be worse during cold weather, stress, hormonal changes or increased activity of the sebaceous glands. <sup>[6, 7, 8]</sup>

## 1.3 Causes of Dandruff

The etiology of dandruff is multifactorial with complex factors associated with microbial colonization, sebaceous gland activity, impaired epidermal barrier function and susceptibility of host. At present there is some indication that dandruff is not caused by inadequate personal hygiene, but rather occurs following a balance of biological and environmental factors that then cause the disruption to normal scalp homeostasis. The three major factors which are always linked to dandruff pathogenesis are colonization with *Malassezia* species, sebum production, and individual susceptibility. <sup>[9, 10]</sup> Of these, *Malassezia globosa* and *Malassezia restricta* are the main fungal species that are linked to dandruff. In these lipid-dependent yeasts, triglycerides are broken down to free fatty acids like oleic acid by lipase enzymes present in the sebum. Oleic acid in susceptible individuals can enter the stratum corneum, break the barrier function of the epidermis, increase local inflammation response and promote the proliferation of keratinocytes, which causes excessive flaking. <sup>[11, 12]</sup> Other causes of dandruff include too oily skin, genetic susceptibility, oxidative stress, too aggressive cosmetics, shampooing too rarely, changing seasons, emotional stress, hormonal imbalance, nutrient deficiencies and immune dysfunction. All of these contribute to the changes in barrier function and microbial balance of the skin on the scalp, which makes them more prone to dandruff. The knowledge of these etiological factors leads to the scientific basis in the development of effective anti-dandruff shampoos with antifungal, keratolytic, anti-inflammatory and sebum-regulating agents. <sup>[13, 14, 15]</sup>

## 1.4 Role of *Malassezia* Species in Dandruff Pathogenesis

*Malassezia* species are lipid-dependent, basidiomycetous yeasts that constitute a dominant Member of the normal human scalp flora. In normal physiological conditions, these fungi are not able to cause disease and co-exist with the host. Changes in sebum composition, integrity of the epidermal barrier and immune response of the host, however, can allow *Malassezia* to become opportunistic pathogens responsible for dandruff and seborrheic dermatitis <sup>[17]</sup>. There are 18 known species of *Malassezia*, the two most common species found in the human scalp are *Malassezia globosa* and *Malassezia restricta*, and they are more strongly correlated with dandruff. These organisms have high lipase and phospholipase activities which break down the triglycerides in the sebum into free fatty acids such as oleic acid and arachidonic acid. These metabolites can affect the stratum corneum barrier, enhance transepidermal water loss, trigger inflammatory mediators and speed up keratinocyte turnover. <sup>[18]</sup>

## 1.5 Anti-Dandruff Shampoos

Anti-dandruff shampoos are a special type of cosmetic and therapeutic shampoo to eliminate dandruff, reduce itching, and aid in the control of microorganisms that play a role in the production of dandruff, such as *Malassezia* species. These formulations can help regulate excessive production of sebum and inflammation while helping to restore homeostasis to the scalp. Anti-dandruff shampoos can be divided into two main categories: herbal shampoos and commercial (herbal/synthetic/medicated) shampoos. Conventional products usually contain antifungal agents (ketoconazole, zinc pyrithione, selenium sulfide, piroctone olamine, or salicylic acid), while herbal shampoos utilize plant based bioactive ingredients that have antifungal, anti-oxidant and anti-inflammatory properties. Herbal anti-dandruff shampoos are gaining popularity among consumers in recent years as the demand for natural, sustainable, and eco-friendly products in personal care products has grown. <sup>[19]</sup>

## 1.6 Herbal Anti-Dandruff Shampoos

Herbal anti-dandruff shampoos are the various formulations that are made up of extracts from medicinal plants, essential oils and naturally occurring phytochemicals that have antifungal, antimicrobial, anti-inflammatory, anti-oxidant and scalp conditioning properties. Commonly used botanicals are neem (*Azadirachta indica*), tea tree (*Melaleuca alternifolia*), tulsi (*Ocimum sanctum*), aloe vera (*Aloe barbadensis*), hibiscus (*Hibiscus rosa-sinensis*), amla (*Phyllanthus emblica*), reetha (*Sapindus mukorossi*), and shikakai (*Acacia concinna*). <sup>[20]</sup> The bioactive compounds present in these medicinal plants, including flavonoids, terpenoids, tannins, alkaloids, saponins, and phenolic compounds, are responsible for their anti-*Malassezia* growth

effect, anti-inflammatory action on the scalp, and beneficial effects on hair health. Herbal anti-dandruff shampoos may contain plant-based ingredients with antibacterial, antioxidant, anti-inflammatory, or scalp-conditioning properties. However, how safe and bearable they are depends on a number of factors, including individual sensitivity, composition, chemical concentration, and formulation quality. These attributes should be demonstrated by appropriate testing rather than being deduced from their natural origin. [21]



**Figure: 1** Medicinal plants

## 1.7 Commercial Anti-Dandruff Shampoos

Commercial anti-dandruff shampoos are industrially produced shampoos with synthetic/pharmaceutical active ingredients that have been clinically proven to have antifungal and keratolytic activity. Common drugs used in therapy are ketoconazole, zinc pyrithione, selenium sulfide, piroctone olamine, salicylic acid, coal tar, and ciclopirox olamine. They effectively inhibit the growth of *Malassezia* species, decrease the scaling of the scalp, calm itching and enhances the condition of the scalp. Standardized manufacturing processes produce commercial shampoos that have superior cleansing efficiency, rich lathering ability, better formulation stability and a longer shelf life. Some synthetic ingredients, however, can become a problem with prolonged or excessive use, causing a scalp to dry out, become irritated, allergic, or to change the texture of the hair in susceptible individuals. [22]

## 1.8 Need for Anti-Dandruff Shampoo

Dandruff is a recurrent, chronic disorder of the scalp which cannot be satisfactorily controlled with normal washing. While surface flakes and excess sebum are being removed with regular shampooing, it does not suppress *Malassezia* proliferation, restore the epidermal barrier, or the inflammatory response. So, special anti-dandruff shampoos need to contain antifungal, keratolytic, anti-inflammatory and sebum-regulating agents for effective long-term management [23]

Medicines used in commercial anti-dandruff shampoos include ketoconazole, zinc pyrithione, selenium sulfide, piroctone olamine, ciclopirox olamine, salicylic acid, and coal tar. The ingredients act on different stages of the pathogenesis of dandruff by decreasing the colonization of the fungi, inhibiting excessive proliferation of the epidermis, promoting the fall off of the

adherent scales and reducing inflammation and itching. Several clinical studies have shown their effectiveness and they are the preferred treatment for moderate to severe dandruff.

[24].

The antimicrobial, antioxidant, anti-inflammatory and scalp conditioning effect of several medicinal plants like *Azadirachta indica* (Neem), *Melaleuca alternifolia* (Tea Tree), *Sapindus mukorossi* (Reetha), *Acacia concinna* (Shikakai), *Phyllanthus emblica* (Amla), *Aloe vera*, *Hibiscus rosa-sinensis* and *Ocimum tenuiflorum* (Tulsi) have been shown in experimental or clinical studies. Aside from the antimicrobial properties which are important for dandruff control, the botanicals can also help to strengthen hair, promote scalp comfort and reduce irritation, balance moisture and help to improve the appearance of the hair. [25, 26]. Additionally, herbal shampoos are eco-friendly and sustainable, reflecting the increasing consumer preference for green beauty products. To minimize the environmental impact and user acceptance, many herbal formulations use biodegradable ingredients and softer surfactant. However, despite these benefits, there are still issues to consider with the standardization of herbal raw material, the variation in the phytochemical composition, quality control, preservation and the lack of good designed clinical trials. Hence, a comparative evaluation of the efficacy, safety and formulation quality of the anti-dandruff products which are commercially available and herbal products made from plants is a systematic evaluation required to determine the relative effectiveness, safety and formulation quality of the products. [27].

## 1.9 Research Gap

Many herbal and commercial anti dandruff shampoos are available in market but very few comparative scientific studies on their physicochemical characters, performance attributes and overall quality under similar experimental conditions are available. Most of the published studies are concerned with the formulation and evaluation of individual herbal shampoos or the clinical efficacy of particular synthetic antifungal agents. A systematic comparison between herbal and commercial products has been little investigated. Moreover, the variability in the herbal ingredients, surfactant systems and composition of formulation makes it difficult to establish standardized quality parameters for comparative assessment. Moreover, increasing consumer preference for herbal, green and sulfate-free personal care products has created a demand for evidence-based research for evaluating the efficacy of herbal shampoos may be just as effective as conventional commercial formulations. Hence, in the present study, we intend to conduct a thorough comparative assessment of herbal and commercial anti-dandruff shampoos based on standardized physicochemical and performance parameters so that trustworthy scientific data can be generated for researchers, manufacturers, healthcare professionals and consumers. [28]

## 2. MATERIALS AND METHODS

### 2.1 Materials

All the plant materials used in the study were of analytical grade and were purchased from authenticated herbal suppliers. The selection of neem (*Azadirachta indica*) leaf powder, tulsi (*Ocimum tenuiflorum*) leaf powder, aloe vera (*Aloe barbadensis* Miller) gel, hibiscus (*Hibiscus rosa-sinensis*) flower powder and tea tree essential oil (*Melaleuca alternifolia*) is based on their reported antifungal, anti-inflammatory, antioxidant and scalp conditioning properties. Prior to use, the shampoo base contained sodium lauryl ether sulfate (SLES), cocamidopropyl betaine (CAPB), glycerin, sodium chloride, citric acid, disodium EDTA, methyl paraben, propyl paraben, fragrance and purified water. All excipients were of pharmaceutical or cosmetic grade quality. For comparison, five commercially available anti-dandruff shampoos were purchased from local market

### 2.2 Selection of Herbal Ingredients and Commercial Shampoo Samples

The choice of herbs and commercial anti-dandruff shampoos was based on the purposive selection of substances that have been reported to be effective against dandruff, have known biological activities, traditional application in hair and scalp treatment and availability for further experimentation and formulation. For example, Neem (*Azadirachta indica*) herb was chosen as a common primary herb ingredient because of the reported antimicrobial and antifungal activities and its traditional application in hair and scalp treatment. The second ingredient, tulsi (*Ocimum tenuiflorum*), was selected due to its antimicrobial, antioxidant and anti-inflammatory activities. Aloe vera (*Aloe barbadensis* Miller) was selected due to its soothing and moisturizing effects. Hibiscus (*Hibiscus rosa-sinensis*) was selected due to the traditional usage in hair treatment. Tea tree essential oil (*Melaleuca alternifolia*) was selected due to the reported antimicrobial and antifungal activities. [29, 30, 31]

In all, six herbal shampoo formulations were developed to evaluate the separate and combined activities of the selected herbal ingredients. In the initial formulations, neem was maintained as a common primary herb ingredient. At the same time, tulsi, aloe vera, hibiscus and tea tree oil were tested separately in combination with neem. Finally, the polyherbal formulation of four herbs mentioned above was made to evaluate if the combined use of them would lead to balanced physicochemical properties and improved shampoo efficacy.<sup>[32]</sup>

In order to conduct comparative evaluation, five commercial anti-dandruff shampoos were selected on the purposeful basis as representatives of different categories of active ingredients used in anti-dandruff shampoos available on the market. The selected commercial samples included shampoos with the active ingredients such as ketoconazole, zinc pyrithione, selenium sulfide, and piroctone olamine. There was also one commercial herbal anti-dandruff shampoo selected. Commercial samples were coded as C1-C5 for the neutrality of comparative evaluation.<sup>[33]</sup>

## 2.3 Herbal Shampoo Formulations

Six herbal anti-dandruff shampoo formulations were prepared with different herbal active ingredients but with similar composition of shampoo base. The selected medicinal plants were selected on the basis of their reported antifungal activity against *Malassezia* species and traditional use in scalp care. The formulation composition of the herbal anti-dandruff shampoo formulations (H1–H6) was developed by changing the type and concentration of herbal active ingredients keeping a common shampoo base. Neem extract was added at 2.0% w/v in H1–H5, and H6 was 1.0% w/v Neem extract. H2 and H6 contained 1.0% w/v of Tulsi extract. H6 and H3 were with 2.0% w/v and 5.0% w/v aloe vera gel. Hibiscus extract was added at 2.0% w/v for H4 and 1.0% w/v for H6 respectively. In H5 and H6 tea tree oil was added at 0.5% w/v. H1 was a formulation based on Neem, H2 was Neem and Tulsi extract, H3 was Neem extract with Aloe vera gel, H4 was Neem and Hibiscus extract, H5 was Neem extract with Tea tree oil and H6 was a polyherbal formulation containing Neem, Tulsi, Aloe vera, Hibiscus, and Tea tree oil. The common shampoo base of all formulations was 25% w/v Sodium Lauryl Ether Sulfate (SLES) and 8% w/v Cocamidopropyl Betaine. 0.10 % w/v disodium EDTA was added as chelating agent and 3 % w/v glycerin was added as humectant. Methyl paraben and Propyl paraben were used as preservatives in concentration of 0.18% and 0.02% w/v respectively. For viscosity control, sodium chloride was added q.s., and citric acid was added in sufficient amounts to make pH 5.5 – 6.0. Fragrance was added in adequate amount to improve acceptability. The solvent used was purified water, and the total volume was 100 ml.

### 2.3.1 Commercial Shampoo Samples

Five commercially marketed anti-dandruff shampoos with different antifungal or anti-dandruff active ingredients were selected for comparison. The samples included Ketoconazole 2%, Zinc pyrithione, Selenium sulphide, Piroctone olamine, Herbal commercial shampoo and a commercial herbal anti-dandruff shampoo. These products were selected to compare their physicochemical properties and anti-dandruff efficacy to those of the manufactured herbal shampoo samples. The commercial products were bought from local pharmacies or retail stores and evaluated using the known experimental protocols.<sup>[33]</sup>

## 2.4 Evaluation Parameters

The organoleptic, physicochemical properties and performances of the prepared herbal shampoo formulations (H1–H6) and commercially available anti-dandruff shampoos (C1–C5) have been carried out based on the standard cosmetic evaluation protocol. All experiments were done in triplicate ( $n = 3$ ) and data was reported as mean  $\pm$  SD.

### 2.4.1 Physical Evaluation

Physical properties of the prepared herbal shampoo formulations (H1–H6) and commercial anti-dandruff shampoos (C1–C5) were evaluated by visual observation in a standard laboratory condition. The colour of all formulations was observed under natural sunlight against a white background. The appearance of the formulations was evaluated based on clarity, transparency, uniformity and presence of any suspended particles or phase separation. The odour of all formulations was evaluated by a panel of volunteers based on organoleptic method in controlled laboratory conditions and was classified as pleasant, acceptable or unacceptable. Homogeneity was checked by visual observation after proper mixing of all formulations to check the presence of any lumps, aggregates, precipitation and phase separation.<sup>[32, 33]</sup>

## 2.4.2 Physicochemical Evaluation [34, 35, 36, 37]

### (a) pH Determination

The pH of all shampoos was determined using a digital pH meter calibrated in a known buffer solution. The concentration of 10% (v/v) shampoo in distilled water was used to determine the pH value at  $25 \pm 2^\circ\text{C}$ .

### (b) Percentage of Solid Content

About 4 g of the shampoo was weighed with precision into a known evaporating dish and heated to dryness in a hot air oven at  $105^\circ\text{C}$  until the weight became constant. The amount of solid matter was determined through this equation: A good shampoo should contain about 20% to 30% total solids.

### (c) Surface Tension

Surface tension was determined using a Du Noüy ring tensiometer at  $25^\circ\text{C}$ . A 10% shampoo solution was prepared with distilled water, and the surface tension was recorded in dynes/cm (or mN/m). Lower surface tension indicates better wetting and cleansing performance.

### (d) Density:

Density was determined using a clean, dry specific gravity bottle (pycnometer). The bottle was filled with shampoo, weighed accurately, and density was calculated as:

### (e) Viscosity

Viscosities of all formulations were measured through a Brookfield Digital Viscometer fitted with the appropriate spindle (for example, spindle No. 64) at  $25 \pm 2^\circ\text{C}$  and 50 rpm. Triplicate measurements were made for each measurement.

### (f) Dirt Dispersion Test

In order to determine the foaming capacity of the soap samples, two drops of the sample were poured into 10 mL of distilled water inside a test tube, and one drop of India ink was added to it. After shaking the test tube 10 times, the quantity of ink left in the foam was observed.

### (g) Wetting Time

Discs of circular canvas of diameter 1 inch having a mass of about 0.44 grams were gently placed on the surface of the 1% shampoo solution. The amount of time taken by the disc to fall was measured with the help of stop watch.

### (h) Foaming Ability

Fifty millilitres of 1% shampoo solution was transferred into a 250 mL graduated cylinder and shaken vigorously 10 times. The total foam volume was recorded immediately after shaking. Higher foam volume indicates good foaming characteristics.

### (i) Foam Stability

The foam volume obtained during the foaming test was measured immediately and again after 5 minutes.

Foam stability (%) was calculated as:

$$\text{Foam Stability (\%)} = \frac{\text{Foam volume after 5 min}}{\text{Initial foam volume}} \times 100$$

## 2.4.3 Performance Evaluation

The performance profiles of the herbal shampoo formulation (H1 to H6) and the commercial anti-dandruff shampoos (C1 to C5) were assessed based on lab testing. The tests were conducted in triplicates, and results were documented in mean  $\pm$  standard deviation (SD).

### (a) Cleansing Ability

A pristine human hair tress (around 5 g) was contaminated artificially using a standard mixture of sebum and dust. The hair tress was then cleaned using 5 mL of shampoo solution (10% v/v). The cleaning efficacy was evaluated through the percentage of soil removal, which is assessed based on the change in the weight of the hair tress before and after washing.<sup>[38]</sup>

The percentage cleansing efficiency was calculated using the following equation:

A higher percentage indicates better cleansing performance.

## **(b) Conditioning Effect**

Hair tresses washed with each shampoo formulation were evaluated by a panel of ten trained volunteers. The following characteristics were assessed:<sup>[39]</sup>

- Softness
- Smoothness
- Manageability
- Shine

## **(c) Ease of Rinsing**

Washing of each hair strand was done with 5 mL of the shampoo solution and then rinsed using tap water kept at  $25 \pm 2^\circ\text{C}$  with controlled flow rate. The rinsing time required to remove all traces of foaming and shampoo from the strand is determined using stopwatch.<sup>[40]</sup>

## **(d) Wet Compatibility**

After each washing and rinse cycle, each wet hair strand was then brushed using a wide-tooth comb. The combing force as well as the formation of hair tangles were assessed using a five-point scale.

### **2.4.4 Literature Based Safety Evaluation**

The potential safety risks associated with the substances used in the herbal and commercial anti-dandruff shampoos were assessed by a study of available scientific literature. The evaluation's primary focus was on reports of skin irritation, allergic contact dermatitis, and other relevant adverse effects associated with the drugs at their intended concentrations and usage conditions. Human clinical evaluation and direct dermatological safety tests were not included in the current investigation. Therefore, the safety debate should not be interpreted as experimental evidence of any formulation's higher tolerance or safety; rather, it is a literature-based assessment of potential risks.<sup>[41]</sup>

### **2.4.5 in Vitro Antifungal Activity**

The antifungal efficacy of the formulated herbal shampoos (H1–H6) and commercially available anti-dandruff shampoos (C1–C5) was assessed in vitro against a verified reference strain of *Malassezia furfur* via the agar-well diffusion technique. The analysis was conducted under controlled microbiological settings, and the antifungal efficacy was evaluated by determining the diameter of the inhibition zone (IZ) surrounding each test specimen.<sup>[42]</sup>

#### **2.4.5.1 Microbial Organisms and Cultivation Parameters**

An authenticated reference strain of *Malassezia furfur* was procured from microbiology laboratory. The organism was cultivated on modified Dixon (mDixon) agar, a lipid-rich culture medium conducive to the proliferation of *Malassezia* species. The culture was cultivated aerobically at  $30^\circ\text{C}$  for 24–48 hrs, until a satisfactory number of actively proliferating colonies were achieved.

The lipid-rich medium was employed as *M. furfur* necessitates appropriate lipid augmentation for optimal growth. All microbiological techniques were conducted in sterile environments.

#### **2.4.5.2 Preparation and Calibration of Inoculum**

A newly cultivated, vigorously proliferating culture of *M. furfur* was utilized for the formulation of the test inoculum. Representative colonies were placed aseptically into an appropriate sterile diluent and vigorously mixed to achieve a

homogeneous yeast suspension. The suspension was modified to align with the inoculum density outlined in the approved institutional microbiological protocol.

The standardized inoculum was utilized promptly for the antifungal assay to reduce fluctuations in microbe density during the experiment. All inoculum preparation processes were conducted under sterile conditions.<sup>[43]</sup>

### 2.4.5.3 Preparation of Test Specimens and Controls

The formulated herbal shampoos (H1–H6) and the commercially produced anti-dandruff shampoos (C1–C5) were assessed under the same experimental parameters. Every test formulation was used at the concentration and sample volume indicated in the experimental framework.

Ketoconazole served as the positive antifungal control at the concentration stated in the experimental protocol. The shampoo base devoid of herbal active components served as the negative/vehicle control. In instances where a commercial ketoconazole shampoo was classified as C1, it was regarded as a test commercial formulation and not treated as an independent positive control unless explicitly indicated in the experimental design.

**All test specimens and controls were assessed in triplicate (n = 3).**

### Agar-Well Diffusion Test

Sterile mDixon agar plates were created and permitted to solidify. The surface of every plate was consistently inoculated with the standardized *M. furfur* suspension employing an aseptic method to achieve a replicable microbial lawn.

Subsequent to inoculation, sterile wells of consistent diameter were created in the agar. A specified and uniform volume of each herbal shampoo formulation, along with the commercial shampoo, positive control, and negative control, was meticulously added to the corresponding wells. Distinct wells were utilized for every test specimen and control to avert cross-contamination. The inoculated plates were subjected to aerobic incubation at 30°C for 24 to 48hrs.

### 2.4.5.4 Assessment of Inhibition Zone

Subsequent to incubation, the plates were scrutinized for the existence of transparent zones encircling the wells. The diameter of the inhibitory zone was quantified in millimeters utilizing a calibrated ruler or digital measuring instrument.

For nearly circular inhibitory zones, two orthogonal diameters were assessed, and the average diameter was computed. The average diameter was noted as the zone of inhibition (ZOI) for the corresponding sample. The outcomes were presented as mean  $\pm$  standard deviation (SD), n = 3. A more extensive inhibitory zone was understood to indicate enhanced antifungal efficacy in the context of the agar diffusion assay. Due to the potential impact of formulation attributes including viscosity, solubility, and composition on diffusion across agar, ZOI values were regarded as a relative indicator of antifungal efficacy rather than a direct assessment of inherent antifungal strength. <sup>[44–45]</sup>

## 2.5. Statistical Analysis

Quantitative experimental results were expressed as mean  $\pm$  standard deviation (SD) of three independent measurements (n = 3), where applicable. For comparisons involving multiple formulations, one-way analysis of variance (ANOVA) may be used when its assumptions are reasonably satisfied. If a significant overall difference is detected, an appropriate post hoc multiple-comparison test should be applied. The significance level should be set at  $p < 0.05$ . The statistical test, software used, and exact p-values should be reported based on the analyses actually performed.

## RESULTS AND DISCUSSION.

### 3.1 Physical Evaluation

All herbal formulations (H1–H6) and anti-dandruff commercial shampoos (C1–C5) had excellent physical properties with no noticeable deficiencies in appearance. All herbal formulations had different colours based on their botanical content. For example, H1 (Neem) and H2 (Neem and Tulsi) were light to dark green because of the presence of chlorophyll-rich extracts, while H4 (Neem and Hibiscus) was light reddish brown in colour because of the natural pigments found in the hibiscus flower. H5 had a unique tea tree smell, while H6 was a darker green because of the presence of multiple herbal extracts. Commercial shampoos were mostly blue, white, orange, and yellow because of the use of cosmetic colourants.

## 3.2 Physicochemical Evaluation Results

### (a) pH Determination

**Table 1.** pH of Herbal and Commercial Anti-Dandruff Shampoos (Mean  $\pm$  SD, n = 3)

| Formulation | Mean $\pm$ SD   | Commercial shampoo        | Mean $\pm$ SD   |
|-------------|-----------------|---------------------------|-----------------|
| H1          | 5.42 $\pm$ 0.02 | Ketoconazole 2%           | 5.84 $\pm$ 0.01 |
| H2          | 5.54 $\pm$ 0.02 | Zinc Pyrithione           | 6.03 $\pm$ 0.01 |
| H3          | 5.62 $\pm$ 0.01 | Selenium Sulfide          | 5.61 $\pm$ 0.01 |
| H4          | 5.70 $\pm$ 0.01 | Piroctone Olamine         | 5.70 $\pm$ 0.01 |
| H5          | 5.73 $\pm$ 0.01 | Herbal Commercial Shampoo | 5.50 $\pm$ 0.01 |
| H6          | 5.81 $\pm$ 0.01 |                           |                 |

### (b) Percentage Solid Content

**Table 2.** Percentage Solid Content

| Formulation | Solid Content (%) | Commercial shampoo        | Solid Content (%) |
|-------------|-------------------|---------------------------|-------------------|
| H1          | 24.00             | Ketoconazole 2%           | 30.23             |
| H2          | 24.70             | Zinc Pyrithione           | 31.51             |
| H3          | 25.54             | Selenium Sulfide          | 30.01             |
| H4          | 25.89             | Piroctone Olamine         | 30.00             |
| H5          | 27.56             | Herbal Commercial Shampoo | 27.00             |
| H6          | 28.09             |                           |                   |

### (c) Surface Tension

**Table 3.** Surface Tension

| Formulation | Mean $\pm$ SD (mN/m) | Commercial shampoo        | Mean $\pm$ SD    |
|-------------|----------------------|---------------------------|------------------|
| H1          | 34.87 $\pm$ 0.10     | Ketoconazole 2%           | 31.25 $\pm$ 0.10 |
| H2          | 35.10 $\pm$ 0.10     | Zinc Pyrithione           | 30.90 $\pm$ 0.10 |
| H3          | 34.5 $\pm$ 0.10      | Selenium Sulfide          | 31.43 $\pm$ 0.10 |
| H4          | 33.28 $\pm$ 0.10     | Piroctone Olamine         | 32.4 $\pm$ 0.10  |
| H5          | 34.6 $\pm$ 0.10      | Herbal Commercial Shampoo | 33.00 $\pm$ 0.10 |
| H6          | 31.90 $\pm$ 0.10     | -                         | -                |

### (d) Density

The density of the herbal shampoo formulations was found to vary between 1.021 g/mL and 1.043 g/mL, implying a relatively uniform and acceptable density of the formulations. The variations in density values might be explained by the difference in the amount and nature of the herbal extract components used in the formulations. On the whole, the density of the formulations is quite uniform. The physicochemical analysis suggests that both the formulated herbal preparations (H1–H6) and the marketed commercial shampoos (C1–C5) are in accordance with the standard quality of anti-dandruff shampoos. All the formulations had an optimal pH value (5.0–6.5), a desirable amount of solids, adequate surface tension for cleansing action and acceptable density, thus assuring consistency and ease of application. Though the marketed commercial shampoos were found to have higher solid content, lower surface tension and higher density because of the use of artificial surfactants and other additives, but still the developed herbal formulations have shown an equal physicochemical property profile.

## (e) Viscosity

**Table 4.** Viscosity of Herbal and Commercial Anti-Dandruff Shampoos

| Code | Mean $\pm$ SD (cP) | Commercial shampoo        | Mean $\pm$ SD (cP) |
|------|--------------------|---------------------------|--------------------|
| H1   | 2956 $\pm$ 8       | Ketoconazole 2%           | 3787 $\pm$ 5       |
| H2   | 3087 $\pm$ 4       | Zinc Pyrithione           | 3798 $\pm$ 4       |
| H3   | 3136 $\pm$ 8       | Selenium Sulfide          | 3876 $\pm$ 7       |
| H4   | 3365 $\pm$ 6       | Piroctone Olamine         | 3745 $\pm$ 6       |
| H5   | 3567 $\pm$ 4       | Herbal Commercial Shampoo | 3568 $\pm$ 5       |
| H6   | 3654 $\pm$ 6       | -                         | -                  |

## (f) Dirt Dispersion Test

It can be concluded from the dirt dispersion test that the herbal shampoo formulations had higher capacity to suspend dirt compared to commercial shampoos. Ink present only slightly in the foam indicates that the dirt particles were suspended in the washing agent and not re-deposited on the hair. The dirt suspension by the herbal formulations was excellent while the commercial formulations did it fairly well to excellently.

## (g) Wetting Time

**Table 5. b**

| Code | Mean $\pm$ SD (s) | Commercial shampoo        | Mean $\pm$ SD (s) |
|------|-------------------|---------------------------|-------------------|
| H1   | 22.6 $\pm$ 0.6    | Ketoconazole 2%           | 14.2 $\pm$ 0.5    |
| H2   | 21.8 $\pm$ 0.6    | Zinc Pyrithione           | 15.6 $\pm$ 0.6    |
| H3   | 20.4 $\pm$ 0.6    | Selenium Sulfide          | 16.8 $\pm$ 0.7    |
| H4   | 18.7 $\pm$ 0.6    | Piroctone Olamine         | 13.4 $\pm$ 0.4    |
| H5   | 17.3 $\pm$ 0.6    | Herbal Commercial Shampoo | 19.1 $\pm$ 0.6    |
| H6   | 15.8 $\pm$ 0.6    |                           |                   |

## (h) Foaming Ability

**Table 6.** Foaming Ability

| Code | Foam Volume (mL) | Commercial shampoo        | Foam Volume (mL) |
|------|------------------|---------------------------|------------------|
| H1   | 139              | Ketoconazole 2%           | 173              |
| H2   | 145              | Zinc Pyrithione           | 177              |
| H3   | 150              | Selenium Sulfide          | 181              |
| H4   | 153              | Piroctone Olamine         | 175              |
| H5   | 160              | Herbal Commercial Shampoo | 166              |
| H6   | 165              | -                         | -                |

## (i) Foam Stability

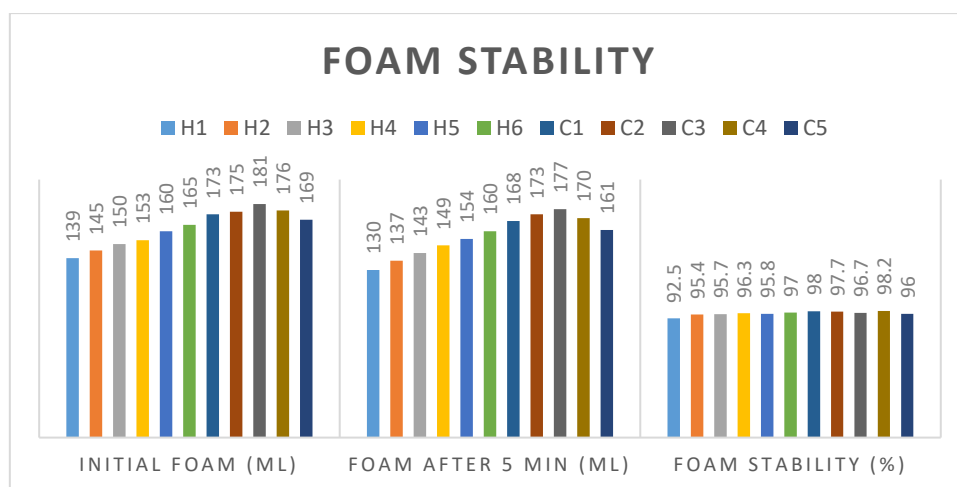


**Chart: 1** foam stability

The developed herbal preparations (H1-H6) had an adequate viscosity level, good dirt dispersibility, appropriate wetting time, and excellent foaming ability, which is similar to that of commercial shampoos (C1-C5). The commercial shampoos had slightly more viscosity, wetting, and foaming ability because of the synthetic surfactants used in them. However, the developed herbal preparations have exhibited better foam stability and lower ink absorption in the dirt dispersibility test. This shows that the developed herbal shampoos have desirable physicochemical properties and can perform similar to commercial anti-dandruff shampoos.

## 3.3 Performance Evaluation

### (a) Cleansing Ability

**Table 7.** Cleansing Ability

| Code | Mean $\pm$ SD (%) | Commercial shampoo        | Mean $\pm$ SD (%) |
|------|-------------------|---------------------------|-------------------|
| H1   | 81.8 $\pm$ 0.4    | Ketoconazole 2%           | 91.4 $\pm$ 0.3    |
| H2   | 83.7 $\pm$ 0.2    | Zinc Pyrithione           | 94.1 $\pm$ 0.2    |
| H3   | 83.5 $\pm$ 0.2    | Selenium Sulfide          | 95.5 $\pm$ 0.2    |
| H4   | 87.4 $\pm$ 0.2    | Piroctone Olamine         | 92.0 $\pm$ 0.2    |
| H5   | 89.5 $\pm$ 0.2    | Herbal Commercial Shampoo | 91.0 $\pm$ 0.2    |
| H6   | 91.4 $\pm$ 0.3    | -                         | -                 |

## (b) Conditioning Effect

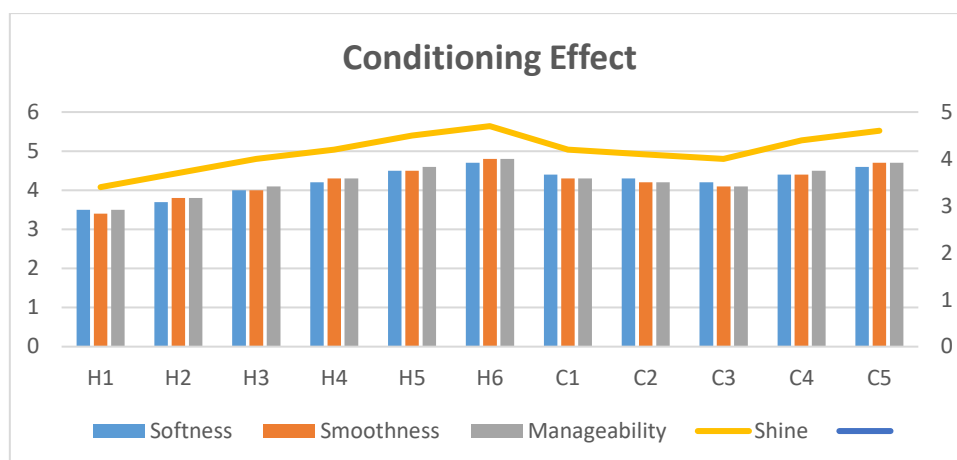


**Chart: 2** Conditioning Effect of shampoo

## (c) Ease of Rinsing

**Table 8.** Ease of Rinsing

| Code | Mean $\pm$ SD (mL) | Commercial shampoo        | Rinsing Time(S) |
|------|--------------------|---------------------------|-----------------|
| H1   | 39.3 $\pm$ 0.6     | Ketoconazole 2%           | 173             |
| H2   | 37.2 $\pm$ 0.6     | Zinc Pyrithione           | 175             |
| H3   | 33.2 $\pm$ 0.6     | Selenium Sulfide          | 181             |
| H4   | 33.8 $\pm$ 0.6     | Piroctone Olamine         | 175             |
| H5   | 31.8 $\pm$ 0.6     | Herbal Commercial Shampoo | 166             |
| H6   | 30.4 $\pm$ 0.6     | -                         | -               |

## (d)Wet Combability

**Table 9.** Wet Combability (5-Point Scale)

| Code/ Commercial Shampoo | Combing Ease | Hair Entanglement |
|--------------------------|--------------|-------------------|
| H1                       | 3.2          | 3.5               |
| H2                       | 3.6          | 3.9               |
| H3                       | 4.2          | 4.1               |
| H4                       | 4.5          | 4.3               |
| H5                       | 4.7          | 4.5               |
| H6                       | 4.7          | 4.7               |
| C1                       | 4.5          | 4.3               |
| C2                       | 4.4          | 4.2               |
| C3                       | 4.6          | 4.1               |
| C4                       | 4.7          | 4.4               |
| C5                       | 4.8          | 4.6               |

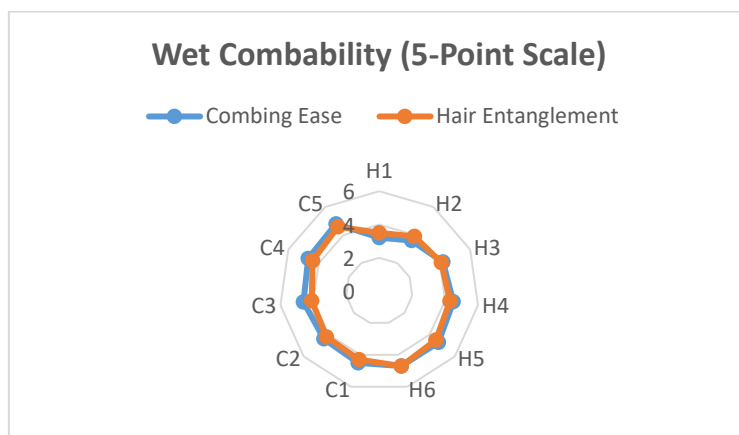


**Chart: 3** Wet Combability

From the above results, it was evident that all the herbal formulations designed were efficient enough in terms of cleansing, conditioning, rinse-off capability, and combing while wet compared to the existing anti-dandruff shampoos. The commercially available shampoos had a better cleaning action and fast rinse off capacity mainly due to the use of synthetic surfactants. Nevertheless, the herbal shampoos especially H5 and H6 had better conditioning and combing capability because of the good effects of the herbal formulation and natural oils.

### 3.4 Safety evaluation

The literature research identified potential safety concerns with the botanical ingredients, surfactants, preservatives, and active substances included in the evaluated shampoos. The use of certain plant-based chemicals for cosmetic or dermatological purposes does not ensure the safety of the final compositions. Similarly, synthetic antifungal agents and surfactants may irritate susceptible individuals or cause other adverse reactions, depending on the ingredient, concentration, and usage conditions. Since no direct dermatological safety testing or human clinical evaluation was conducted, it was not possible to determine the relative safety of the commercial and herbal shampoos by experimentation. Further assessment using appropriate safety-testing methods would be required to determine the final formulations' tolerability.

**Table 10.** Antifungal activity of herbal and commercial anti-dandruff shampoo formulations against *M. furfur*

| Sample                             | Zone of inhibition (mm), Mean $\pm$ SD |
|------------------------------------|----------------------------------------|
| Negative control (Shampoo base)    | 0.00 $\pm$ 0.00                        |
| Positive control (Ketoconazole 2%) | 25.63 $\pm$ 0.58                       |
| H1                                 | 12.47 $\pm$ 0.50                       |
| H2                                 | 14.23 $\pm$ 0.42                       |
| H3                                 | 13.16 $\pm$ 0.38                       |
| H4                                 | 12.91 $\pm$ 0.44                       |
| H5                                 | 17.36 $\pm$ 0.51                       |
| H6                                 | 19.42 $\pm$ 0.51                       |
| C1                                 | 23.87 $\pm$ 0.45                       |
| C2                                 | 20.74 $\pm$ 0.42                       |
| C3                                 | 19.86 $\pm$ 0.51                       |
| C4                                 | 21.63 $\pm$ 0.47                       |
| C5                                 | 15.72 $\pm$ 0.45                       |

### Interpretation of the Results

The results indicate that all prepared herbal formulations exhibited measurable antifungal activity against *M. furfur*, although the magnitude of inhibition varied among the formulations. The negative control showed no appreciable inhibition, indicating that the shampoo vehicle itself had little or no antifungal effect under the test conditions.

Among the prepared herbal formulations, H6 (polyherbal formulation) demonstrated the highest antifungal activity, with a ZOI of approximately  $19.42 \pm 0.51$  mm, followed by H5 containing neem and tea tree oil ( $17.36 \pm 0.51$  mm). H2 containing neem and tulsi exhibited moderate activity ( $14.23 \pm 0.42$  mm), while H3 and H4 showed ZOIs of approximately  $13.16 \pm 0.38$  mm and  $12.91 \pm 0.44$  mm, respectively. H1 containing neem alone produced a ZOI of  $12.47 \pm 0.50$  mm.

Among the commercial formulations, C1 containing ketoconazole 2% showed the greatest inhibition ( $23.87 \pm 0.45$  mm), which was comparable to the positive ketoconazole control ( $25.63 \pm 0.58$  mm). C4 containing piroctone olamine and C2 containing zinc pyrithione showed ZOIs of  $21.63 \pm 0.47$  mm and  $20.74 \pm 0.42$  mm, respectively. C3 containing selenium sulfide demonstrated  $19.86 \pm 0.51$  mm, whereas the commercial herbal shampoo C5 showed comparatively lower activity ( $15.72 \pm 0.45$  mm).

Overall, the findings suggest that the polyherbal formulation H6 possessed the strongest antifungal activity among the developed herbal shampoos, while the synthetic antifungal-containing commercial formulations exhibited greater inhibition overall. The enhanced activity of H6 may be attributed to the combined contribution of the selected herbal ingredients, particularly neem, tulsi, aloe vera, hibiscus and tea tree oil, which may provide complementary antifungal effects.

## LIMITATION

The present study had particular limitations. Only six herbal and five commercial anti-dandruff shampoo formulations were evaluated; therefore, the findings may not represent the entirety of available therapies. The antifungal efficacy was evaluated in vitro against *Malassezia furfur* using an agar diffusion method, and the results may not accurately represent clinical effectiveness on the human scalp. The zone of inhibition may also be influenced by formulation properties such as viscosity, solubility, and diffusion traits. The study excluded clinical evaluation in human participants to determine dandruff severity, itching, scalp discomfort, or long-term efficacy. Moreover, a comprehensive quantitative standardization of the bioactive constituents of the herbal extracts was not performed. Variations in the source, extraction methods, and chemical composition of herbal constituents may therefore influence reproducibility. The enduring safety, sensitization, and extended stability in actual commercial storage conditions were not comprehensively evaluated. Further investigation into clinical, microbiological, phytochemical, and long-term stability aspects is essential to ascertain the clinical effectiveness, safety, and reliability of the studied herbal anti-dandruff shampoos.

## CONCLUSION.

The current study has been able to successfully evaluate prepared herbal anti-dandruff shampoo formulations by comparing them to commercially available anti-dandruff shampoos through standard physical, physicochemical, performance, and safety evaluation parameters. All the herbal formulations proved to have satisfactory organoleptic attributes, ideal pH, viscosity, foaming capacity, wetting ability, and cleansing efficacy, thus making them fit for the purpose of regular cleansing of scalp skin. The polyherbal formulation (H6) containing neem, tulsi, aloe vera, hibiscus, and tea tree oil demonstrated the greatest overall antifungal activity among the produced herbal formulations tested in vitro. In a few assessment tests, it also showed favorable performance characteristics. Nevertheless, the data do not demonstrate a synergistic interaction between the herbal ingredients because no specific synergy experiment was conducted.

It is clear from the observations made in the current study that the commercial shampoos have better foaming capacity and cleansing efficacy than the herbal formulations, which may be due to the addition of optimized synthetic surfactant systems along with antifungal pharmaceutical agents. Despite having slight disadvantages, the herbal formulations had comparable physicochemical properties, but offered the advantage of plant active compounds known to have antifungal, anti-inflammatory, and antioxidant properties. Moreover, the review of the literature has suggested that the herbal ingredients do not have high levels of irritation and dermatological side effects when used in their recommended concentrations.

In general, it appears that proper formulation of the herbal anti-dandruff shampoos makes them have performance comparable to the commercial shampoos by reducing the need for synthetic chemicals. The current study confirms the increasing trend of developing herbal formulations and contributes scientifically to developing effective and safe cosmetic products. In future, research work should involve in vitro antifungal testing against *Malassezia*, stability testing, and consumer trial studies.

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## Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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