World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

A COMPREHENSIVE REVIEW OF A POLYHERBAL ANTI-DANDRUFF SHAMPOO_ FORMULATION, EVALUATION, AND THERAPEUTIC POTENTIAL OF AEGLE MARMELOS, ALOE VERA, AND ACACIA CONCINNA, SHIKEKAI

Vishvajit Mane*, Pranav Humbe, Pranav Chavan, Pooja Dhumale, P.A. Mane Patil

Eklavya College of Pharmacy, Tasgaon.

***Corresponding Author: Vishvajit Mane**

Eklavya College of Pharmacy, Tasgaon. **DOI:** https://doi.org/10.5281/zenodo.17749387

How to cite this Article: Vishvajit Mane*, Pranav Humbe, Pranav Chavan, Pooja Dhumale, P.A. Mane Patil. (2025). A COMPREHENSIVE REVIEW OF A POLYHERBAL ANTI-DANDRUFF SHAMPOO_ FORMULATION, EVALUATION, AND THERAPEUTIC POTENTIAL OF AEGLE MARMELOS, ALOE VERA, AND ACACIA CONCINNA, SHIKEKAI. World Journal of Pharmaceutical and Life Science, 11(12), 131–147.

Article Received on 03/11/2025 **Article Revised on 24/11/2025** **Article Published on 01/12/2025**

ABSTRACT

Dandruff, or *Pityriasis capitis*, is a highly prevalent scalp condition primarily linked to the overgrowth of lipophilic *Malassezia* yeasts, notably *Malassezia globosa*.[1] While conventional synthetic antifungal agents like zinc pyrithione (ZPT) and ketoconazole are effective, increasing consumer preference for natural, eco-friendly alternatives necessitates the development of efficacious herbal solutions.[3] This study focuses on designing, formulating, and rigorously evaluating a novel polyherbal liquid shampoo incorporating standardized hydroalcoholic extracts of *Aegle marmelos*, *Acacia concinna*, *Curcuma longa*, and *Aloe vera*. The rationale centers on synergistic activity, combining multiple mechanisms including fungicidal action (curcuminoids, bael components), potent anti- inflammatory effects (turmeric, aloe), natural detergency (saponins from shikakai), and antioxidant protection. Optimal hydroalcoholic extraction parameters were determined to maximize bioactive compound yield. The formulated shampoo underwent comprehensive physicochemical testing, including pH assessment, detergency, and foam stability. Crucially, anti-dandruff efficacy was quantified by determining the minimum inhibitory concentration (MIC) against *Malassezia globosa* in an olive oil supplemented medium. Safety was confirmed using the gold standard *in vitro* Reconstructed Human Epidermis (RHE) model (OECD Test Guideline 439). The findings are expected to validate the formulated product as a stable, safe, and significantly effective natural alternative for scalp health management, warranting future clinical trials.

INTRODUCTION

Etiology of Dandruff and the Need for Natural Solutions

Dandruff is a globally common inflammatory skin condition of the scalp, characterized by pruritus, erythema, and the shedding of scales. The pathogenesis of dandruff involves complex interactions between host factors (sebum composition, immune response), environmental variables, and microbial colonization.[2] The primary microbial drivers are the lipophilic yeasts of the genus *Malassezia*, particularly *M. globosa* and *M. restricta*, which metabolize scalp sebum into irritant byproducts.[1]

Traditional pharmacological treatments rely on synthetic antifungal agents and keratinolytic compounds. However, these compounds, while effective in the short term, are frequently associated with diminished consumer acceptance, potential local side effects such as increased scaling or irritation, and undesirable environmental accumulation.[4] This context has driven substantial innovation in "Green Cosmetics," fostering demand for plant-based formulations that are biodegradable, safe, and capable of delivering therapeutic benefits without reliance on synthetic additives.[3]

Rationale for Polyherbal Synergy

The development of polyherbal systems represents a sophisticated approach in dermo cosmetics, moving beyond the limitations of single-agent synthetic treatments.[3] This methodology allows for the simultaneous integration of multiple bioactive compounds, facilitating a comprehensive therapeutic

attack on dandruff etiology.[7] A successful antidandruff strategy must not only inhibit fungal proliferation but also manage the secondary symptoms of irritation, inflammation, and sebum imbalance.[8]

The selected combination of *Aegle marmelos*, *Acacia concinna*, *Curcuma longa*, and *Aloe vera* is justified by the phytochemistry of these botanicals, which collectively provide fungicidal, anti-inflammatory, antioxidant, and natural cleansing properties.[10] The formulation is strategically designed to provide a multi-mechanism action. For instance, combining agents that target the fungal cells directly (*Curcuma longa*, *Aegle marmelos*) with agents that address the underlying environmental factors, such as inflammation and oxidative stress (*Aloe vera*, *Aegle marmelos*), yields a holistic scalp treatment.[12] Furthermore, incorporating conditioning and moisturizing agents ensures that the primary cleansing action does not result in the hair cuticle damage, dryness, or dullness often associated with less refined shampoo bases. This integrative formulation approach is anticipated to overcome microbial adaptation and enhance overall scalp health beyond simple fungal inhibition.[6]

Dandruff

Dandruff is a chronic scalp disorder characterized by excessive scaling, itching, and redness of the scalp. It occurs when epidermal cells are shed in large aggregates. This condition, defined as the abnormal shedding of dead skin cells from the scalp, is primarily associated with the fungal species *Malassezia restricta* and *Malassezia globosa*. *Malassezia*, previously known as *Pityrosporum*, is a lipophilic yeast responsible for various infections of the skin and scalp. Under normal physiological conditions, scalp cell turnover occurs gradually and is invisible to the naked eye, typically completing a renewal cycle every month. However, when this process accelerates, it disrupts the scalp's normal balance, leading to the visible flaking known as dandruff. Environmental factors such as warm and humid climates, overcrowding, and inadequate personal hygiene provide optimal conditions for the proliferation of *Malassezia*. Dandruff affects approximately 5% of the global population, most commonly appearing after puberty, particularly between the ages of 20 and 30, and it tends to occur more frequently in males than in females.[61]

Classification of Dandruff

Dandruff can be broadly classified into two primary categories based on its clinical presentation and associated scalp conditions:

(A) Dry dandruff
(B) Oily dandruff

A. Dry Dandruff (Pityriasis simplex)

Dry dandruff, medically termed *Pityriasis simplex capillitii*, is characterized by the excessive shedding of fine, white or grayish scales from the scalp. The condition typically lacks significant inflammation or erythema and is not commonly associated with notable hair loss. The desquamation usually initiates at the central region of the scalp and gradually extends to the frontal, parietal, and occipital zones. The primary contributing factors include decreased scalp hydration, environmental dryness, and inadequate sebaceous secretion, resulting in a brittle scalp surface prone to flaking.

B. *Oily Dandruff (Pityriasis steatoides)*

Oily dandruff, or *Pityriasis steatoides*, represents a more severe clinical variant frequently linked to excessive sebum production from hyperactive sebaceous glands. This form often manifests during puberty, coinciding with hormonal fluctuations that increase sebum secretion. The condition is characterized by greasy, adherent, yellowish scales that accumulate on an inflamed scalp surface. These oily scales may cause irritation and pruritus and are often accompanied by secondary hair fall due to follicular blockage and inflammation. Commonly affected areas include the scalp, post-auricular region, sternum, and axillae.[3,4]

Causes of Dandruff

Pityrosporum ovale (P. ovale), a fungus naturally present on the scalp and other skin regions, is one of the primary microorganisms associated with dandruff formation. Under normal conditions, this fungus remains harmless. However, factors such as hormonal fluctuations, stress, and changes in weather can increase scalp oil production, creating an environment that promotes the excessive growth of *P. ovale*.

Dandruff develops when the fungus proliferates abnormally, leading to irritation of the scalp, accelerated shedding of skin cells, and weakening of hair follicles. The underlying mechanism of dandruff formation is believed to involve the production of lipase enzymes by *Malassezia* species. These enzymes convert sebum into oleic acid, which penetrates the epidermis and triggers increased skin cell turnover in susceptible individuals. Consequently, this process results in redness, itching, and the formation of dandruff flakes.

- Dry skin
- Irritated, oily skin
- Infrequent shampooing

- **Other skin conditions**

1. Eczema
2. Psoriasis

- Seborrheic dermatitis caused by *Malassezia* (yeast-like fungus)
- Sensitivity to hair care products (contact dermatitis)[6]

Fig. 1: Condition That Affect the Scalp.

Hair Anatomy

> Hair originates from hair follicles located in the fatty layer of the scalp. Contrary to the common belief that hair grows individually, follicles usually grow in clusters of 1–4 hairs, known as "follicular units."

> At the base of each follicle lies the hair bulb, which is responsible for hair growth. Hair follicles receive nutrients from blood vessels in the dermis. Cells within the bulb divide and develop, forming the hair shaft.

> While still beneath the epidermis, the hair remains soft. Once it emerges through the epidermis, the outer layer hardens into keratin, giving hair its strength.

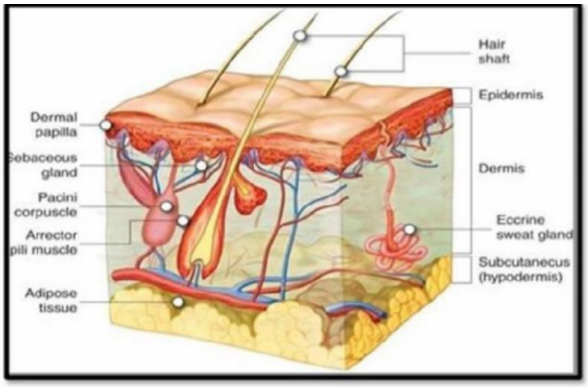

Fig. 2: Hair Anatomy.

Parts of the Hair

1. **Dermal Papillae:** The dermal papilla regulates the hair growth cycle and overall hair growth. It contains androgen receptors that respond to the hormone DHT.
2. **Matrix:** Surrounding the dermal papilla, the matrix contains all the active cells required for hair growth and the formation of different hair components, including the outer root sheath, inner root sheath, and hair shaft. Together, the matrix and dermal papilla form the hair bulb.
3. **Outer Root Sheath:** Also called the trichelemmal layer, the outer root sheath is the keratinized outermost layer of the hair. It covers the hair follicle within the dermis and extends to the epidermis, providing an opening for the hair to emerge.
4. **Inner Root Sheath:** The inner root sheath consists of three layers: Henle's layer, Huxley's layer, and the cuticle. Henle's and Huxley's layers form capsular structures that anchor together to stabilize the hair. The cuticle, closest to the hair shaft, is made of dead, hardened cells, offering protection to the hair shaft. Together, these layers secure the hair and support its growth.
5. **Hair Shaft:** The hair shaft is the part of the hair that extends fully above the skin's surface. It is composed of three layers:

- **Medulla:** The innermost layer, irregular and unstructured, which may not always be present.
- **Cortex:** The middle layer, highly organized and made of keratin, provides strength, durability, and water absorption. It also contains melanin, which determines hair color based on the type, quantity, and distribution of melanin granules

6. **Cuticle:** The outer protective layer, connected to the inner root sheath. It is a single molecular layer of lipids that helps repel water and shields the inner layers.[15]

Herbal Shampoos

Herbal shampoos are cosmetic formulations derived primarily from plant-based ingredients intended for effective hair and scalp cleansing. These formulations utilize naturally sourced surfactants, extracts, and essential oils that facilitate the removal of dandruff, excess sebum, dirt, and environmental pollutants without causing adverse effects commonly associated with synthetic agents. Unlike conventional chemical shampoos, herbal variants emphasize biocompatibility, minimal toxicity, and additional therapeutic benefits such as scalp nourishment, antifungal protection, and restoration of natural hair vitality. Consequently, they are considered a safer and more sustainable alternative in contemporary hair care and cosmeceutical practices.[7]

CLASSIFICATION OF SHAMPOOS

- Powder shampoo
- Liquid shampoo
- Aerosol shampoo
- Jelly shampoo
- Cream shampoo
- Keratin shampoo
- Specialized shampoo
- Volumizing shampoo.

1. **POWDER SHAMPOO:** This type of shampoo is supplied in a dry powder form. In the past, dry soaps were used in their preparation, but today, dry synthetic detergents are preferred. During formulation, the activity of the ingredients can be reduced by adding water or another solvent, particularly when preparing medicated shampoos. Due to the inconvenience and handling difficulties associated with their use, powder shampoos are rarely employed in modern formulations.

2. **LIQUID SHAMPOO:** Liquid shampoos are the most used formulations, typically appearing as clear aqueous solutions. They are generally prepared using low cloud point detergents and may include transparent variants. Owing to their ease of application and versatility, liquid shampoos dominate the commercial shampoo market.

3. **AEROSOL SHAMPOO:** Aerosol shampoos are packaged in pressurized containers, making them aerosol-based products. They require special formulations, processing, and packaging methods because an additional propellant is incorporated. The chosen propellant must be compatible with the cleansing agents and must not hinder their activity. When the valve on the container is pressed, the shampoo is dispensed as foam. Hence, aerosol shampoos are often referred to as "foam-type shampoos."

4. **JELLY SHAMPOO:** These shampoos are characterized by their thick, translucent gel-like consistency and are produced using gelling agents such as cellulose. The main ingredient is a detergent, which may be used alone or in combination with soap. The concentration of detergent can be adjusted to achieve the desired gel consistency. Gel shampoos can also be produced by thickening clear liquid shampoos with methyl cellulose. They are widely utilized in hairdressing and beauty salons.

5. **CREAM SHAMPOO:** Cream shampoos, also known as lotion shampoos, are opaque variants of clear liquid shampoos. An opacifying agent is incorporated and dissolved with the help of solubilizing agents like magnesium stearate. These formulations are rich and conditioning, providing both cleansing and moisturizing benefits to the hair.

6. **KERATIN SHAMPOO:** Keratin shampoos are formulated with keratin oil to moisturize and nourish the hair. They help make hair appear smooth, shiny, and manageable while reducing frizzy and controlling flyaway's. Moreover, these shampoos protect the hair from damage caused by styling tools such as flat irons and blow dryers, enhancing overall hair strength and texture.

7. **SPECIALISED SHAMPOO:** Specialized shampoos are designed to meet the specific needs of various consumers, such as those with dandruff, color-treated hair, gluten or wheat sensitivities, or preference for natural ingredients. Formulations for infants and children, such as "baby shampoos," are milder and less irritating to the scalp and eyes.

8. **VOLUMIZING SHAMPOO:** Volumizing shampoos are formulated to give hair a fuller, bouncier, and more voluminous appearance. These shampoos work by enhancing hair texture rather than altering strand thickness. They are designed to be lightweight so as not to weigh the hair down, ultimately providing increased body and lift.[7]

IDEAL PROPERTIES OF HERBAL SHAMPOO

1. It should effectively cleanse the hair by removing dirt, oil, and other impurities.
2. It must produce adequate foam to ensure uniform application and thorough cleaning.
3. The shampoo should be easily rinsed off with water, leaving no residue
4. It should make the hair soft, smooth, shiny, and manageable without causing dryness.
5. It should leave a pleasant and long-lasting fragrance on the hair.
6. It must be mild on the hands, preventing dryness or irritation during use.
7. It should be dermatologically safe, causing no adverse effects or skin irritation.[4]

ADVANTAGES OF HERBAL SHAMPOO

1. It is a non-toxic shampoo.
2. It is easily available.
3. It is inexpensive and economically affordable.
4. It is eco-friendly.
5. Herbal shampoo does not cause redness, itching, irritation, or any harmful effects
6. Herbal shampoo contains pure organic ingredients and does not include any additive surfactants.[5]

FUNCTIONS OF HERBAL SHAMPOO

1. Herbal shampoos help soothe and calm irritated scalps, reducing inflammation and itching.
2. Certain herbs, such as tea tree oil and neem, possess antifungal and antibacterial properties that help control infections and promote healthy scalp.
3. Herbal shampoos can provide relief from symptoms of psoriasis and eczema, including redness, scaling, and itching.

4. Natural shampoos effectively cleanse the hair and scalp by removing dirt, excess oil, and impurities.
5. Herbal shampoos help maintain the scalp's pH balance, minimizing irritation and supporting a healthy scalp environment.[4]

Treatment of Dandruff and Role of Herbal Shampoos

Conventional dandruff treatment focuses on suppressing the scalp fungus and reducing flaking. Common topical medications include antifungal agents (e.g. ketoconazole 2% shampoo) and keratolytics (e.g. salicylic acid)[2] Coal tar, selenium sulfide, zinc pyrithione and other medicated treatments are also widely used.[20] The goal is to clear existing scales, relieve itching, and maintain remission through regular shampooing.[20,2] Polyherbal shampoos aim to complement or replace these synthetics by combining multiple natural activities. For example, *Aegle marmelos* (bael) leaves contain antifungal marmelosin and are traditionally used to fight dandruff and strengthen hair. *Shikakai* pods are rich in natural saponins and have anti-fungal and anti-inflammatory properties.[21,22,23] *Aloe vera* gel soothes and hydrates the scalp. *Turmeric* (curcumin) is also valued for its broad antimicrobial and anti-inflammatory effects (used in folklore for scalp health). In practice, these herbs may help reduce *Malassezia* growth and calm scalp irritation. Studies report that polyherbal formulations can markedly decrease dandruff and improve scalp conditions.[8,9]

LITERARTURE REVIEW

Kumar et al. (2025) conducted a detailed investigation on the development and evaluation of an herbal-based shampoo formulated to treat dandruff and fungal scalp infections. Their study emphasized the rising preference for natural alternatives over synthetic antifungal shampoos due to concerns about irritation, long-term side effects, and environmental impact associated with chemical agents like ketoconazole and zinc pyrithione. The authors incorporated multiple plant extracts—such as curry leaves, aloe vera, ginger, garlic, onion, mint, and rice water chosen for their proven antimicrobial, antifungal, antioxidant, and conditioning properties. According to Kumar and colleagues, these herbal ingredients provide therapeutic benefits through bioactive compounds including sulfur derivatives, flavonoids, alkaloids, and phenolic antioxidants. Their formulation was assessed through standard physicochemical tests, including pH measurement, dirt dispersion, foam stability, and solid content analysis. Results showed that the shampoo maintained an ideal pH range (4.5–5.5) and exhibited effective cleansing and conditioning properties. The authors also evaluated antifungal activity using the agar well diffusion method against *Malassezia furfur*, a primary dandruff- causing fungus. The herbal formulation demonstrated notable inhibition zones, confirming its antifungal potential, although slightly less than the pharmaceutical control (ketoconazole). Additionally, a four-week clinical assessment revealed a significant reduction in scalp flaking, itching, and irritation among participants, supporting the therapeutic promise of the polyherbal shampoo. Kumar et al. concluded that herbal-based shampoos offer a safe and effective alternative to conventional chemical formulations. However, they emphasized the need for larger clinical studies, extract standardization, and advanced antifungal testing to strengthen future evidence.[5]

Yoginath B. Mule (et al. 2021) studied that the study presents a simple, efficient, and eco-friendly method for synthesizing 3-acetyl quinolines using the aqueous extract of Acacia concinna pods as a green surfactant-type catalyst. The extract effectively promoted the condensation reaction between α-amino aryl ketones and active methylene compounds. This method provided excellent product yields ranging from 90–98% under mild and environmentally safe conditions. The use of a natural, biodegradable catalyst reduces the need for hazardous chemicals. Additionally, the process is cost-effective, sustainable, and easy to perform. Due to these advantages, it holds great potential for large-scale industrial applications in green chemistry.[27]

Saravanasingh K. (et al. 2016) In their study on the antimicrobial potential of *Aegle marmelos*, the authors investigated the plant's antibacterial and antifungal activities using different solvent extracts. According to the researchers, *Aegle marmelos*—a widely used medicinal plant in Ayurveda—contains bioactive constituents such as alkaloids, flavonoids, tannins, and phenolic compounds, which contribute significantly to its antimicrobial properties. The authors reported that both leaf and fruit extracts demonstrated notable inhibitory effects against several pathogenic microorganisms. Their results showed stronger activity in ethanol and methanol extracts compared to aqueous extracts, suggesting that organic solvents extract a higher concentration of active phytochemicals. The study revealed effective antibacterial activity against common pathogens such as *Staphylococcus aureus* and *Escherichia coli*, highlighting the broad- spectrum potential of the plant Similarly, antifungal testing showed that *Aegle marmelos* extracts inhibited the growth of fungal species including *Aspergillus* and *Candida*, which aligns with earlier findings reported in medicinal plant research. The authors emphasized that the antifungal activity is likely due to the presence of coumarins, lignans, and other phenolic molecules known for disrupting fungal cell membranes.[24]

More AG (et al. 2022) formulated a polyherbal antidandruff shampoo incorporating Neem, Bhringraj, Reetha, Shikakai, Fenugreek, Hibiscus, Tulsi and Aloe vera. The authors found their formulation to have a stable pH of 6.7, high dirt-dispersion capacity, and favorable viscosity and foaming properties. Most notably, they observed a **19.6 mm zone of inhibition** against *M. furfur*, demonstrating strong antifungal efficacy. Their stability study showed the formulation remained unchanged for a month under various

temperatures, further supporting its suitability for commercial development.[44]

Sharma R (et al. 2011) conducted an in-vitro investigation to evaluate the antifungal potential of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) essential oils against *Malassezia furfur*, the primary causative organism of *Pityriasis versicolor*. In their study, fresh rhizomes of both plants were hydro distilled using a Clevenger apparatus to obtain pure essential oils. The antifungal activity was assessed using two standard methods: the disc diffusion technique and the microdilution method for determining minimum inhibitory concentration (MIC). The authors reported that turmeric oil produced an inhibition zone of 55 mm, ginger oil produced 37.5 mm, and their combination exhibited the highest zone of 65 mm, surpassing the effects of conventional antibiotics such as gentamycin and streptomycin. MIC values further confirmed this activity, with turmeric oil inhibiting growth at 0.1 µl/ml, ginger oil at 0.03 µl/ml, and the combined oils at 0.02 µl/ml. The mixture demonstrated synergistic action, showing greater efficacy than single oils. Overall, the study conducted by Sharma et al. provides strong evidence that turmeric and ginger essential oils possess significant antifungal activity and may serve as potential natural therapeutic agents for managing superficial mycotic infections such as *Pityriasis versicolor*.[19]

Deshmukh S (et al. 2025) conducted a comprehensive study focusing on the development and evaluation of a polyherbal shampoo incorporating seven Ayurvedic botanicals: *Zingiber officinale* (ginger), *Phyllanthus emblica* (amla), *Acacia concinna* (shikakai), *Hibiscus rosa-sinensis* (hibiscus), *Eclipta alba* (bhringraj), *Cassia angustifolia* (senna), and *Aloe barbadensis* (aloe vera). The authors selected these herbs due to their well-documented antimicrobial, cleansing, conditioning, antioxidant, and scalp- rejuvenating properties. In their work, they prepared extracts through maceration and decoction methods, followed by phytochemical screening, which confirmed the presence of key bioactive constituents such as saponins, flavonoids, alkaloids, glycosides, tannins, and terpenoids, and phenolic compounds. These phytoconstituents are known to contribute to essential shampoo attributes, including foaming, cleansing, conditioning, and scalp health. A total of seven formulations (F1–F7) were developed using a gelatin–xanthan gum gel base combined with herbal extracts, rose oil, lemon juice, surfactant (SLS), and natural preservatives. The formulations were subjected to standard evaluation parameters, including pH, viscosity, foam generation and stability, dirt dispersion, washability, solid content, and organoleptic properties. Among all prototypes, formulation F5 demonstrated the most desirable characteristics, with a pH of 5.16, viscosity of 946 mPa·s, high foam stability (90 mL after 1 minute), and complete dirt dispersion. The authors noted that F5 showed a balanced sensory profile, including pleasant aroma, honey-gold appearance, and smooth texture, reflecting commercial-grade quality. In conclusion, the synergistic use of multiple herbal ingredients can significantly enhance shampoo performance without relying on synthetic additives. Their findings align with previously reported literature emphasizing the effectiveness of maintaining scalp integrity. The study validates polyherbal shampoo as a promising natural alternative in personal care, supporting further advancements in eco-conscious and dermatologically compatible herbal products.[39]

Key Phytochemical Classes and Their Antidandruff Function

The therapeutic activity of the chosen polyherbal blend stems from three principal classes of secondary metabolites: saponins, curcuminoids, and general polyphenols (flavonoids and tannins).

Saponins and Natural Detergency

Saponins, highly abundant in *Acacia concinna* (Shikakai), are natural triterpenoid glycosides functioning as effective, mild surfactants.[16] These compounds generate foam and facilitate the removal of dirt, oil, and impurities from the scalp and hair fibers, a critical functional requirement for any shampoo.[17] Studies indicate that the presence of sufficient saponin content is essential for acceptable foaming ability and consumer acceptance, as formulations lacking this compound demonstrate poor foaming capacity.[18] Beyond detergency, the mild cleansing action of Shikakai saponins helps cleanse the scalp without stripping natural oils, maintaining a healthier scalp environment and potentially discouraging microbial overgrowth due to a mildly acidic pH.[16]

Curcuminoids and Potent Antifungal Specificity

Curcuma longa is a powerhouse of bioactive compounds, chief among them being curcuminoids, responsible for its antimicrobial and potent anti-inflammatory properties.[11] Curcuminoids exhibit strong antifungal activity, specifically demonstrating powerful inhibition against *Malassezia furfur* with a low reported minimum inhibitory concentration (MIC) of 0.1 µl/ml for the essential oil.[19] The mechanism of action includes the disruption of the fungal cell membrane integrity and the inhibition of ergosterol synthesis, a critical component of the fungal cell structure.[20] This specific targeting capability positions *C. longa* as the core fungicidal agent within the polyherbal system.

Flavonoids, Tannins, and Protective Roles

Flavonoids, tannins, and phenolic compounds are broadly distributed across all four plant extracts.[12] These polyphenols confer significant antioxidant and broad-spectrum antimicrobial activity.[12] Antioxidants are critical for protecting hair follicles from oxidative damage induced by environmental stressors, which contribute to aging and hair loss.[13] Furthermore, the broad antimicrobial nature of tannins and flavonoids

complements the targeted antifungal action of curcuminoids, helping to inhibit the growth of harmful bacteria (such as *Staphylococcus aureus* implicated in secondary scalp infections) and fungi.[12]

Synergy in Antifungal and Scalp Management Strategies

The combination of ingredients allows the formulation to address the unique ecological requirements of *Malassezia* species. *Malassezia* yeasts are obligatory lipophiles, requiring exogenous lipids (sebum or olive oil in culture) for growth.[2] Thus, a comprehensive antidandruff formulation must execute two concurrent actions: physically cleanse excess sebum and simultaneously inhibit fungal proliferation.

The incorporation of *Aloe vera* provides an essential, non-fungicidal component to this strategy. *Aloe vera* contains enzymes that effectively break fats and strip the hair of excess oil or sebum. By reducing the available lipid nutrient source on the scalp, the *Aloe vera* component directly reinforces the efficacy of the chemical antifungals (curcuminoids and *Aegle* components). This synergy essentially starves the *Malassezia* of its required nutrient source while the potent antifungals ensure cell death. Concurrently, the anti- inflammatory properties of *Aloe vera* and *Curcuma longa* significantly soothe the scalp, reducing the scaliness and pruritus associated with seborrheic dermatitis, thereby managing the visible symptoms while the polyherbal actives address the microbial root cause.[9]

AIM AND OBJECTIVE

Aim of the Study

The aim of this research is to formulate, optimize, and rigorously evaluate a stable, safe, and efficacious polyherbal liquid shampoo incorporating standardized extracts of *Aegle marmelos*, *Acacia concinna*, *Curcuma longa*, and *Aloe vera* for the holistic management of dandruff and associated scalp conditions.

Objectives

1. To optimize the extraction methodology (solvent ratio and technique) for the four herbs to ensure maximal co-extraction of key active compounds, including high yields of antifungals and saponins.
2. To design and prepare quantitative polyherbal shampoo formulations (F1, F2, F3) varying the concentration of the standardized extract and the natural surfactant base.
3. To conduct a comprehensive physicochemical and performance evaluation (pH, viscosity, surface tension, detergency, foaming ability, and stability) of the trial formulations to select an optimum formula (F-Optimized).
4. To quantify the anti-dandruff efficacy of F-Optimized by determining the Minimum Inhibitory Concentration (MIC) against *Malassezia globosa* using standardized *in vitro* assay protocols.
5. To assess the safety profile of F-Optimized using the modern *in vitro* skin irritation test (Reconstructed Human Epidermis model) to classify the product according to international safety guidelines.

PLAN OF WORK

Plan of Work (Phased Approach)

The investigation will proceed in four sequential phases to ensure systematic development and validation:

Phase I: Extract Preparation and Standardization

Raw materials will be collected, authenticated, cleaned, shade-dried, and finely powdered. The optimal hydroalcoholic solvent ratio will be determined and utilized with a suitable extraction technique (e.g., Soxhlet or reflux). The crude extract will be filtered, concentrated, and standardized for use in the formulation.[29] Initial phytochemical screening, particularly for saponin and flavonoid content, will be performed at this stage.

Phase II: Formulation Trials

A base formulation containing glucoside surfactants, thickening agents (e.g., HPMC or Xanthan Gum), and conditioning agents (*Aloe vera* and Glycerin) will be prepared. Three trial formulations (F1, F2, F3) will be prepared by incorporating varying concentrations of the standardized polyherbal extract and adjusting the Active Surfactant Matter (ASM) percentage.

Phase III: Evaluation and Optimization

The formulations will be subjected to comprehensive physicochemical tests, including visual assessment, pH stability, rheological evaluation (viscosity), wetting time, detergency testing, and foam analysis (ability and stability).[15] Based on optimal performance and stability metrics, the superior formulation (F-Optimized) will be selected for advanced biological testing.

Phase IV: Efficacy and Safety Testing

The F-Optimized formulation will undergo rigorous *in vitro* testing, including the quantitative anti-*Malassezia* MIC assay and the internationally recognized Reconstructed Human Epidermis (RHE) skin irritation test (OECD TG 439).[25]

PLANT PROFILE

5.1 *Aegle marmelos* (Bael)

Fig. 5.1: Aegle Marmelos.

Table 5.1: Taxonomical classification.[10]

KINGDOM	PLANTAE
DIVISION	MAGNOLIPHYTA
FAMILY	MAGNOLIOPSIDA
ORDER	SAPINDALES
GENUS	RUTACEAE
SPECIES	AURANTIOIDEAE
BOTNICAL NAME	AEGLE
SYNONYME	A. MARMELOS
REGION	BAEL PATRA, BAEL

Aegle marmelos (L.) Correa, commonly known as Bael or wood apple, belongs to the Rutaceae family and is highly valued in traditional Ayurvedic medicine.[26] Pharmacological studies confirm its wide range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects.[10]

Phytochemistry and Dermatological Benefits

The plant, particularly the peel and leaves, is rich in bioactive compounds such as phenolic acids (gallic, vanillic, chlorogenic acids), flavonoids(rutin), tannins, coumarins (e.g. marmin, marmelosin, scopoletin, psoralen and related furocoumarins), and alkaloids (notably *aegeline*, *aegelenine*, *dictamine*, etc.). These constituents contribute significantly to the plant's efficacy in dermatological applications.[12] The high flavonoid and polyphenol content provides enhanced antioxidant protection, neutralizing free radicals that cause oxidative damage and contribute to scalp aging and hair follicle degradation.[8]

Specific Fungal Activity

Aegle marmelos demonstrates significant fungicidal activity. Ethanolic and water extracts of the leaves have been shown to inhibit the growth of various clinical isolates of dermatophytic fungi (e.g., *T. mentagrophytes*, *M. canis*) at minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) as low as 200 µg/mL.[14] Furthermore, crude extracts exhibit maximum antifungal activity against *Candida albicans* (MFC 15.6 mg/mL).[24] Although specific MIC data against *Malassezia globosa* requires confirmation, the proven broad-spectrum fungicidal activity validates its role as a key fungistatic agent against dandruff causing yeasts.

5.2 Acacia concinna (Shikakai)

Fig. 5.2: Acacia Concinna.

Table 5.2: Taxonomical classification of acacia concinna.[10]

KINGDOM	PLANTAE
DIVISION	MAGNOLIOPHYTA (ANGIOSPERMS)
FAMILY	FABACEAE
ORDER	FABALES
GENUS	ACACIA
SPECIES	ACACIA CONCINNA
BOTNICAL NAME	ACACIA CONCINNA
SYNONYME	SAPTALA, SOAP POD

Acacia concinna (Shikakai) has an extensive history of use as a natural alternative to synthetic shampoos, primarily due to its non-stripping cleansing action.[17]

Cleansing and Conditioning Mechanisms

The primary functional constituents are triterpenoid saponins, which provide effective detergency and cleansing by removing dirt, oil, and impurities.[16] Unlike harsh synthetic surfactants, Shikakai achieves this without excessive removal of natural protective oils, preserving the scalp's moisture balance.[17] Shikakai also offers substantial nutritional value, containing vitamins A, C, D, E, and K, as well as antioxidants that nourish the scalp and hair follicles, thereby promoting healthy growth and reducing hair fall.[13]

Dandruff Efficacy

The herb's cleansing and anti-inflammatory properties are critical in dandruff management, soothing scalp irritation, and reducing itching and flaking.[17] Studies indicate that *Acacia concinna* extracts possess specific antimicrobial and antifungal activity, particularly against *Malassezia* species, achieving effective inhibition comparable to or exceeding some synthetic formulations.[16] The presence of oxalic, tartaric, and ascorbic acids also helps create a mildly acidic environment, which naturally discourages microbial overgrowth.[16]

5.3 Curcuma longa (Turmeric)

Fig. 5.3: Curcuma Longa Root.

Table 5.3: Taxonomical Classification of Curcuma Longa Root.[13]

KINGDOM	PLANTAE
DIVISION	MAGNOLIOPHYTA (ANGIOSPERMS)
FAMILY	FABACEAE
ORDER	ZINGIBERALES
GENUS	CURCUMA
SPECIES	LONGA
BOTNICAL NAME	CURCUMA LONGA
SYNONYME	HARIDRA, HALDI

Curcuma longa, the source of turmeric, is a plant in the ginger family long utilized in Ayurvedic and Chinese medicine for treating skin, digestive, and inflammatory disorders.[11]

Key Therapeutic Constituents

The main bioactive compound is curcumin, which imparts potent antimicrobial and highly effective anti-inflammatory properties.[11] These anti-inflammatory effects are crucial, as dandruff often involves an underlying inflammatory response.[2] The key bioactive pigments in turmeric are **curcuminoids** – principally *curcumin* (~77% of curcuminoids), desmethoxycurcumin, and bisdemethoxycurcumin – as well as volatile **essential oils**. Curcumin itself is a bright yellow diarylheptanoid polyphenol.[5] Chemically, curcumin contains two phenolic rings connected by a diketone (β-diketone) linker; it exists in keto and enol tautomeric forms. This molecule exhibits potent antioxidant and anti-inflammatory effects. It has also shown broad antimicrobial activity in vitro (including against skin and scalp fungi), which makes it a useful adjunct in dandruff formulations.[13]

Specific Antifungal Efficacy

The essential oil derived from *C. longa* exhibits exceptionally strong antifungal activity against *Malassezia furfur*, reporting an MIC of 0.1 µl/ml.[19] Broader research confirms its antifungal spectrum against numerous fungal species, mediated by the destruction of the fungal cell membrane and suppression of ergosterol synthesis. This potent, quantified activity against the specific causative agent of dandruff ensures that *Curcuma longa* serves as the cornerstone of the formulation's antifungal efficacy.[20]

5.4 Aloe *vera* (L.)

Fig. 5.4: Alovera Leaf.

Table 5.4: Taxonomical classification of Alovera plant.

Kingdom	Plantae
Division	Magnoliophyta
Claas	Liliopsida
Order	Liliales
Family	Liliaceae
Subfamily	Asphodeloideae
Genus	Aloe
Species	A. Vera

Aloe vera is globally recognized for its moisturizing, soothing, and wound-healing properties.[9] It plays a critical supporting role in the polyherbal shampoo, mitigating the potential harshness of cleansing agents.

Conditioning and Scalp Soothing

The gel is rich in polysaccharides, amino acids, and vitamins (A, B12, C, E) that contribute to moisturizing and conditioning the hair and scalp, reducing irritation caused by dryness or flaking.[9] Most importantly for dandruff management, *Aloe vera* contains enzymes that break down fats, helping to control excessive sebum production and greasiness.[9] This action is integral to the overall anti-dandruff strategy, as it effectively limits the available nutrients for the lipophilic *Malassezia* yeast.[2] Its anti-inflammatory effects have also been shown to significantly decrease the scaliness and itchiness characteristic of seborrheic dermatitis.[9] Table 4 summarizes the synergistic contribution of the selected botanicals.

Table 5.4: Key Phytochemicals and Pharmacological Rationale of Selected Ingredients.

Plant (Botanical Name)	Primary Active Constituents	Antidandruff Mechanism	Supporting Evidence
Aegle marmelos	Flavonoids, Coumarins, Essential Oils	Antifungal (broad spectrum), Anti- inflammatory, Antioxidant	Fungicidal activity against dermatophytes and *C. albicans*[14]
***Acacia concinna* (Shikakai)**	Triterpenoid Saponins, Vitamins	Natural Detergency, Cleansing, *Malassezia* Inhibition	Cleanses without stripping oil; specific antidandruff fungi activity.[16]
***Curcuma longa* (Turmeric)**	Curcuminoids, Volatile Oils	Potent Specific Antifungal, Anti- inflammatory	Strong activity against *M. furfur* (MIC 0.1 µl/ml for oil)[19]
Aloe vera	Polysaccharides, Enzymes, Amino Acids	Moisturizing, Soothing, Sebum Control	Reduces scaliness, itching, and controls greasiness.[9]

6. METHOD OF EXTRACTION AND PREPARATION

1. Maceration

In this method, a solvent (menstruum) is poured over finely powdered plant material such as leaves, stem bark, or root bark until it is fully submerged. The container is then sealed and left undisturbed for three days. During this period, the mixture is regularly stirred or shaken if stored in a bottle to promote effective extraction. After the extraction is complete, the liquid extract (micelle) is separated from the solid residue (marc) by filtration or decantation. The micelle is then subjected to evaporation, either in an oven or over a water bath, to remove the menstruum.

Fig. 6.1: Maceration Process.

2. Infusion

In this technique, the plant material is ground into a fine powder and placed in a clean container. The extraction solvent is then added until it saturates and covers the surface of the powdered material for a brief period. This method is effective for extracting bioactive compounds that dissolve easily. Additionally, it is suitable for preparing fresh extracts for immediate use. The typical ratio of solvent to sample ranges from 4:1 to 16:1, depending on the desired application.

Fig. 6.2: Infusion Process.

3. Decoction

This traditional method is commonly used to extract water-soluble and heat-stable compounds from raw herbal materials by boiling them in water for about 15 minutes. Once boiled, the mixture is allowed to cool, then filtered. Cold water is added afterward to bring it to the desired volume. Tougher plant parts like roots, bark, and seeds are usually used in this process, and it's helpful to crush or grind them beforehand to improve extraction. To make a decoction, the herbs are gently simmered in water for around 30 minutes or until half the water has boiled away. The resulting liquid is then strained and can be used either as-is or diluted as needed.

Fig. 6.3: Decoction Process.

3. Soxhlet Extraction

The powdered solid sample is placed inside a thimble made of filter paper and then inserted into the Soxhlet apparatus. This setup is connected to a round-bottom flask (RB flask) containing the chosen solvent and fitted with a reflux condenser. As the solvent in the flask is gently heated, its vapors rise through the side tube, condense in the condenser, and drip onto the thimble holding the sample. The solvent gradually fills the Soxhlet chamber, dissolving the extractable compounds. Once the chamber is full, the solvent automatically siphons back into the flask, carrying the dissolved

substances with it. This cycle continues repeatedly until the extraction is complete.

Fig. 6.4: Soxhlet Extraction Process.

4. Digestion

Digestion is a form of maceration that involves gentle heating to improve extraction. By warming the mixture, the plant material softens, and the solvent becomes more effective at pulling out the active compounds. This method is ideal for herbal substances that can tolerate a bit of heat without breaking down. The added warmth helps reduce the solvent's thickness (viscosity), making it easier to extract secondary metabolites from the plant. It's especially useful when working with plant materials that dissolve easily.

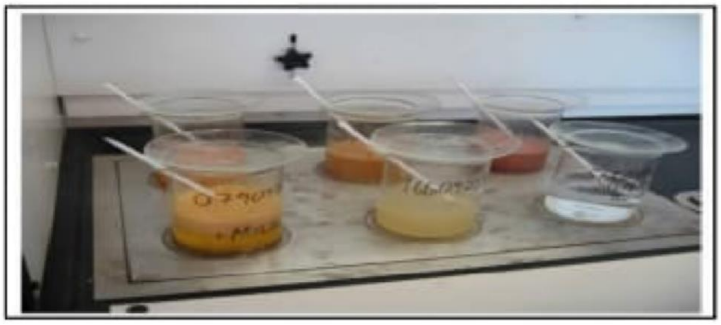

Fig. 6.5: Digestion Process.

Selection of Raw Material and Pre-Treatment

Appropriate parts of the plants (*A. marmelos* leaves/peel, *C. longa* rhizome, *A. concinna* pods, and *A. vera* gel/leaf) must be procured from reputable sources, authenticated, and prepared for extraction. The preparation involves cleaning, shade-drying to prevent decomposition of thermolabile components, and subsequent pulverization into a fine powder using a mortar and pestle.[29] This meticulous pre-treatment is critical as it maximizes the surface area, facilitating efficient mass transfer during the extraction process.

Optimization of Extraction Solvent and Technique

The choice of solvent is paramount, as the polarity determines which therapeutic molecules are successfully extracted from the botanical matrix.[32] To achieve a broad- spectrum extract containing both highly polar saponins and semi-polar compounds such as curcuminoids and certain lipophilic flavonoids, a hydroalcoholic mixture is required.[33] Research suggests that an aqueous alcoholic mixture, often around 80% methanol or ethanol, provides the best balance, maximizing the yield of both total flavonoids and saponins.[35]

The conventional wisdom in extraction science presents a challenge regarding extraction conditions for different molecule classes. While Soxhlet extraction or reflux techniques— which utilize heat and continuous solvent cycling—offer superior extraction efficiency due to enhanced mass transfer compared to simple cold maceration[29], the process temperature can be detrimental to certain compounds. For instance, studies on black bean seed coats demonstrate that while flavonoids are stable and their extraction is improved with heat (e.g., 45°C), the highest yield of saponins is achieved at lower temperatures (e.g., 25°C).[35]

Consequently, direct application of high-heat Soxhlet extraction to all materials could compromise the saponin content of the Shikakai, thus reducing the functional detergency and conditioning of the final shampoo. To

mitigate this risk, two strategies can be considered: 1) Sequential extraction, where the Shikakai is subjected to a cold or room- temperature maceration first to optimize saponin recovery, followed by Soxhlet/reflux of the remaining materials; or 2) Optimization of the hydroalcoholic Soxhlet/reflux parameters to employ the lowest effective temperature (e.g., 40–45°C) to balance high flavonoid/curcuminoid extraction yield with acceptable saponin recovery. The final choice of technique and subsequent initial phytochemical screening must quantitatively verify the simultaneous presence of functional saponins and therapeutic actives.

Preparation for Concentrated Polyherbal Extract

Following the optimized extraction, the resulting crude liquid extract must be filtered to remove particulate matter.[29] The solvent (hydroalcoholic mixture) is then evaporated under controlled conditions, preferably using a rotary evaporator at a low, regulated temperature (e.g., 40°C), to obtain a concentrated, high-potency dry extract powder.[29] This standardized polyherbal extract powder serves as the active pharmaceutical ingredient (API) in the subsequent liquid shampoo formulation.

7. FORMULATION DESIGN AND PREPARATION OF THE SHAMPOO

1. *Selection of Excipients and Base*

The formulated polyherbal shampoo must achieve optimal cleansing, stability, and therapeutic efficacy while maintaining a mild profile.

2. *Cleansing System*

The cleansing system integrates *Acacia concinna* saponins (natural detergent) with mild, nonionic surfactants such as Decyl Glucoside or Lauryl Glucoside.[36] These glucoside surfactants are favored in natural formulations due to their good biodegradability and low irritation potential. The total Active Surfactant Matter (ASM) concentration must be carefully controlled; for a standard shampoo, an ASM concentration of 10–15% is typically targeted, with the lower end preferred for dry or sensitive hair types.[38] Balancing the Shikakai saponin content with the secondary glucoside surfactant ensures satisfactory foaming and cleansing performance while minimizing the harshness associated with conventional anionic surfactants like Sodium Lauryl Sulfate (SLS).[18]

3. *Conditioning, Thickening, and Preservation*

Viscosity is managed using natural thickeners such as Hydroxypropyl Methylcellulose (HPMC) or Xanthan Gum, dissolved in warm distilled water to form a uniform gel base prior to the incorporation of other ingredients.[39] *Aloe vera* extract is included specifically for its conditioning effects, moisturizing the hair shaft and counteracting any potential drying effects of the surfactants.[15]

Because the formulation contains a high percentage of water and active botanical extracts, a broad-spectrum preservation system is critical to prevent microbial contamination and spoilage.[40] Although Phenoxyethanol is a well-regarded, low-toxicity synthetic preservative with broad efficacy,[41] a strictly "Green Cosmetic" approach may utilize natural or naturally derived systems such as Leucidal Liquid Complete, Potassium Sorbate, or Sodium Benzoate, often combined with antioxidant boosters like Rosemary Seed Extract to enhance stability.[42]

4. *Quantitative Formulation Development Trials*

Trial formulations F1, F2, and F3 will be developed to determine the optimum ratio of active extracts to the surfactant base. Formulation F3, detailed below, represents the optimized hypothesis, featuring a high concentration of active herbal extract and a balanced cleansing system to maximize efficacy and stability.

Table 7.1: Proposed Formulation Design (Exemplary Trial F3).

Ingredient	Function	Concentration (% w/w)	Justification
Polyherbal Standardized Extract	Active Antidandruff Agent	10.0%	Optimized efficacy target derived from literature MIC data.[18]
***Acacia concinna* Saponin Extract**	Primary Natural Detergent/Condition er	5.0%	Provides natural Detergency and detangling properties.[16]
Decyl Glucoside (50% AM)	Secondary Mild Surfactant	15.0% (7.5% ASM)	Contributes to moderate total ASM (12.5% total)
***Aloe vera* Gel (1:10 Conc.)**	Conditioning, Soothing, Moisturizing	3.0%	Hydrating effect, sebum control, and anti-irritation.[9]
Xanthan Gum or HPMC	Viscosity Modifier/Thickener	1.0%	Establishes desired flow properties and Stability.[39]
Preservative (e.g., Phenoxyethanol)	Stability/Antimicro-bial	0.8%	Essential for ensuring product shelf life and safety.[40]
Citric Acid Solution (10%)	pH Adjuster	q.s. to pH 6.0	Adjusts pH for scalp compatibility (5.5-7.0)[45]
Distilled Water	Vehicle	q.s. to 100%	Base solvent

8. EVALUATION OF HERBAL SHAMPOOS

1. SUBSTANCES

- Herbal ingredients such *as Aloe vera, Beal leaves, shikekai* and *curcuma longa* which are well known for their soothing, cleansing, and moisturizing properties.
- **Free from Harsh Chemicals:** Ideally, an effective herbal shampoo should be free from harmful synthetic additives such as *sulphates*, *parabens*, *silicones*, and *artificial fragrances*, as these can cause scalp irritation and long-term hair damage.
- **Active Ingredients:** Active components play a crucial role in targeting specific scalp and hair concerns, including dandruff, dryness, and excess oil. Common beneficial natural activities include *Onion*, *Rice water*, *Aloe vera*, and *Ginger*, which promote scalp health and enhance hair strength.[4]

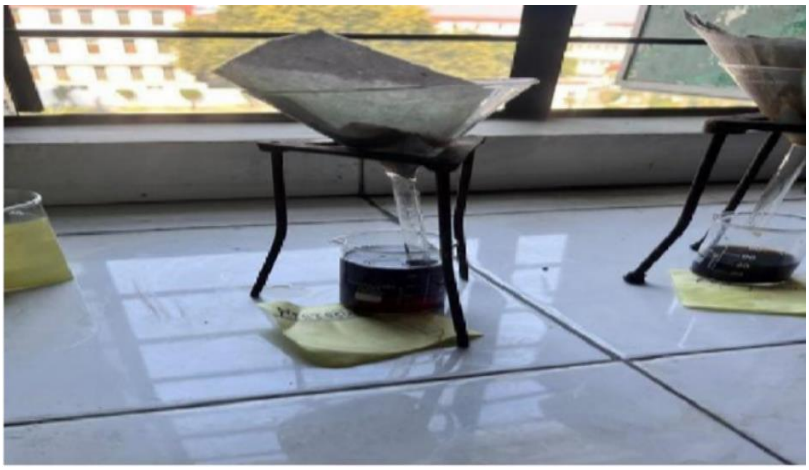

Fig. 8.1: Extraction Process.

1. Foam and Foam Stability

Although foaming action is not directly indicative of cleansing efficiency, the formation of foam in shampoo formulations is important for consumer acceptance. Some non-ionic surfactants demonstrate excellent cleansing properties but produce minimal or no foam, while effective wetting agents often yield unstable foams. The Ross-Miles foam column method is a widely accepted technique for assessing foam height and stability. In this method, 200 mL of shampoo solution is allowed to fall through an orifice into a glass column containing 50 mL of the same shampoo solution. The height of the resulting foam column is measured at specific time intervals—immediately after passage and again after 5 minutes. The foam height is considered proportional to foam volume. This method can also be employed to study the influence of hard water on foaming properties.

2. *Detergency and Cleaning Action*

Since the primary function of shampoo is cleansing, the detergency test serves as an essential indicator of its cleaning ability. The cleaning action is commonly evaluated using grease-coated wool yarns, as human hair clippings often yield inconsistent results and are therefore unsuitable. In the Barnet and Powers method, 5 g of wool yarn coated with grease is immersed in 200 mL of water containing 1 g of shampoo in a flask. The temperature is maintained at 35°C, and the flask is shaken 50 times per minute for 4 minutes. The sample is then removed, dried, and weighed to determine the percentage of soil removed under controlled experimental conditions.

3. *Wetting Action*

The Draves-Clarkson test is a standard method used to assess the wetting efficiency of surfactant-based formulations. Originally developed for evaluating products designed to aid in dying cotton yarns and fabrics, it has been adapted for studying the wetting action of shampoos. Although there is no direct correlation between fabric dyeing and shampoo performance, this test provides valuable insight into the surface activity and penetration efficiency of the formulation.

4. *Oral Toxicity*

Oral toxicity is expressed in terms of the lethal dose (LD_5□)—the quantity of material (in g/kg body weight) required to kill 50% of the test animals. Typically, rats are used for this test. Fasting animals are administered the formulation orally using a stomach tube. A lower LD_5□ value indicates higher toxicity. Soap-based shampoos exhibit optimal effectiveness within a pH range of 9.0–10.0, while synthetic detergent-based shampoos are effective between pH 6.0–9.0. Therefore, it is recommended to adjust the pH of shampoos within these ranges during formulation. The pH can be accurately determined using a pH meter and adjusted as needed with acid or alkali.

5. *Eye Irritation Test*

For the ocular irritation study, albino rabbits are typically used. Approximately 1% shampoo solution is instilled into the eyes of six rabbits, with eyelids gently held open using clips. The eyes are then examined at specific intervals over an observation period of 4 hours. The

severity of ocular irritation is assessed based on symptoms such as eyelid swelling, iris inflammation, ulceration, hemorrhaging, or, in severe cases, loss of vision.

6. *Determination of pH*

The pH of a 10% shampoo solution prepared in distilled water was measured at room temperature (25°C) to ensure compatibility with scalp and hair physiology.

Fig. 8.2: determination of pH.

7. Percentage of Solid Contents

A clean, dry evaporating dish was accurately weighed, followed by the addition of 4 g of shampoo. The combined weight was recorded, and the dish was placed on a hot plate until the liquid portion evaporated completely. After cooling, the dish was reweighed, and the solid content percentage was calculated from the difference in weights.

Fig. 8.3: solid content.

8. Anti-Microbial Activity

The well diffusion method was employed to evaluate the antimicrobial efficacy of the formulated herbal shampoo against dandruff-causing microorganisms. On separate solidified Muller Hinton Agar (MHA) plates, a bacterial and fungal lawn was prepared by spreading 0.1 mL of each culture uniformly across the surface. A well was created at the center of each plate using a sterilized borer, and 0.5 mL of the herbal shampoo was carefully introduced into the wells. The fungal culture plates were incubated at room temperature for 2–4 days, whereas the bacterial culture plates were incubated at 37°C for 24 hours. Following incubation, the zones of inhibition were observed and measured to determine the antimicrobial activity of the formulation.[6,7]

Table 8.1: Key Evaluation Protocols and Metrics.

Evaluation Parameter	Methodology	Acceptance Criteria (Ideal)	Purpose in Efficacy/Safety
pH Determination	10% solution, calibrated pH meter[15]	5.5 – 7.0	Ensures scalp compatibility and minimizes cuticle damage.
Detergency/Cleans- ing	Dirt Dispersion Test (India Ink/Artificial Sebum)	Effective dispersion (Light/Moderate)	Confirms functional removal of oil and debris/sebum.
Foam Stability	Cylinder Shake Test (time-based[46]	Stable volume is over 5 minutes	Essential for consumer acceptance and sensory quality.
Anti-Malassezia MIC	Agar/Broth Microdilution (Olive Oil supplementation)[18]	Low MIC (comparable to synthetic control)	Scientific validation of the antidandruff claim against the causative agent.
Skin Irritation	Reconstructed Human Epidermis (RHE) Model (OECD TG 439)[31]	Greater than 50% Cell Viability	Ensures user safety and classification as a Non-Irritant.

SAFETY ASSESSMENT

The assessment of consumer safety is mandated for all cosmetic preparations.

In Vitro Skin Irritation Test: The ethical and scientifically validated method for assessing potential irritancy is the *in vitro* assay utilizing the Reconstructed Human Epidermis (RHE) model (e.g., EpiSkin™).[53] This model is compliant with OECD Test Guideline No. 439, offering a human-relevant alternative to the traditional Draize rabbit test, whose concordance with human responses for mild-to-moderate irritants is often low.[55]

Procedure: The test substance (F-Optimized) is applied topically to the RHE tissue model.[53] Following a controlled exposure time, tissue viability is quantified using the MTT reduction assay.[53] An irritant substance is typically classified as one that reduces the tissue viability to 50% or less relative to the negative control. The goal is for F-Optimized to demonstrate minimal cytotoxicity, achieving greater than 50% cell viability, thereby classifying it as a Nonirritant according to the UN Globally Harmonized System (GHS) Category 2 criteria.[31]

CONCLUSION AND FUTURE PROSPECTS

The comprehensive formulation strategy leveraging *Aegle marmelos*, *Acacia concinna*, *Curcuma longa*, and *Aloe vera* is highly scientifically justified by the synergistic Multi mechanism approach. The combination is designed not merely to inhibit *Malassezia* proliferation but also to address the underlying inflammatory, oxidative, and sebaceous factors contributing to dandruff etiology. The strategic inclusion of *Aloe vera* enzymes, which reduce the lipophilic nutrient source for *Malassezia*, complements the direct fungicidal effects of curcuminoids and *Aegle marmelos* extracts, providing a robust solution for scalp health.

Based on the proposed methodology, the optimized hydroalcoholic extraction is expected to yield a high concentration of saponins, flavonoids, and curcuminoids. The subsequent formulation (F-Optimized) is projected to possess ideal physicochemical properties, exhibiting an optimal pH, satisfactory viscosity, high detergency, and acceptable foam stability.[49] Crucially, the rigorous *in vitro* evaluation is anticipated to demonstrate that the F-Optimized shampoo retains significant anti-*Malassezia* efficacy (low MIC) comparable to synthetic benchmarks, confirming the successful integration of the actives into the cosmetic matrix.[50] Furthermore, the RHE model testing should confirm the formulation's excellent safety profile, meeting the demands for safe, natural cosmetics.

This research provides substantial scientific validation for the development of a novel polyherbal shampoo, aligning with the rising demand for "green pharmacy" solutions.[6] To fully translate these findings into clinical practice, further scientific investigation is warranted. This includes conducting *in vivo* clinical trials using human subjects to confirm efficacy in reducing the dandruff severity index (DASI scores). Long-term stability studies under accelerated conditions are necessary to ensure the shelf life of the final product. Finally, detailed analytical work focused on the isolation and characterization of specific marker compounds responsible for *Malassezia* inhibition within the final shampoo matrix should be undertaken to fully elucidate the mechanism of synergistic action.[24]

11. REFERENCES

1. Ishwarya J, Shajiya S. In vitro antidandruff activity of polyherbal ecofriendly herb care cosmetics with commercial dandruff shampoos. Int J Res Anal Rev (IJRAR). 2023 Apr; 10(2): 738– 766.
2. Ahmed H, Diaz I, Cai C, Yin H, Zuniga A, Sandoval F. Evaluation of a natural anti- dandruff technology in shampoo formulation via in-vivo and in-vitro methods. J Clin Investigat Dermatol, 2023; 11(1): 6.
3. Deshmukh S, Dewangan A, Choudhary R. Formulation and evaluation of polyherbal based shampoo. Int J Pharmacogn Herb Drug Technol, 2025; 2(8): 39–53.
4. Anusha Potluri, Asma Shaheda S.K., Neeharika Rallapally, Durrivel S., Harish G. A Review on Herbs Used In Anti-Dandruff Shampoo and Its Evaluation Parameters. Res. J. Topical and Cosmetic Sci., Jan.–June 2013; 4(1): 05-13.
5. Kumar A, Gautam VK, Pal SS, Singh S. Development and assessment of a herbal- based shampoo for antidandruff and antifungal efficacy. IOSR J Pharm Biol Sci., 2025; 20(3 Ser. 1): 14–28. doi:10.9790/3008-2003011428.
6. Inderjeet, Kukkar R, Sharma A, Sharma M. A comprehensive review on herbal anti- dandruff shampoo. Int J Novel Res Dev (IJNRD), 2024; 9(2): 720–728.
7. Lal K, Sonkar D, Tripathi R, Yadav R. A review on herbal anti-dandruff shampoo. World J Pharm Res., 2024; 13(2): 540–556.
8. Kolhe R, Waghchaure A, Jain U, Anap B, Salunke N. Formulation and evaluation of peel-off mask by Aegle marmelos. World J Pharm Life Sci., 2024; 10(12): 97–99.
9. Dive A, Pandav A, Chougule N. Aloe-Vera in hair cosmetics: a comprehensive overview. Int J Innov Res Technol (IJIRT), 2024; 10(8): 136–145.
10. Kanaujiya R, Naaz F. A review on phytochemical and pharmacological activity of Aegle marmelos. Int J Pharm Health Care Res., 2025; 13(1): 37–45.
11. Bhowmik D, Chiranjib, Sampath Kumar KP, Chandira M, Jayakar B. Turmeric: A Herbal and Traditional Medicine. Arch Appl Sci Res., 2009; 1(2): 86-108.
12. Sable S. Aegle Marmelos Peel-Off Mask: A Natural Approach to Skin Care with Antioxidant, Antimicrobial, And Anti-Inflammatory Benefits. Int J Pharm Sci., 2025; 3(3): 149-158. 152.
13. Pailwan JS, Pore AV, Bais SK. Review on: Cosmetic

Importance of Shikakai. Int J Pharm Herb Technol, 2024; 2(4): 2330-2339.
14. Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pac J Trop Biomed, 2011; 1(4): 309-312.
15. Sravanthi K, Kavitha N, Sowmya K, Naazneen S, Vaishnavi U, Anil CH. A Review on Formulation and Evaluation of Herbal Anti-Dandruff Shampoo. Int J Pharm Res Appl., 2021; 6(3): 1300-1311.
16. Acharya SK. Shikakai (Acacia concinna) in dermatology: Potential uses and therapeutic benefits for skin disorders. Int J Prog Res Eng Manag Sci., 2024; 4(6): 1221-1227.
17. Gupta A, Tiwari P, Verma R, Gupta A. A Review Paper - Formulation and Evaluation of Herbal Shampoo. Int J Pharm Res Appl, 2025; 10(4): 706-715.
18. Bakr RO, Amer RI, Fayed MAA, Ragab TIM. A completely polyherbal conditioning and antioxidant shampoo: A phytochemical study and pharmaceutical evaluation. J Pharm Bioallied Sci., 2019; 11(2): 105-115.
19. Sharma R, Sharma G, Sharma M. Additive and inhibitory effect of antifungal activity of Curcuma longa (Turmeric) and Zingiber officinale (Ginger) essential oils against Pityriasis versicolor infections. J Med Plants Res., Dec. 30, 2011; 5(32): 6987-6990.
20. Chen C, Long L, Zhang F, Chen Q, Chen C, Yu X, Liu Q, Bao J, Long Z. Antifungal activity, main active components and mechanism of Curcuma longa extract against Fusarium graminearum. PLoS ONE, 2018; 13(3): e0194284: 1-19.
21. Rahman ABZN, Patwary MF, Ahmed S, Hossen M, Hossain MH, Islam MS, Rahman R, Dutta S, Sultana KF, Hossain MS, Ahmed F, Dhar SB. Antidermatophytic activity of Curcuma longa against Trichophyton spp.: compound identification and molecular docking to lanosterol 14α-demethylase. BMC Complement Med Ther., 2025; 25: 321, 2-15.
22. Monika S, Thirumal M, Kumar PR. Phytochemical and biological review of Aegle marmelos Linn. Future Sci OA., 2023; 9(3): FSO849. 1-26.
23. Bhamare G, Nangude M, More M, Mude S, Nayak H, Padawale S, Patil G. Formulation & evaluation of Aegle marmelos cream scrub. Int J Pharm Sci., 2025; 3(9): 1198–1206.
24. Saravanasingh K, Frdrik PG, Ramamurthy M. A study on antibacterial and antifungal activities of extracts of medicinal plant Aegle marmelos. Int J Adv Res Biol Sci., 2016; 3(2): 321-328.
25. Tessema TD, Wendante YS, Teffera B. Experimental dataset on leaf-based extracted antidandruff shampoo derived from Aloe Vera, Ocimum Sanctum, and Withania Somnifera: Advancing Ethiopian local herbs via formulation. Data Brief, 2024; 57: 110937: 1-13.
26. Kamble SR, Vyas L, Sen AK, Uppalwar S. Aegle marmelos: A comprehensive review of its phytochemical, pharmacological and health benefits. Int J Innov Sci Res Technol, 2024; 9(12): 35-42.
27. Mule YB, Bhale PS, Chavan HV, Shringare SN, Bandgar BP. Aqueous extract of Acacia concinna pods: an efficient, eco-friendly catalyst for the synthesis of 3- acetyl/carboxylate quinolines at room temperature. Int J Innov Res Sci Eng Technol, 2021; 10(6): 7814–7821.
28. Rahman S, Parvin R. Therapeutic potential of Aegle marmelos (L.)-An overview. Asian Pac J Trop Dis., Feb. 2014; 4(1): 71-77.
29. Zambare KK, Gonge SB, Shewale GB, Pawar PS. Preparation and evaluation of polyherbal shampoo. SBSPM's B. Pharmacy College, Ambajogai – 431517. Dist. Beed., Maharashtra, India: 23-28.
30. Grigore A, Vulturescu V, Neagu G, Ungureanu P, Panteli M, Rasit I. Antioxidant/anti-inflammatory evaluation of a polyherbal formula. Pharmaceuticals, 2022; 15(1): 114: 1–16.
31. OECD. Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method. OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects. Paris: OECD Publishing, Jun. 25, 2025; 1-73.
32. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioall Sci., 2020; 12(1): 1-10.
33. Anusha V, Vineela M, Priyanka O, Mangilal T. Formulation and evaluation of poly herbal cosmetic face cream. Indo Am J Pharm Sci., 2017; 4(08): 2724-2730.
34. Gai QY, Jiao J, Wang X, Luo M, Gu CB, McClements DJ, et al. Selectivity of current extraction techniques for flavonoids from plant materials. Processes, 2020; 8(10): 1222.
35. Oliveira PM, et al. Composition of flavonoid-rich and saponin-rich extracts obtained from black bean seed coat with the optimal conditions found in the Box-Behnken design. ResearchGate, 2013.
36. Sharma R, et al. Formulation and evaluation of a novel polyherbal shampoo. Indian J Pharm Sci., 2022; 84(2): 249-257.
37. De S, Malik S, Ghosh A, Saha R, Saha B. A review on natural surfactants. RSC Adv., 2015; 5: 65757-65767.
38. Joglekar T, Joshi S, Joshi D, Joshi V, Kadam R, Shelar S, Chakorkar S. Natural foaming agents: A review. Int J Creative Research Thoughts, 2023; 11(5): 192-203.
39. Deshmukh S, Dewangan A, Choudhary R. Formulation and evaluation of polyherbal based shampoo. Int J Pharmacogn Herb Drug Technol, 2025; 2(8): 39-53.
40. Poddebniak P, Kalinowska-Lis U. A survey of preservatives used in cosmetic products. Appl Sci., 2024; 14: 1581.
41. Uzdrowska K, Grąśka-Ponikowska M. Preservatives in cosmetics technology. Aesth Cosmetol Med., 2023; 12(2): 73-78.

42. Madnani N, Khan K. Hair cosmetics. Indian J Dermatol Venereol Leprol, 2013; 79: 654-667.
43. Bezerra KGO, Meira HM, Veras BO, Stamford TCM, Fernandes EL, Converti A, Rufino RD, Sarubbo LA. Application of plant surfactants as cleaning agents in shampoo formulations. Processes, 2023; 11: 879.
44. More AG, Pote PD, Kore PS, Garhwani YD. Formulation and evaluation of polyherbal anti-dandruff shampoo. Int J Ayurvedic Med., 2022; 13(2): 365-369.
45. Patidar DK. Polyherbal Anti-dandruff Shampoo: Basic Concept, Benefits, and Challenges. AJP [Internet], 2018 Nov. 5 [cited 2025 Nov. 5]; 12(03).
46. Kulaksiz Piskin B, Seyhan GV. Natural preservatives. Int J Tradit Complement Med Res., 2021; 2(3): 184-192.
47. Rasul, M. G. Conventional Extraction Methods Use in Medicinal Plants, their Advantages and Disadvantages. International Journal of Basic Sciences and Applied Computing, 2018; 2(6): 10–14.
48. Abubakar, A. R., & Haque, M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy & Bioallied Sciences, 2020; 12(1): 1–10.
49. Kancharla K, Lakshmi Prasanna B, Aparna Evi M, Nagadevi G, Rajeswari S. Formulation and Evaluation of Polyherbal Shampoo. Human Journals – International Journal of Pharmaceutical and Phytopharmacological Research (IJPPR), Aug. 2018; 13(1): 251-268.
50. Das S, Alam F, Sethi AK, Judder MI, Kalita P, Sarkar D. Development of polyherbal anti-dandruff formulation: an approach to green cosmetics. J Pharm Negative Results, 2022; 13(7): 2757-2764.
51. Nagalingam, A. Drug delivery aspects of herbal medicines. In A. Nagalingam & K. Watanabe (Eds.), Japanese Kampo Medicines for the Treatment of Common Diseases: Focus on Inflammation, 2017; 143–164.
52. Sinha D, Mukherjee S, Chowdhury S. Methods of extraction of phytochemicals. In Isolation, characterization, and therapeutic applications of natural bioactive compounds, 2022; 250-279. IGI Global Scientific Publishing.
53. European Centre for the Validation of Alternative Methods (ECVAM). DB-ALM Protocol No. 131: EpiSkin™ Skin Irritation Test (15 min – 42 h). Ispra (Italy): European Commission, Joint Research Centre, 2012; 40.
54. M.D. Luque de Castro, C. Priego. Soxhlet extraction: past and panacea. J Chromatogr A., April 2010; 1217(16): 2383–2389.
55. Raabe HA, Costin GE, Allen DG, Lowit A, Corvaro M, O'Dell L, Breeden-Alemi J, Page K, Perron M, Silva TF, Westerink W, Baker E, Sullivan K. Human relevance of in vivo and in vitro skin irritation tests for hazard classification of pesticides. Cutaneous Ocul Toxicol, 2025; 44(1): 1–21.