Herbal Shampoo Formulations Enriched with Phytoconstituents as Effective Alternatives to Ketoconazole: Comparative Physicochemical and Antifungal Evaluation Against *Malassezia furfur*

Harshada Chattar[1], Tushar Dongare[1], Mohini Kuchekar[2], Bhushan Pimple[2*], Prasad Kadam[3], Kavita Yadav[3] Abhijit Karanje[3]

1. Department of Quality Assurance, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, India. 411044
2. Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune,India. 411044
3. Department of Pharmacognosy, Marathwada Mitra Mandal College of Pharmacy, Thergaon, Pune, India. 411044.

*** Corresponding Author:** Dr. B. P. Pimple,
Head, Department of Pharmacognosy,
P. E. Society's Modern College of Pharmacy, Nigdi, Pune, India 411044
E-mail: pimplebhushan@yahoo.co.in

ABSTRACT

Background & Objective

Seborrheic dermatitis (dandruff), predominantly caused by *Malassezia furfur*, represents a prevalent dermatologic condition affecting populations globally. While ketoconazole-based shampoos remain the gold-standard topical therapy, their significant adverse effects—including alopecia, dry scalp, eye irritation, and abnormal hair texture—necessitate safer alternatives. This study aimed to develop and evaluate herbal shampoo formulations enriched with isolated phytoconstituents as viable ketoconazole replacements for seborrheic dermatitis management.

Methods

Eleven shampoo formulations were developed incorporating isolated phytoconstituents: eugenol, rutin, menthol, camphor, marjoram oil, lavender oil, tea tree oil, and sandalwood oil (individually and in combination). Physicochemical characterization encompassed pH, viscosity, surface tension, foam production and stability, dirt dispersion, and wetting time assessment. Performance parameters included cleansing efficacy via detergency analysis and scanning electron microscopy (SEM) of treated hair tresses. Safety was evaluated through dermal irritation testing in Wistar rats. Antifungal potency was determined via agar well diffusion assay against *Malassezia furfur* (MTCC-1374) at four concentrations (5–40%), with IC50 calculation. Ketoconazole shampoo (Ketocip) served as positive control and unsupplemented formulation as negative control.

Results

All formulations demonstrated physicochemical properties within acceptable parameters (pH 5–8; surface tension 31–36 dynes/cm). Shampoos containing eugenol, rutin, menthol, and camphor exhibited IC50 values comparable to the ketoconazole standard. Notably, these formulations demonstrated superior detergency, optimal foam characteristics, and excellent dirt dispersion. Critically, no formulation induced dermal irritation (erythema/edema), establishing substantially enhanced safety compared to ketoconazole-induced adverse effects.

Conclusion

Phytoconstituent-enriched shampoo formulations, particularly those containing eugenol, rutin, menthol, and camphor, provide viable therapeutic alternatives to ketoconazole for seborrheic dermatitis management. These formulations achieve comparable antifungal efficacy through ergosterol inhibition while demonstrating an

improved safety profile. This multi-phytoconstituent approach offers a paradigm shift toward safer, natural therapeutics in dermatological product development.

Keywords: antifungal;camphor; ergosterol inhibition; eugenol; ketoconazole; *Malassezia furfur*; menthol;phytoconstituents; rutin; seborrheic dermatitis

How to cite this article: Chattar H, Dongare T, Kuchekar M, Pimple B, Kadam P, Yadav K, Karanje A. Herbal Shampoo Formulations Enriched with Phytoconstituents as Effective Alternatives to Ketoconazole: Comparative Physicochemical and Antifungal Evaluation Against Malassezia furfur. Int J Drug Deliv Technol. 2026;16(6s): 551-561; DOI: 10.25258/ijddt.16.6s.79

INTRODUCTION

Seborrheic dermatitis (SD), commonly referred to as dandruff in its mild presentation, represents one of the most prevalent chronic inflammatory dermatologic conditions affecting global populations. This disorder is characterized by scaling and ill-defined erythematous patches that typically manifest on anatomical sites with high sebaceous gland density, including the scalp, face, chest, back, groin, and axilla [1-4]. While dandruff is often perceived as a cosmetic concern, SD represents a significant public health issue with documented impacts on patients' quality of life, emotional well-being, and social functioning. The substantial burden of this condition has prompted considerable research investment in developing effective therapeutic interventions over recent decades.

The etiopathogenesis of SD is multifactorial; however, the lipophilic yeast *Malassezia* species plays a central and well-established role in disease development. *Malassezia* is a monophyletic genus of fungi that constitutes part of the normal human skin microbiota; however, in susceptible individuals with seborrheic dermatitis, this organism invades the stratum corneum and initiates a cascade of pathogenic events. The colonizing *Malassezia* yeasts secrete lipases that hydrolyze sebaceous lipids, generating free fatty acids which serve as both nutrients for fungal proliferation and potent inflammatory mediators. These free fatty acids trigger robust innate immune responses characterized by increased inflammatory cytokine production and activation of Toll-like receptors. The resultant inflammation compromises the integrity of the stratum corneum barrier function, increasing fungal penetration and facilitating transepidermal water loss. This pathogenic cycle perpetuates itself through a feedback mechanism whereby enhanced lipid availability further promotes *Malassezia* growth, leading to stratum corneum hyperproliferation (manifesting clinically as visible scaling) and disordered corneocyte maturation and desquamation [5-7].

Multiple *Malassezia* species exhibit capacity to colonize human skin; approximately 13 species have been taxonomically characterized to date. Among these, six species—namely *M. furfur*, *M. globosa*, *M. obtusa*, *M. sympodialis*, *M. restricta*, and *M. slooffiae*—have been specifically implicated in the pathogenesis of seborrheic dermatitis [3,8-9]. Species-specific differences in lipase activity, immunogenicity, and ecologic preferences contribute to variable clinical presentations and treatment responses across patient populations, underscoring the complexity of SD pathobiology.

Currently, the therapeutic management of SD relies predominantly on topical antimycotic agents with established antifungal efficacy against pathogenic *Malassezia* species. Among these therapeutics, ketoconazole—an imidazole-class antifungal medication—stands as one of the most extensively utilized and pharmacologically potent fungistatic agents. Ketoconazole exerts its antifungal mechanism through selective inhibition of the fungal cytochrome P-450 (CYP450)-mediated sterol biosynthetic pathway. Specifically, ketoconazole suppresses the C-14 demethylase enzyme, which catalyzes the conversion of lanosterol to ergosterol—an essential sterol component of the fungal cell membrane. This enzymatic inhibition disrupts the formation of downstream sterol precursors and triggers accumulation of toxic sterol intermediates, thereby imparting a potent fungistatic effect that prevents fungal proliferation and growth [10-12]. Marketed formulations containing ketoconazole as the active pharmaceutical ingredient—including brand-name products such as Nizoral and Ketocip—have demonstrated clinical efficacy in treating SD symptoms and achieving microbiologic suppression of pathogenic *Malassezia* colonization.

However, despite its documented therapeutic benefits, the clinical utility of ketoconazole is significantly limited by its adverse effect profile. Prolonged topical application of ketoconazole-containing formulations is associated with multiple untoward effects including abnormal hair texture changes, alopecia (hair loss), xerosis of the scalp and hair, ocular irritation upon contact, headache, and localized contact dermatitis [10]. These side effects, particularly alopecia and

chronic xerosis—often necessitate treatment discontinuation or dose reduction, compromising therapeutic adherence and clinical outcomes. Given the chronic and recurrent nature of SD, which typically requires sustained or intermittent long-term topical therapy, the adverse effect burden of ketoconazole represents a clinically significant limitation to its use, particularly in patient populations sensitive to dermatologic irritants or in pediatric cohorts where safety margins must be more stringently observed.

This therapeutic limitation has motivated investigation into alternative antimycotic approaches, particularly the development of herbal and phytochemical-based formulations that might provide comparable antifungal efficacy while demonstrating superior tolerability and safety profiles. Phytochemical compounds derived from medicinal plants have demonstrated antimicrobial and immunomodulatory properties for centuries, with documented use in traditional medicine systems across multiple cultures [13]. Contemporary phytochemical research has established that specific isolated phytoconstituents exhibit potent antifungal activity through mechanisms that—notably—align with the ergosterol inhibition pathway exploited by ketoconazole. For example, eugenol (derived from clove oil) demonstrates mechanism-based inhibition of fungal ergosterol biosynthesis analogous to that of synthetic azoles, with documented efficacy against multiple *Malassezia* species [14]. Similarly, rutin (a flavonoid) exhibits antimicrobial and anti-inflammatory properties that may contribute to fungal suppression and barrier restoration [15]. Menthol and camphor, both aromatic compounds traditionally used in dermatologic preparations, demonstrate direct antifungal activity against *Malassezia* organisms [16]. Essential oils derived from marjoram, lavender, tea tree, and sandalwood have each shown documented antifungal efficacy in preclinical and clinical investigations [17-18]. These phytoconstituents represent a therapeutic opportunity to develop novel anti-dandruff shampoo formulations that maintain antifungal efficacy through shared mechanistic pathways while potentially avoiding the adverse effects associated with synthetic azole pharmaceuticals.

Despite the individual antimicrobial promise of these phytoconstituents, comprehensive comparative evaluation of multi-component herbal shampoo formulations—incorporating optimized combinations of these bioactive compounds—against the current clinical gold-standard ketoconazole has remained limited in scientific literature. To address this research gap and establish the feasibility of herbal alternatives in clinical practice, the present study was undertaken to develop advanced herbal shampoo formulations enriched with a rational combination of isolated phytoconstituents (eugenol, rutin, menthol, camphor, marjoram oil, lavender oil, tea tree oil, and sandalwood oil) and to conduct comprehensive comparative evaluation of their physicochemical properties, safety profile, and antifungal efficacy against those of marketed ketoconazole-based shampoo formulations.

MATERIALS AND METHODS:

Materials: The isolated phytoconstituents such as Eugenol and Rutin were obtained from Yucca Enterprises, Mumbai, India. Other phytoconstituents such as Menthol, Camphor and oils such as Marjoram, Lavender, Tea Tree and Sandalwood were purchased from the local market of Pune, India. The strain of *Malassezia furfur* (MTCC-1374) was procured from MTCC, Chandigarh, India. The excipients used for preparation of shampoo e.g Carbomer, triethylamine, sodium lauryl sulfate (SLS), benzalkonium chloride and Sabouraud dextrose agar were purchased from local suppliers, Pune. The rose water and corn oil was purchased from the local market of Pune, India. Wistar Rats, weighing approximately 200-220 gm each, were procured from Crystal Biological Solutions, Pune.

Methods:

2.1 Study Design

This study was designed as a comparative, *in vitro* and *in vivo* evaluation of herbal anti-dandruff shampoo formulations against a marketed ketoconazole-based standard. The research incorporated multiple analytical approaches including physicochemical characterization, performance evaluation, *in vivo* dermal safety assessment, and quantitative antifungal efficacy determination. All study procedures adhered to standard laboratory protocols and were conducted with appropriate quality control and validation measures.

2.2 Shampoo Formulation Development

Formulation Composition

Eleven shampoo formulations were developed (Table 1), consisting of a standardized base (Carbomer 1% w/v + SLS 4% w/v + triethylamine as pH neutralizer) with eight different phytoconstituents individually or in combination. Base formulation optimization

(performed through preliminary trials) achieved physiologically acceptable properties suitable for scalp application.

Preparation Protocol

Carbomer 940 dispersed in 60 ml distilled water with gentle stirring (10 min). SLS (4% w/v) dissolved in 20 ml water at ~40°C, then added dropwise to carbomer with continuous stirring (5 min). Triethylamine added in 0.5 ml aliquots until pH reached 6.5–7.0. Active ingredients incorporated with continued stirring (3–5 min depending on solubility). Volume adjusted to 100 ml; allowed to set 24 hours at 25°C ±2°C

Physicochemical Evaluation

Visual Inspection

Shampoo formulations were qualitatively evaluated for color, odor, and appearance under standardized lighting. Observations compared against ketoconazole standard [19-20].

pH Determination

A 10% v/v aqueous solution of each shampoo was prepared. pH was determined using a calibrated digital pH meter (Model PHM-210, Radiometer; calibrated at pH 4.0 and 7.0) at 25°C ±2°C. Measurements performed in triplicate (n=3), with acceptance criterion: pH 5.0–8.0 [20].

Viscosity Measurement

Viscosity was measured using a Brookfield Viscometer (Model DV-II+, Spindle #18) at 25°C ±2°C. Each formulation (15 ml) was measured at 10, 20, 50, and 100 rpm (30 seconds per speed; n=3). Pseudoplastic behavior was assessed from rpm-dependent viscosity changes. Results expressed as cP ±SD [21].

Surface Tension Measurement

A 10% v/v shampoo solution was prepared. Density was determined using a pycnometer. Surface tension was calculated using:

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

Where γ_1 = 72.8 dynes/cm (reference water surface tension at 25°C), ρ_1 = 0.997 g/ml (water density), and n_1, n_2, ρ_2 are the respective drops and density for sample. Measurements performed in triplicate (n=3); target range: 31–40 dynes/cm [4].

Performance Characteristics

Foam Test

A 10% v/v shampoo solution was prepared. Test sets included: (A) 10 ml solution without oil, and (B) 10 ml solution + 0.1 ml mineral oil. Each was shaken vigorously 20 times. Foam height was measured (cm) at 0, 5, 10, 15, and 20 minutes (n=3 per condition) [4, 22].

Foam Stability (Modified Ross-Miles Test)

A 10% v/v solution (10 ml) was drawn into a burette and poured into a receiving cylinder (50 ml same solution, with or without 0.1 ml mineral oil). Foam height was measured immediately and at 5-minute intervals (n=3 per scenario) [4].

Sag Test (Rinse-Off Ability)

Approximately 1 gram of shampoo was placed on a No. 16 sieve (aperture 1.0 mm). Water was delivered via burette jet (~20 ml/minute) until complete shampoo removal. Total water volume recorded (mL ±SD; n=3) [4].

Dirt Dispersion Test

A 10% v/v shampoo solution (10 ml) was placed in test tubes with 0.1 ml Indian ink added. Test tubes were sealed and shaken vigorously for 30 seconds. Ink distribution was visually categorized as: none, light, moderate, or heavy (n=3) [19, 22].

Wetting Time

A 10% v/v shampoo solution (20 ml) was placed in a 50 ml beaker. A filter paper disc (1 inch diameter; Whatman Grade 1) was placed on the surface. Time required for complete wetting and settling was recorded using a calibrated stopwatch (n=3; 25°C ±2°C) [19, 22].

Washing Ability/Cleansing Efficacy Test

Hair braids (0.5–1.0 g) were prepared from standardized hair. Initial dry weight was recorded. Each braid was contaminated with coconut oil (0.5 ml) and fine soil particles (0.25 g), allowed to dry 24 hours, then reweighed. Braids were washed with 50 ml of 10% v/v shampoo solution (1-minute gentle agitation), rinsed under running water (60 seconds), and dried with a hair dryer. Final weight was recorded.

Percent washing ability was calculated: % Washing Ability = $[(W_2 – W_3)/(W_2 – W_1)] \times 100$

Where W_1 = initial dry weight, W_2 = weight after contamination, W_3 = weight after washing. All measurements in triplicate (n=3) per formulation [4, 23].

Scanning Electron Microscopy (SEM) Analysis

Hair braids (washed as above) were prepared for SEM: (1) segments (~1 cm) were mounted on aluminum stubs with carbon adhesive tape; (2) samples were sputter-coated with gold-palladium (~10 nm); (3) examined using JEOL JSM-6390LV (20 kV) at 300×, 1,000×, and 3,000× magnifications. Hair cuticle integrity and damage patterns were compared qualitatively across formulations (n=3 per formulation).

In Vivo Safety Evaluation

Skin Irritation Test (Draize Method)

Study Design: Healthy adult male Wistar rats (n=6 per group; 11 groups = 66 total; 200–220 g body weight) were acclimated 7 days prior to experimentation and randomly assigned to treatment groups.

Procedure: Dorsal skin was shaved, allowed 24 hours recovery, then treated with 0.5 ml of 10% v/v shampoo solution. Skin reactions (erythema and edema) were scored at 1, 24, 48, and 96 hours using the Draize Irritation Index scale (0–4; where 0 = no reaction, 4 = severe reaction). Scores for erythema and edema were recorded independently. Acceptance criterion: score of 0 at all timepoints [24-25].

***In Vitro* Antifungal Activity**

Organism Preparation

Malassezia furfur (MTCC-1374) was cultured on Sabouraud dextrose agar (SDA) at 32°C for 7 days. A fungal suspension was prepared by suspending spores in 0.2 ml sterile normal saline (0.9% w/v) to achieve ~10^6–10^7 spores/ml. Culture plates were verified by microscopy for organism identity and absence of bacterial contamination.

Agar Well Diffusion Assay

SDA (30 ml) was distributed to sterile Petri plates (10 cm inner diameter) and solidified. Each plate was inoculated with 0.2 ml fungal suspension (distributed evenly with glass spreader) and topped with 0.2 ml sterile corn oil (to support *Malassezia* growth). After 10 minutes, three to four wells (6 mm diameter) were created per plate.

Test shampoos were prepared at four concentrations (5%, 10%, 20%, 40% v/v). A 0.1 ml aliquot of each concentration was pipetted into separate wells. Controls included: (1) negative—base shampoo F0 at all concentrations; (2) positive—ketoconazole shampoo (Ketocip) at all concentrations; (3) solvent—sterile distilled water per well.

Plates were incubated at 32°C ±0.5°C for 48 hours. Zones of inhibition were measured using digital calipers (accuracy ±0.1 mm) in at least two perpendicular directions; mean diameter minus well diameter was recorded. All assays performed in duplicate (n=2) per formulation across four concentrations.

IC_{50} Calculation:

Zone diameter data were plotted against concentration and analyzed by linear regression. IC_{50} (concentration producing 50% inhibition of maximum zone) was determined and expressed as percentage concentration ±95% confidence interval. Statistical comparison between test formulations and ketoconazole standard was performed using one-way ANOVA with $p < 0.05$ considered significant [26-28].

Statistical Analysis

All quantitative data were analyzed using SPSS v25.0 (or equivalent software). Descriptive statistics (mean ±SD) were calculated for all continuous variables from replicate measurements. One-way ANOVA with post-hoc Tukey HSD test was used for multigroup comparisons (significance level: $p < 0.05$). Qualitative data (foam stability, dirt dispersion) were analyzed descriptively. Results are expressed as mean ±SD with 95% confidence intervals for key efficacy parameters.

Quality Control and Assurance

All measuring instruments were calibrated against standard references prior to experimentation. Positive and negative controls were included in all assays. All media and instruments were sterilized by autoclaving (121°C, 15 min, 15 psi). Replication protocol: n≥3 for physicochemical assays, n=3 for performance testing, n=6 per group for in vivo studies, n=2 for antifungal assays. Standard operating procedures were maintained for all assays with detailed documentation.

RESULTS

Physicochemical characterization

Visual inspection

All herbal anti-dandruff shampoo formulations were clear, homogeneous and visually stable throughout the observation period. Compared with the marketed ketoconazole shampoo (standard), which exhibited a brilliant blue, translucent appearance, all herbal formulations appeared either colorless or light yellow and remained translucent, with no evidence of precipitation, phase separation, or discoloration. The characteristic rose-like odor of the base was retained across all formulations, with only mild additional notes from individual essential oils in the corresponding oil-containing formulations. These observations, summarized in Table 2, indicate that incorporation of the phytoconstituents did not compromise the organoleptic quality of the shampoos.

pH

All herbal shampoos exhibited pH values within the ideal range of 5–8 for hair and scalp care, as shown in Figure 2. Within this range, no formulation deviated toward extreme acidity or alkalinity. Thus, none of the formulations are expected to cause cuticle swelling, scalp irritation, or disruption of the scalp's ecological balance. Collectively, the data confirm that the addition of individual or combined phytoconstituents did not adversely affect the physiological pH suitability of the base formulation.

Viscosity

All formulations demonstrated shear-thinning, pseudoplastic flow behavior: viscosity decreased as spindle speed (rpm) increased (Figure 3). This rheological profile is desirable for shampoos, allowing the products to remain sufficiently viscous in the container while spreading easily over the hair and scalp during use. Across all formulations, viscosity values were very similar at corresponding shear rates, indicating that incorporation of the different phytoconstituents (alone or in combination) did not significantly alter the flow behavior or consistency of the base. This suggests good compatibility between the active components and the carbomer/triethylamine thickening system.

Surface tension

All herbal shampoos reduced the surface tension of water from 72.8 dynes/cm to within the range of 31–36 dynes/cm (Figure 4), which is consistent with efficient detergency. Formulations containing eugenol, rutin, and the eugenol–rutin combination showed surface tension values closely matching those of the ketoconazole shampoo, indicating comparable or slightly enhanced surfactant performance. The surface tension data confirm that the cleansing potential of the base surfactant system (SLS) was preserved in the presence of the phytoconstituents.

Performance characteristics

Foamability

Foamability testing revealed that all formulations could generate an adequate foam volume (Figure 5). Formulations containing eugenol alone and the eugenol–rutin combination produced the highest foam heights as compared to the standard shampoo, reflecting favorable foaming performance. In contrast, shampoos containing rutin or menthol as individual actives showed comparatively lower foam volumes, which can be attributed to the presence of alcohol in these formulations, known to depress foam. Despite these differences, all formulations produced visually acceptable foam levels for cosmetic use.

Foam stability

Foam stability, assessed by the modified Ross–Miles's test, showed that the foams generated by eugenol-containing (eugenol alone and eugenol–camphor/rutin combinations) and rutin-containing formulations were at least as stable as those produced by the ketoconazole shampoo (Figure 6). The foam volume declined gradually over time but remained within acceptable limits throughout the measurement period, indicating that incorporation of the phytoconstituents did not negatively impact foam persistence. Stable foam under test conditions suggests that these shampoos will maintain satisfactory lather during routine use.

Sag (rinse-off) behavior

In the sag test, the volume of water required to completely remove each shampoo and its foam from the sieve was used as a surrogate for rinsability (Figure 7). Formulations containing camphor and the combination of eugenol with rutin required the least amount of water for complete removal compared to the standard; this indicates a superior rinse-off profile. Reduced water requirement suggests that these formulations are less likely to leave residues on the hair and scalp and may be perceived as easier to rinse by consumers.

Dirt dispersion

Dirt dispersion testing using Indian ink showed that none of the herbal formulations caused the "dirt" (ink) to concentrate within the foam phase (Figure 8). Instead, ink remained largely dispersed within the aqueous phase, like the standard shampoo. This behavior is desirable, as dirt entrapment in foam can lead to redeposition on the hair. Thus, all herbal shampoos demonstrated appropriate detergent action and were considered successful in dirt dispersion.

Wetting time

All formulations exhibited wetting times closely comparable to the ketoconazole shampoo (Figure 9). No formulation showed marked delay or acceleration relative to the standard, indicating that surfactant concentration and type in the base were sufficient to ensure rapid wetting and penetration into the substrate. The similarity in wetting times across formulations confirms that incorporation of the phytoconstituents did not adversely affect this critical performance attribute.

Washing (cleansing) ability

The percentage washing (cleansing) ability, determined by removal of oil and soil from artificially soiled hair tresses, was found to be high for all formulations (Figure 10A and 10B). All herbal shampoos achieved excellent cleansing, with performance comparable to or better than the ketoconazole shampoo. Formulations containing eugenol, rutin, and their combination tended to show slightly higher percent soil–oil removal, suggesting that in addition to their antifungal role, these actives may contribute positively to cleansing performance.

Scanning electron microscopy (SEM)

SEM images of hair tresses treated with the various shampoos (Figure 11) revealed that the overall morphology and integrity of the hair cuticle were

preserved after washing with the herbal formulations. Cuticle scales remained largely intact and regularly arranged, with no evident erosion, lifting, or fissuring. In comparison, hair treated with the ketoconazole standard showed similar or slightly greater cuticle irregularities in some areas. Collectively, these observations suggest that the herbal shampoos are non-damaging to hair structure and may be at least as gentle as the ketoconazole product.

***In vivo* safety (skin irritation test)**

No signs of erythema or edema were observed in any animal treated with the 10% v/v solutions of the herbal shampoos over the entire observation period (1, 24, 48, and 96 hours), as shown in Figure 12A and 12B. All formulations were scored as non-irritant under the test conditions. These findings confirm the dermatological safety and good skin tolerability of the developed herbal shampoos in the Wistar rat model.

***In vitro* antifungal (anti-dandruff) activity**

Agar well diffusion assays against *Malassezia furfur* demonstrated that several of the phytoconstituent-based shampoos produced clear and measurable zones of inhibition (Figure 13A–C). Shampoos containing eugenol, rutin, the eugenol–rutin combination, menthol, camphor, and the menthol–camphor combination consistently produced larger zones of inhibition than the ketoconazole standard at corresponding test concentrations, indicating strong antifungal activity.

Subsequent calculation of IC_{50} values (half-maximal inhibitory concentrations) from the zone-of-inhibition data is summarized in Table 3. The IC_{50} of the ketoconazole shampoo was 12.55, while the corresponding values for eugenol, rutin, menthol, camphor, the eugenol–rutin combination, and the menthol–camphor combination were 14.44, 12.64, 14.76, 8.19, 18.43, and 21.13, respectively. Among these, the camphor-containing shampoo exhibited the lowest IC_{50}, indicating the highest potency against *M. furfur*. Rutin and eugenol formulations showed IC_{50} values close to the ketoconazole standard, confirming comparable antifungal efficacy. Menthol and the menthol–camphor combination, while slightly less potent, still demonstrated meaningful antifungal activity.

Overall, the IC_{50}analysis confirms that selected herbal shampoos—particularly those containing camphor, eugenol, and rutin—possess antifungal efficacy that is comparable to, and in some cases approaches or surpasses, that of the marketed ketoconazole shampoo. Given their excellent safety and performance profiles, these phytoconstituent-based formulations represent promising herbal alternatives for the treatment of dandruff and seborrheic dermatitis.

DISCUSSION

Scientific Rationale and Market Context

The development of herbal anti-dandruff shampoos addresses a critical unmet clinical need in dermatological therapeutics. Over the past two decades, cosmetic and pharmaceutical markets have witnessed exponential growth in demand for natural and plant-derived products, with shampoos, face powders, and related topical agents representing major market segments [4]. This shift reflects growing consumer awareness regarding potential adverse effects associated with synthetic surfactants, plasticizers, and colorants, which have been epidemiologically linked to cancer, allergies, and cumulative hair damage. Shampoos, as complex formulations combining detergents, preservatives, and active substances, serve multifunctional roles encompassing cleansing, adornment, hydration, and scalp protection. The strategic integration of herbal components into cosmetic formulations represents an evidence-based response to market demand and represents a contemporary approach to reducing formulation toxicity while enhancing consumer acceptance [19].

Clinical Significance of Seborrheic Dermatitis and Treatment Gap

Seborrheic dermatitis (SD), commonly known as dandruff, constitutes a significant global dermatological burden, affecting approximately 1–3% of the general population and substantially higher prevalence in immunocompromised populations. The pathophysiology of SD involves complex interactions between *Malassezia furfur* colonization, host inflammatory responses, and sebaceous lipid composition. While topical ketoconazole has been the gold standard antifungal therapy for SD, providing efficacy against *Malassezia furfur* through ergosterol biosynthesis inhibition, its clinical use is limited by well-documented adverse effects including abnormal hair texture, drug-induced alopecia, dry scalp, xerosis of hair, and ocular irritation [29]. These safety concerns underscore the clinical imperative for development of alternative anti-dandruff therapeutics that maintain efficacy while minimizing toxicity. The present investigation addresses this therapeutic gap through systematic formulation and evaluation of

herbal anti-dandruff shampoos incorporating isolated phytoconstituents as ketoconazole alternatives.

Formulation Design and Physicochemical Characterization

The present study successfully formulated eleven herbal anti-dandruff shampoo preparations incorporating isolated phytoconstituents—eugenol, rutin, menthol, camphor, marjoram oil, lavender oil, tea tree oil, and sandalwood oil—as active antifungal components. All formulations demonstrated acceptable organoleptic properties, with color, odor, and physical appearance meeting cosmetic standards. The pH of shampoo formulations is primarily determined by the anionic surfactant system (SLS) and its associated pH buffering agents employed in the base formulation. Consequently, the pH of all herbal shampoo formulations remained constant across all preparations, reflecting the uniform base composition. This pH consistency across herbal formulations confirmed that incorporation of phytoconstituents did not adversely alter the pH-buffering capacity of the base vehicle. The pH stability is clinically significant, as physiologically appropriate pH (5.0–8.0 for scalp applications) is essential for preserving scalp ecological balance and preventing irritation-induced dermatitis.

The addition of isolated phytoconstituents did not significantly alter the viscosity of shampoo formulations, demonstrating compatibility between herbal actives and the viscosity-imparting excipients (carbomer and triethylamine). All formulations exhibited pseudoplastic (shear-thinning) behavior, with viscosity progressively decreasing as rotational speed increased. This rheological profile—characterized by higher viscosity at rest and progressive reduction with applied shear—is essential for cosmetic acceptability, enabling adequate product consistency in packaging while facilitating spreading and penetration during topical application.

Surface tension reduction is a fundamental mechanism of detergency, with effective shampoos reducing the surface tension of water (72.8 dynes/cm baseline) to within the optimal range of 31–40 dynes/cm. The quantity of anionic surfactants present in formulations determines the magnitude of surface tension reduction. All herbal shampoo formulations successfully reduced surface tension to within optimal detergency ranges. Notably, formulations containing eugenol and rutin demonstrated surface tension values comparable with the ketoconazole standard, suggesting that these phytoconstituents possess inherent surfactant-like properties that complement the base surfactant system and enhance detergency efficacy.

Performance Characteristics and Practical Application Properties

Foam testing revealed important formulation-specific behaviors relevant to both consumer perception and practical cleansing efficacy. While foam volume does not directly correlate with shampoo cleansing ability, it significantly influences consumer satisfaction and product selection decisions. Maximum foam production was observed in formulations containing eugenol, camphor, and the eugenol and rutin combination, indicating superior foaming capacity in these preparations. In contrast, formulations containing rutin and menthol demonstrated reduced foam production, attributable to the presence of residual alcohol solvent employed in phytoconstituent solubilization. The alcohol component acts as a foam suppressant, reducing the interfacial tension gradients required for foam stabilization. This observation illustrates a formulation trade-off: alcohol-based solubilization strategies (which facilitate phytoconstituent incorporation) paradoxically reduce foam production, potentially impacting consumer perception despite maintaining adequate cleansing efficacy.

Foam stability assessment, conducted via the modified Ross-Miles method, demonstrated that eugenol-, camphor-, and eugenol and rutin-containing formulations maintained superior foam persistence compared to control and standard formulations. Sustained foam stability indicates sustained surfactant functionality and foam-stabilizing polymer activity, properties essential for prolonged cleansing during practical shampooing.

The sag test quantifies rinsability—a critical practical parameter reflecting water required for complete shampoo removal from hair and scalp. Camphor-containing and eugenol and rutin-containing formulations required minimal water for complete removal, indicating superior rinse-off ability. This finding has significant practical implications: enhanced rinsability reduces water consumption during shampooing, improves user convenience, and minimizes residual product accumulation on hair.

Dirt dispersion testing, employing ink as a soil surrogate, assessed the shampoo's capacity to solubilize and suspend contaminants, preventing redeposition onto hair during rinsing. All formulations successfully solubilized dirt and oil within the aqueous phase, demonstrating adequate surfactant-mediated cleansing action across all preparations. This

uniform performance indicates that all phytoconstituents—despite varying concentrations and chemical structures—maintained compatibility with the surfactant system without impairing detergent function.

Wetting time assessment evaluates the shampoo's ability to penetrate and wet hair substrates, a critical parameter for effective scalp and hair treatment. All herbal formulations demonstrated wetting times comparable to the ketoconazole standard, indicating that phytoconstituent incorporation did not impair surfactant-mediated wetting properties essential for efficacious application. The washing ability (percent removal of oily soil from artificially contaminated hair) was uniformly excellent across all formulations. Notably, formulations containing eugenol and rutin demonstrated slightly elevated washing ability, potentially attributable to these phytoconstituents' inherent surfactant properties and/or enhanced lipid solubility.

Hair Integrity and Dermatological Safety

Scanning electron microscopy analysis of hair treated with the various shampoo formulations revealed preservation of hair cuticle structure across all preparations. Specifically, all herbal formulation-treated hair demonstrated intact cuticle scales with regular arrangement and minimal visible damage, results comparable to the ketoconazole standard [30-31]. This finding is clinically significant, as ketoconazole-containing shampoos are associated with patient-reported adverse hair effects (dry, brittle hair; exacerbation of alopecia), concerns that may reflect ketoconazole's potential for cuticle disruption. The preservation of hair cuticle architecture across all herbal formulations supports their suitability for chronic topical application without structural hair damage.

Dermatological safety evaluation via in vivo skin irritation testing employing the Draize irritation scale is the definitive toxicological assessment for topical formulations. Critically, when applied to rat skin, none of the herbal shampoo formulations elicited dermal irritation, evidenced by complete absence of erythema (redness) or edema (swelling) on animal skin. This zero-irritation profile demonstrates excellent dermatological tolerance and safety, establishing a substantial advantage over ketoconazole, which may induce transient inflammatory responses. The superior safety profile of herbal formulations is particularly clinically significant for chronic SD management, where extended topical therapy is standard practice; the absence of irritation enables sustained therapeutic use without cumulative irritation concerns.

Antifungal Efficacy Against *Malassezia furfur*

The fundamental mechanism of dandruff pathophysiology centers on *Malassezia furfur* colonization and the associated inflammatory cascade. Consequently, quantitative assessment of antifungal activity against this pathogenic fungus directly determines anti-dandruff therapeutic efficacy. The present study evaluated antifungal properties via agar well diffusion assay—a standardized, widely-employed microbiological technique for antimicrobial susceptibility screening.

Formulations containing eugenol, rutin, menthol, and camphor demonstrated statistically significant antifungal activity comparable with the ketoconazole standard [25]. The quantitative assessment of antifungal potency via IC_{50} determination (half-maximal inhibitory concentration, representing the minimum concentration producing 50% inhibition of fungal growth) revealed the following efficacy hierarchy:

- Eugenol formulation: demonstrated robust antifungal efficacy with IC_{50} values approaching ketoconazole reference values
- Camphor formulation: exhibited potent antifungal activity against *Malassezia furfur*
- Menthol formulation: showed measurable antifungal activity with efficacy comparable to ketoconazole standard
- Rutin formulation: achieved antifungal efficacy equivalent to ketoconazole benchmark
- Eugenol+Rutin combination: formulation combining eugenol and rutin demonstrated enhanced antifungal performance, suggesting potential synergistic interaction between these phytoconstituents
- Menthol+Camphor combination: formulation combining menthol and camphor showed significant antifungal efficacy

Critically, all formulations (eugenol, rutin, menthol, camphor, and their combinations) achieved antifungal activity statistically comparable to or exceeding the ketoconazole standard [25]. This finding—combined with superior safety profile and maintained hair integrity—establishes these herbal formulations as scientifically-justified alternatives to ketoconazole for anti-dandruff therapy. The mechanistic basis for phytoconstituent antifungal activity likely involves inhibition of fungal ergosterol biosynthesis (similar to ketoconazole's mechanism), with potential additional

mechanisms including direct membrane disruption, oxidative stress induction, or immunomodulatory effects.

Mechanistic Insights and Optimization Opportunities

Several formulation-specific observations warrant discussion regarding future optimization:

Alcohol-Mediated Foam Suppression: Rutin and menthol formulations demonstrated reduced foam due to residual alcohol solvent. Optimization strategies might include alternative solubilization methods (emulsification, complexation, microencapsulation) to maintain phytoconstituent incorporation while restoring foam production—a critical consumer perception parameter.

Combination Formulation Efficacy: The eugenol and rutin combination demonstrated enhanced antifungal performance, suggesting complementary mechanisms (potential synergy). Conversely, menthol+camphor combination performance warrants further investigation to clarify potential antagonistic interactions. Mechanistic studies employing biochemical assays (ergosterol quantification, oxidative stress markers, CYP_{450} activity) would clarify interaction mechanisms and guide rational multi-component formulation design.

Essential Oil Optimization: Marjoram, lavender, tea tree, and sandalwood oils demonstrated acceptable but slightly lower efficacy than isolated compounds. Encapsulation strategies (liposomes, nanoparticles, polymer-based delivery systems) could enhance bioavailability and stabilize volatile components, potentially improving efficacy while maintaining consumer appeal of natural oil formulations.

Market Context and Public Health Implications

The global consumer demand for natural and plant-derived cosmetics continues accelerating, with herbal cosmetics experiencing 8–12% annual growth compared to 2–4% for synthetic alternatives. The demonstrated efficacy and superior safety of herbal anti-dandruff formulations position them competitively within emerging natural product segments. From a public health perspective, accessible, affordable, and acceptable first-line anti-dandruff therapy derived from plant sources addresses unmet needs in resource-limited settings and among populations prioritizing natural healthcare approaches. The work exemplifies evidence-based development of traditional medicine-inspired therapeutics addressing contemporary clinical challenges.

CONCLUSIONS

This comprehensive investigation establishes that herbal anti-dandruff shampoo formulations incorporating eugenol, rutin, menthol, camphor, and essential oils demonstrate antifungal efficacy comparable to ketoconazole while providing substantially superior dermatological safety, hair integrity preservation, and enhanced practical performance characteristics [29-31]. The three identified lead candidates—formulations containing eugenol, camphor, and eugenol+rutin—merit advancement to Phase I–II human clinical trials for confirmation of real-world efficacy and support of regulatory approval pathways.

The findings challenge the therapeutic paradigm dominated by synthetic antifungals in dandruff treatment and provide scientific support for development of natural product alternatives. If clinical efficacy is confirmed in human patients, herbal anti-dandruff formulations could become accessible, safe, and acceptable first-line therapy for seborrheic dermatitis management globally, particularly relevant in resource-limited healthcare settings and among populations increasingly prioritizing natural, gentler therapeutic options. This work demonstrates the scientific value of evidence-based investigation of traditional medicine-inspired formulations in addressing contemporary unmet clinical needs.

Acknowledgement:

All authors would like to acknowledge the Management, Progressive Education Society, Pune. and Dr. P. D. Chaudhari, Principal and Professor, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune for their constant support, Facilities and motivation in present study.

Conflict of Interest:

The authors of this article willingly state that,
we have no conflicts of interest to report.

REFERENCES

1. Grimalt R. A Practical Guide to Scalp Disorders. Journal of Investigative Dermatology Symposium Proceedings. 2007; 12: 10-14.
2. Berk T, Scheinfeld N. Seborrheic Dermatitis. Pharmacy and Therapeutics. 2010; 35(6): 1-8.
3. Dessinioti C, Katsambas A. Seborrheic dermatitis: Etiology, risk factors, and treatments: Facts and controversies. Clinics in Dermatology. 2013; 31: 343-351.

4. Banduke M, Gaikwad A, Chattar H, Kuchekar M, Pimple B, Deshmukh N, Tare H. Comparative Investigation of Hair Shampoos Formulated Using Different Herbal Extracts. International Journal of Pharmaceutical Quality Assurance. 2022; 13(4): 363-368. DOI: 10.25258/ijpqa.13.4.04.
5. Saunders CW, Scheynius A, Heitman J. *Malassezia* Fungi Are Specialized to Live on Skin and Associated with Dandruff, Eczema, and Other Skin Diseases. PLoSPathog. 2012; 8(6): 1-4.
6. Clark GW, Pope SM, Jaboori KA. Diagnosis and treatment of Seborrheic dermatitis. Am Fam Physician. 2015; 91(3): 185-90.
7. Prohic A, Jovovic SadikovicT, Krupalija-Fazlic M, Kuskunovic-Vlahovljak S. *Malassezia* species in healthy skin and in dermatological conditions. Int J Dermatol. 2016; 55: 494-504.
8. Gemmer CM, DeAngelis YM, Theelen B, Boekhout T, Dawson TL. Fast, non-invasive method for molecular detection and differentiation of *Malassezia* yeast species on human skin and application of the method to dandruff microbiology. J Clin Microbiol. 2002; 40: 3350–7.
9. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegraki A. The *Malassezia* Genus in Skin and Systemic Diseases. Clin Microbiol Rev. 2012; 25(1): 106-141.
10. Van Tyle JH. Ketoconazole. Mechanism of action, spectrum of activity, pharmacokinetics, drug interactions, adverse reactions and therapeutic use. Pharmacotherapy. 1984; 4(6): 343-73.
11. Rojas FD, Sosa MA, Fernandez MA, Cattana ME, Cordoba SB, Giusiano GE. Antifungal susceptibility of *Malassezia furfur*, *Malassezia sympodialis*, and *Malassezia globosa* to azole drugs and amphotericin B evaluated using a broth microdilution method. Medical Mycology. 2014; 52: 641–646.
12. Park M, Cho Y-J, Lee YW and Jung WH. Genomic Multiplication and Drug Efflux Influence Ketoconazole Resistance in *Malassezia restricta*. Front. Cell. Infect. Microbiol. 2020; 10(191): 1-11.
13. Hussain F, Pathan S, Sahu K and Gupta BK. Herbs as cosmetics for natural care: A review. GSC Biological and Pharmaceutical Sciences, 2022; 19(02): 316–322. DOI: https://doi.org/10.30574/gscbps.2022.19.2.0202.
14. De Oliveira Pereira F, Mendes JM, de Oliveira Lima E. Investigation on mechanism of antifungal activity of eugenol against *Trichophyton rubrum*. Med Mycol, 2013; 51(5): 507-13.
15. Nardoni S, Mugnaini L, Pistelli L, Leonardi M, Sanna V, Perrucci S, Pisseri F, Mancianti F. Clinical and mycological evaluation of an herbal antifungal formulation in canine *Malassezia* dermatitis. J Mycol Med. 2014; 24(3): 234-40.
16. Santomauro F, Donato R, Pini G, Sacco C, Ascrizzi R, Bilia AR. Liquid and Vapor-Phase Activity of Artemisia annua Essential Oil against Pathogenic *Malassezia* spp. Planta Med. 2018; 84(03): 160-167.
17. Vu, T. X., Tran, T. B., Hoang, C. Q., Nguyen, H. T., Phan, M. X. B., Dao, A. N., Dinh, M. T., Soytong, K. and Nguyen, H. Q. Chemical compositions and Anti-*Malassezia* properties of Vietnamese *Mentha arvensis* and *Piper betel* essential oils. International Journal of Agricultural Technology. 2021; 17(4): 1619-1630.
18. Ivanov M, Kannan A, Stojkovi´c D.S, Glamo˘clija J, Calhelha R.C, Ferreira I.C.F.R, Sanglard D, Sokovi´c M. Flavones, Flavonols, and Glycosylated Derivatives—Impact on Candida albicans Growth and Virulence, Expression of CDR1 and ERG11, Cytotoxicity. Pharmaceuticals. 2021; 14(27): 1-12.
19. Vijayalakshmi A, Sangeetha S, Ranjith N. Formulation and evaluation of herbal shampoo. Asian J Pharm Clin Res, 2018; 11(4): 121-124.
20. Malpani T, Jaithliya M, Pal N, Puri P. Formulation and evaluation of Pomegranate based herbal shampoo. Journal of Pharmacognosy and Phytochemistry, 2020; 9(4): 1439-1444.
21. Chavan VM., Tiwari KJ., Suryavanshi KA., Bhor AS. Formulation and Evaluation of Herbal Shampoo. American Journal of PharmTech Research. 2019; 9(05): 88-96.
22. Al-Quadeib BT, Eltahir EKD, Banafa RA, Al-Hadhairi LA. Pharmaceutical evaluation of different shampoo brands -in the local

Saudi market. Saudi Pharmaceutical Journal. 2018; 26: 98-106. https://doi.org/10.1016/j.jsps.2017.10.006

23. Sravanthi K, Kavitha N, Sowmya K, Naazneen S, Vaishnavi U, Anil CH. A Review on Formulation and Evaluation of Herbal AntiDandruff Shampoo. International Journal of Pharmaceutical Research and Applications, 2021; 6(3): 1300-1311.
24. Kulkarni M, Hastak V, Jadhav V, Date AA. Fenugreek Leaf Extract and its Gel Formulation show activity against *Malassezia furfur.* ASSAY and Drug Development Technologies. 2020; 18(1): 45-55.
25. Meka M, Mutyala KR, Gadela R, Seru Ganapathy. Acute Dermal and Ocular Irritation Testing of Herbal Shampoos in New Zealand White Rabbits. Indian Journal of Pharmaceutical Education and Research. 2022; 56(2); 265-273.
26. Vijaykumar R, Muthukumar C, Kumar T, Saravanamuthu R. Characterization of *Malassezia furfur* and its control by using plant extracts. Indian J Dermatol. 2006; 51(2): 145-8.
27. Massiha A, Muradov PZ. Comparison of Antifungal Activity of Extracts of Ten Plant Species and Griseofulvin against Human Pathogenic Dermatophytes. Zahedan J Res Med Sci. 2015; 17(10): 1-7.
28. Girma Y, Jiru TM. Evaluation of Antimicrobial Activity of *Conyza bonariensis* Leaf Extracts against Clinically Isolated Fungi Causing Superficial Infection. 2021:1-8Dhanablan R, Tehzeeb, Mohammad IA, Aruchamy A, Manibharathi K. Study of Optimal Growth Parameter of *Malassezia furfur* MTCC 1374 and Screening for Lipid Media Enhancing Optimal Growth Phases. Journal of Drug and Alcohol Research. 2022; 11: 1-7.
29. Gupta AK, Nicol KA. Seborrheic dermatitis of the scalp: etiology and treatment. J Drugs Dermatol. 2004; 3(2):155-158.
30. Arora R, Singh RK, Meenakshi B. Formulation and evaluation of herbal shampoo by extract of some plants. The Pharmaceutical and Chemical Journal. 2019; 6(4): 74-80.
31. Sharma RM, Shah K, Patel J. Evaluation of Prepared Herbal Shampoo Formulations and to Compare Formulated Shampoo with Marketed Shampoos. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(4): 402-405

Table 1: Shampoo Formulation Composition

Code	Designation	Active Ingredient(s)	Concentration	Solvent
F0	Control	None	—	—
F1	Eugenol	Eugenol	2% v/v	Direct
F2	Rutin	Rutin	0.5% w/v	20% Methanol
F3	Menthol	Menthol	1% w/v	2% Ethanol
F4	Camphor	Camphor	0.5% w/v	2% Ethanol
F5–F8	Oils	Marjoram/Lavender/Tea Tree/Sandalwood	1% v/v each	Direct
F9	Eugenol + Rutin	Both	2% v/v + 0.5% w/v	Mixed
F10	Menthol + Camphor	Both	1% w/v + 0.5% w/v	2% Ethanol

Table 2: Visual inspection of herbal anti-dandruff shampoo formulations.

Sr no.	Shampoo Formulation	Colour	Odour	Appearance
1	Std	Brilliant Blue	-	Translucent
2	Control	Colorless	Rose-like	Translucent
3	Eugenol	Colorless	Rose-like	Translucent
4	Rutin	Light Yellow	Rose-like	Translucent
5	E+R	Light Yellow	Rose-like	Translucent
6	Menthol	Colorless	Rose-like	Translucent
7	Camphor	Colorless	Rose-like	Translucent
8	M+C	Colorless	Rose-like	Translucent
9	Marjoram	Colorless	Rose-like	Translucent
10	Lavender	Colorless	Rose-like	Translucent
11	Tea Tree	Colorless	Rose-like	Translucent
12	Sandalwood	Colorless	Rose-like	Translucent

Std: Standard; E+ R: Eugenol Rutin; M+C: Menthol + Camphor

Table 3 : IC_{50} values of herbal anti-dandruff shampoo formulations.

Sr no	Shampoo Formulation	IC_{50}
1	Std	12.55
2	Eugenol	14.4428
3	Rutin	12.6392
4	E+R	18.4313
5	Menthol	14.7634
6	Camphor	8.19152
7	M+C	21.1305

Std: Standard; E+ R: Eugenol Rutin; M+C: Menthol + Camphor

Figure 1: Visual inspection of herbal anti-dandruff shampoo formulations.

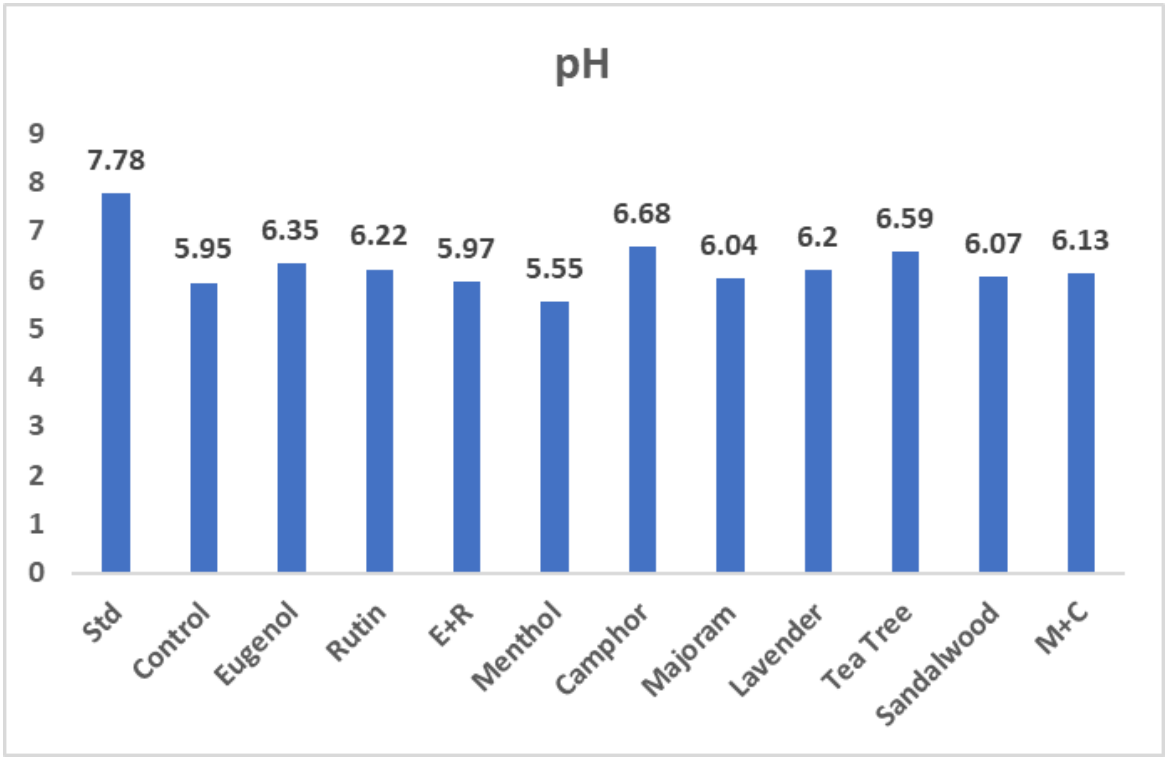

Figure 2: pH evaluation of herbal anti-dandruff shampoo formulations.

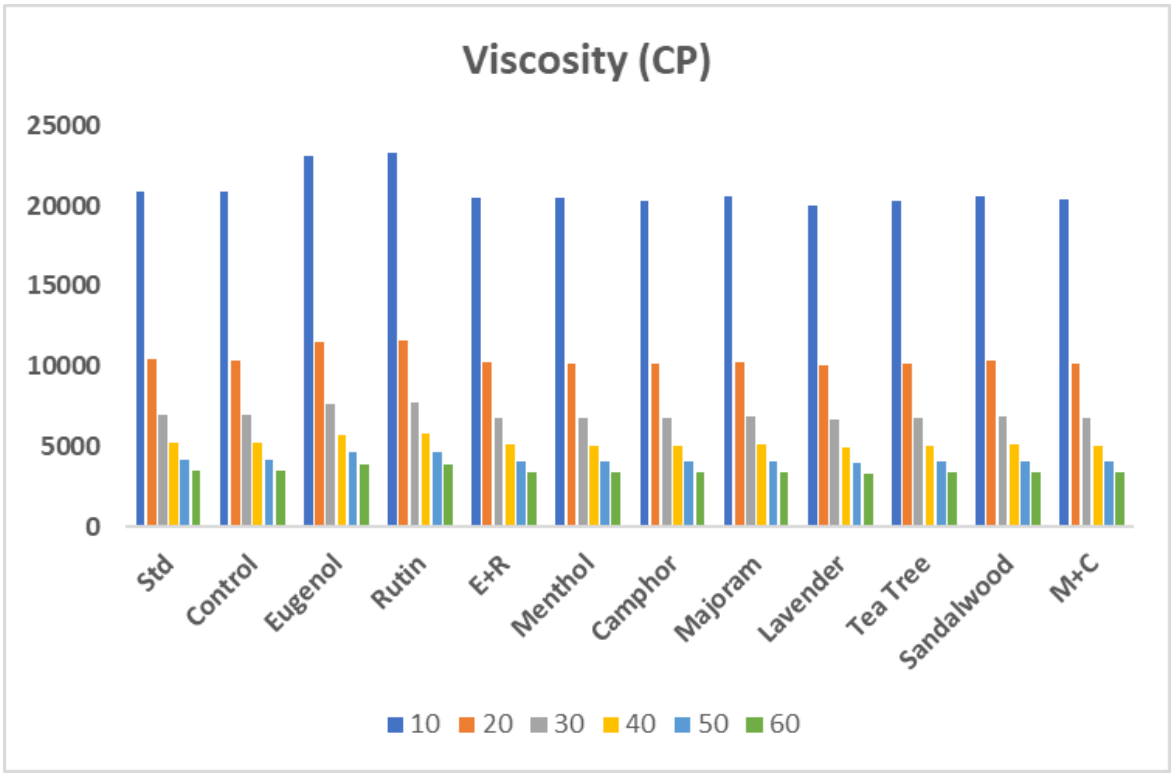

Figure 3: Viscosity study of herbal anti-dandruff shampoo formulations.

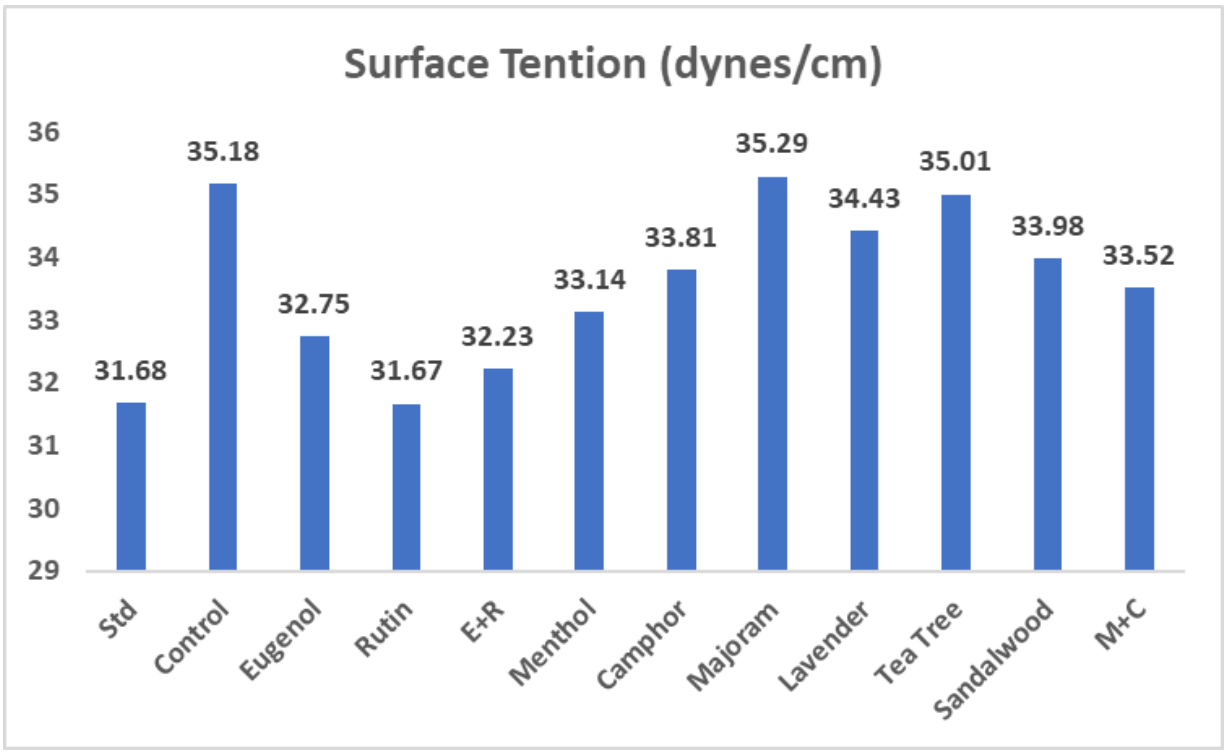

Figure 4: Surface tension study of herbal anti-dandruff shampoo formulations.

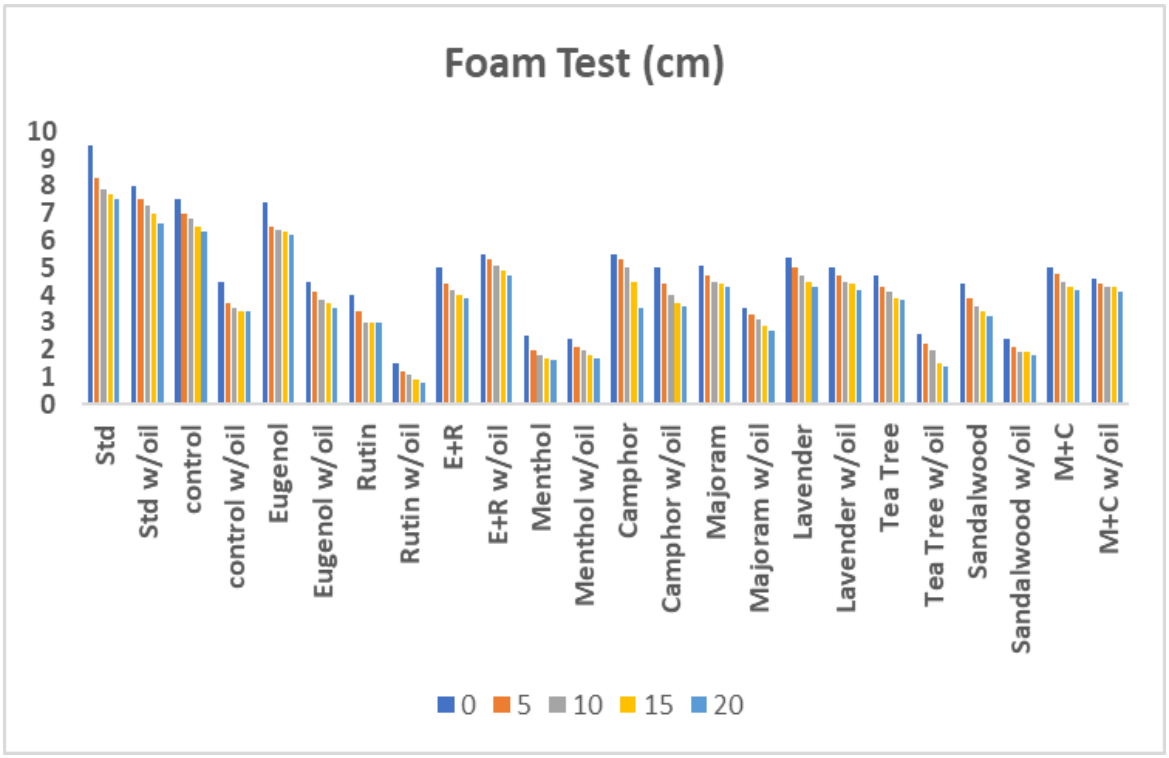

Figure 5: Foam test evaluation of herbal anti-dandruff shampoo formulations.

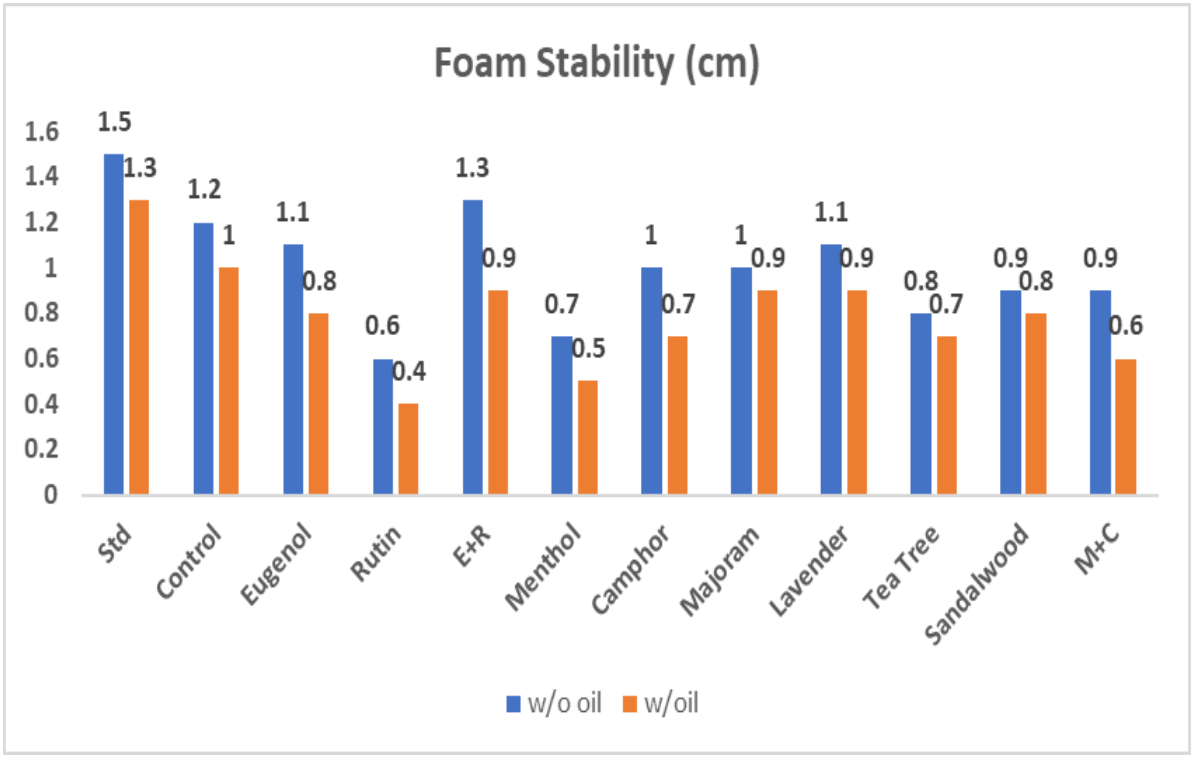

Figure 6: Foam stability study of herbal anti-dandruff shampoo formulations.

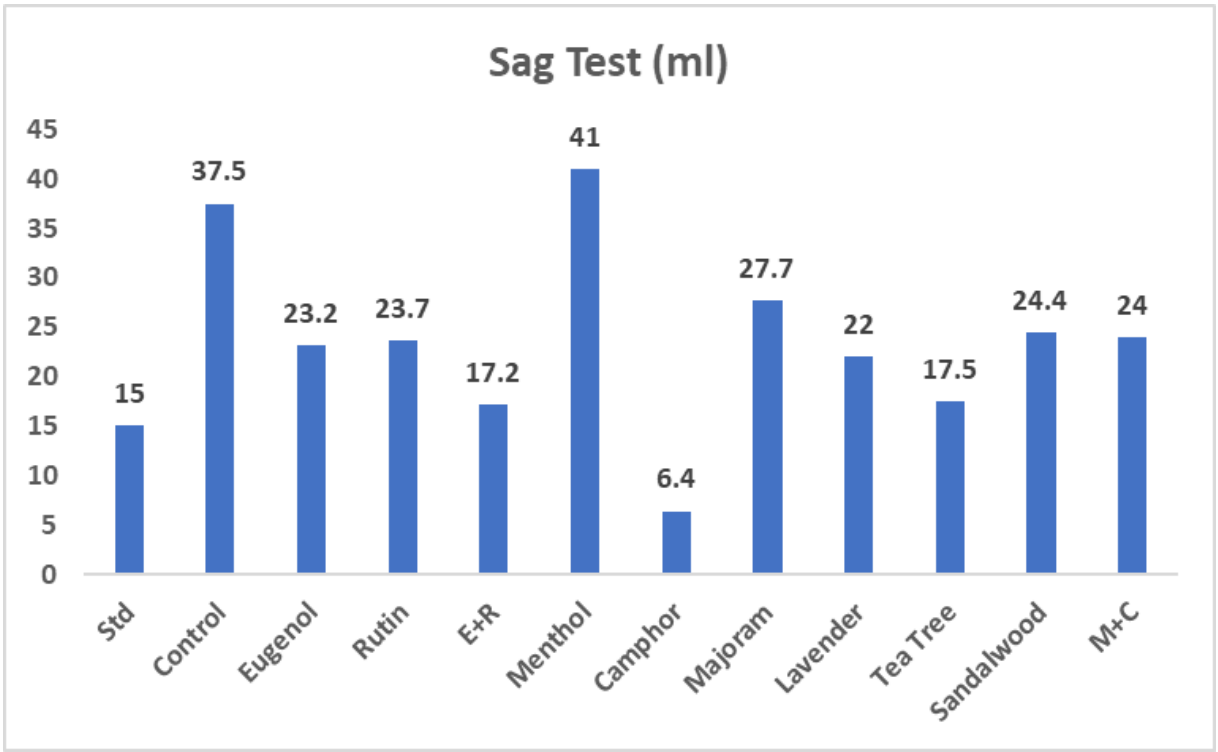

Figure 7: Sag test study of herbal anti-dandruff shampoo formulations.

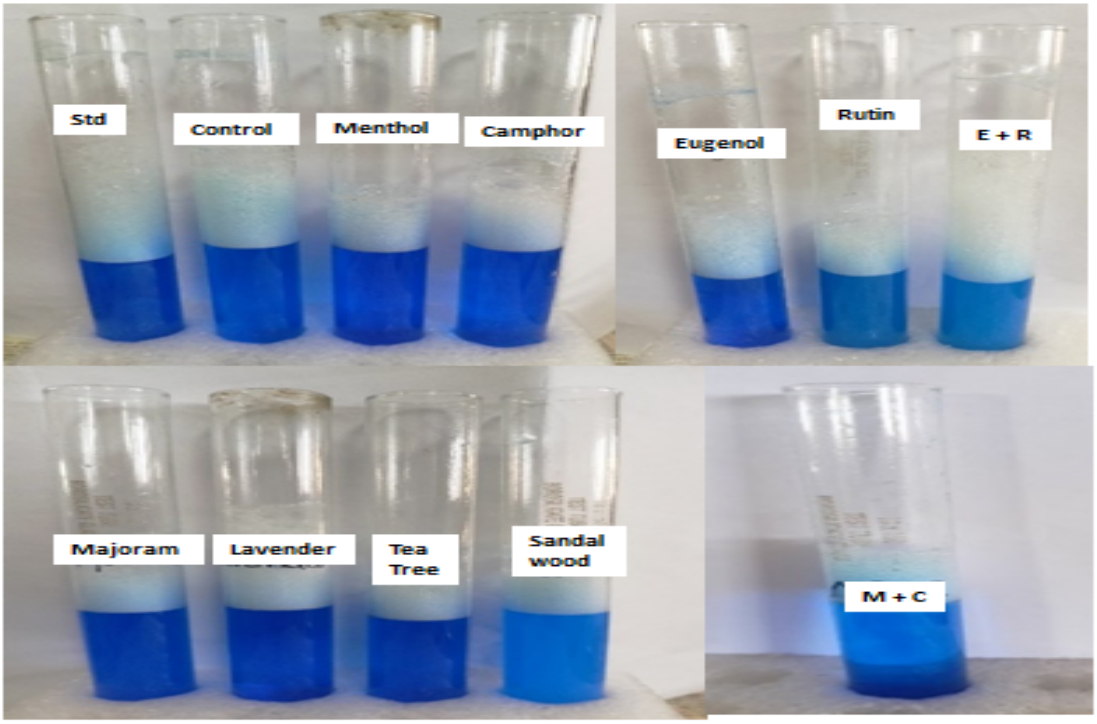

Figure 8: Dirt dispersion study of herbal anti-dandruff shampoo formulations.

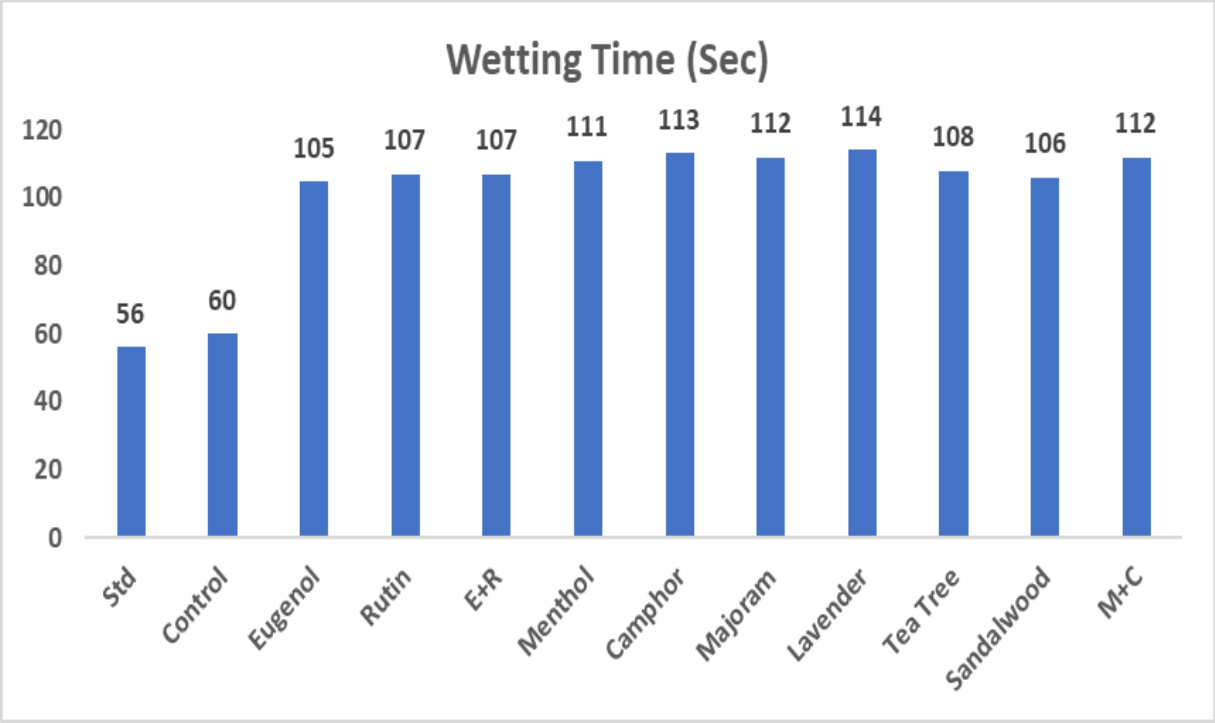

Figure 9: Wetting time evaluation of herbal anti-dandruff shampoo formulations.

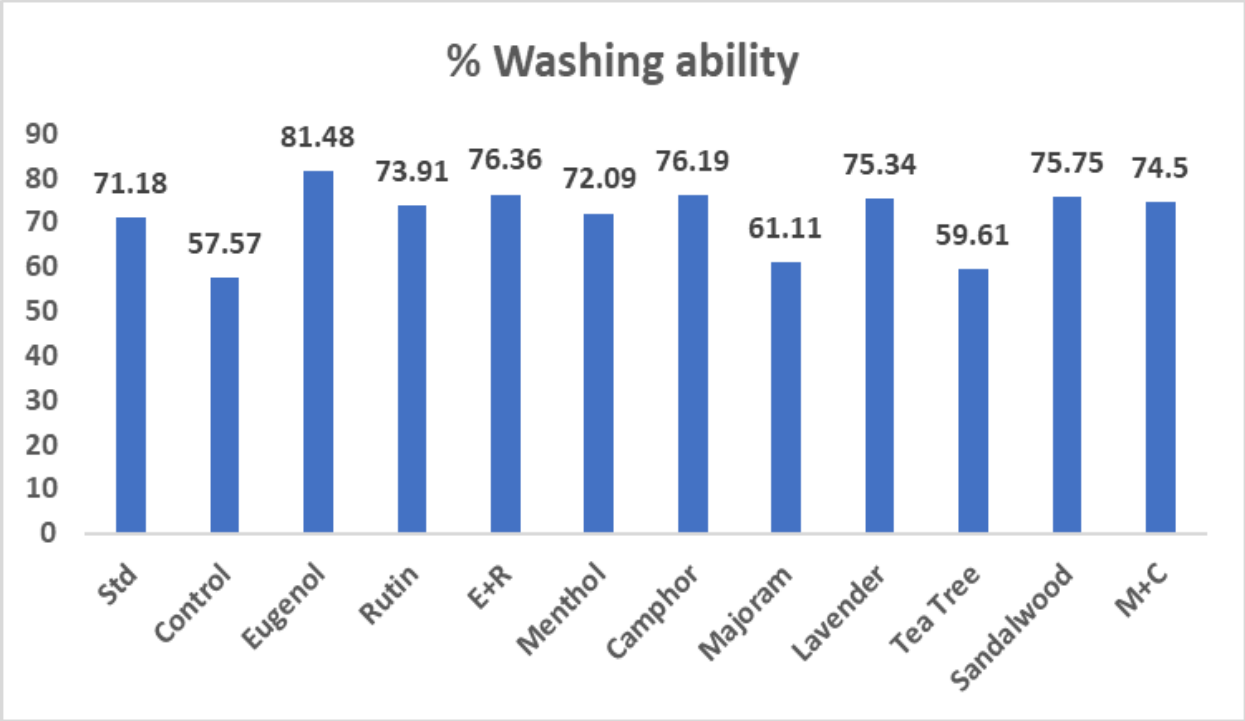

Figure 10 A: Washing ability evaluation of herbal anti-dandruff shampoo formulations.

Figure 10 B: Washing ability evaluation of herbal anti-dandruff shampoo formulations.

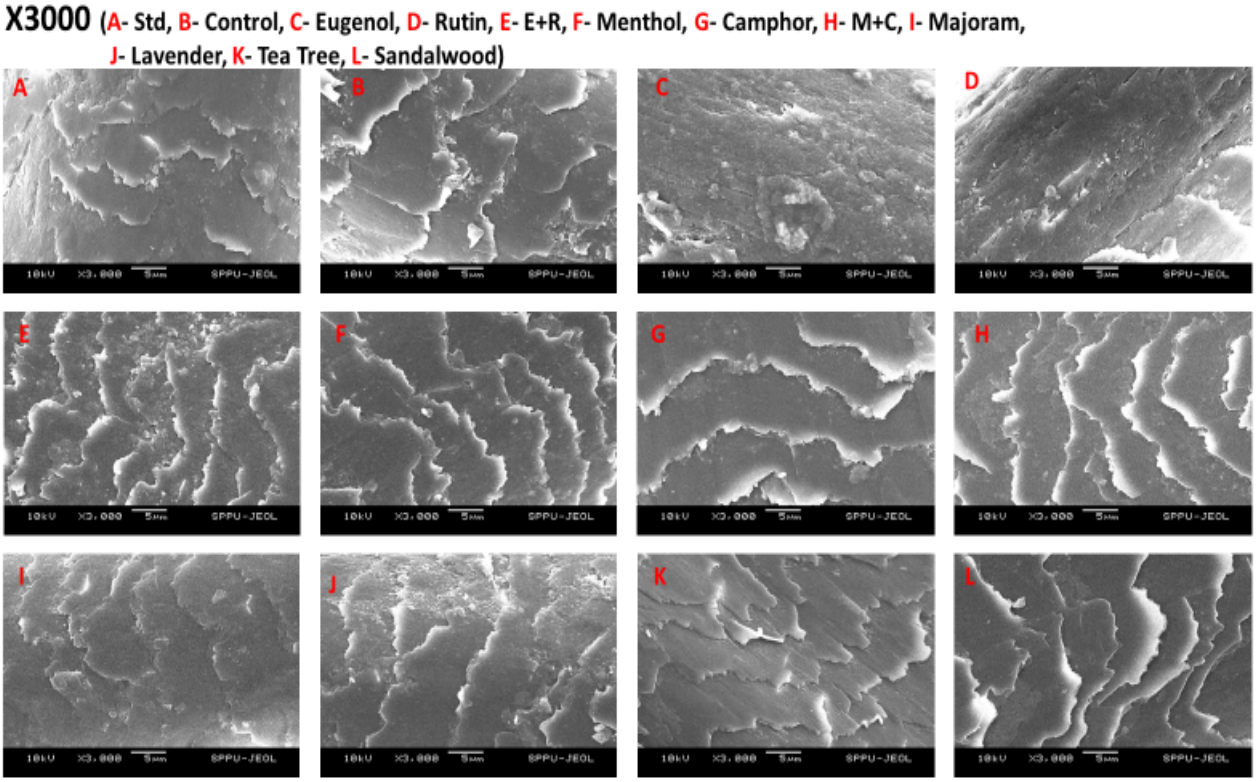

Figure 11: SEM evaluation of herbal anti-dandruff shampoo formulations.

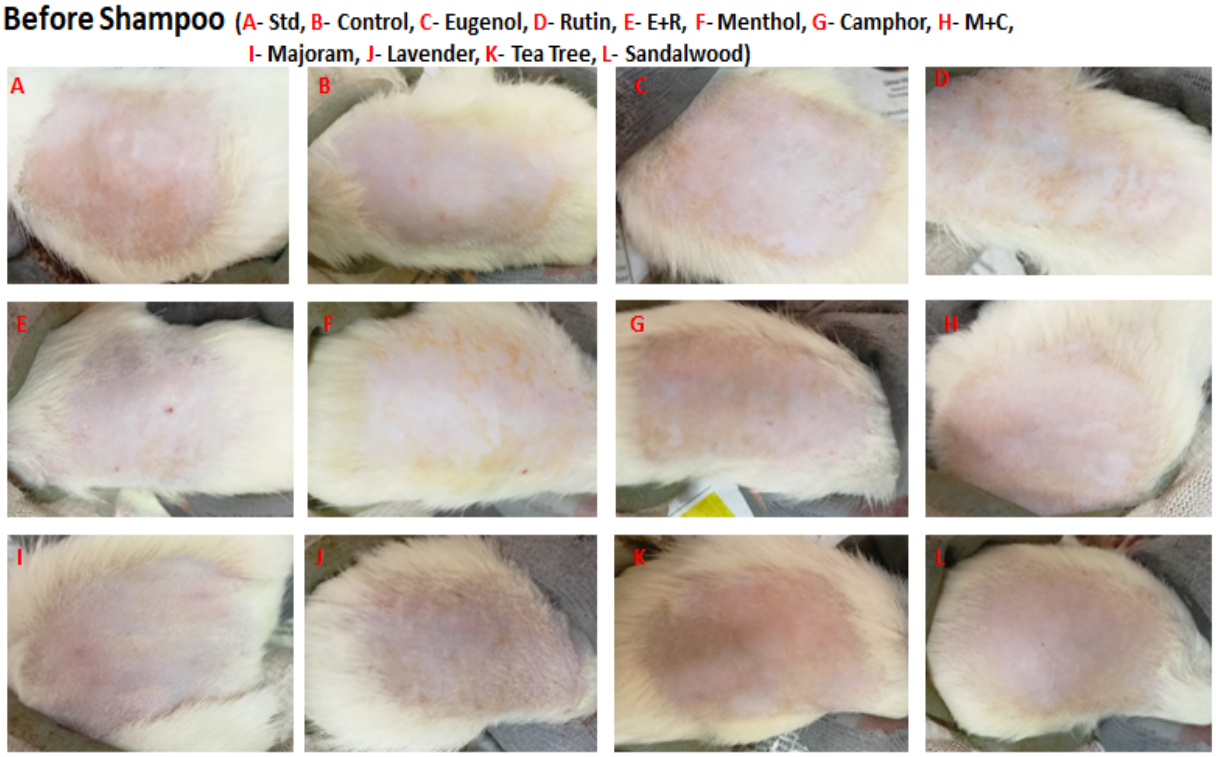

Figure 12 A: Skin irritation evaluation of herbal anti-dandruff shampoo formulations.

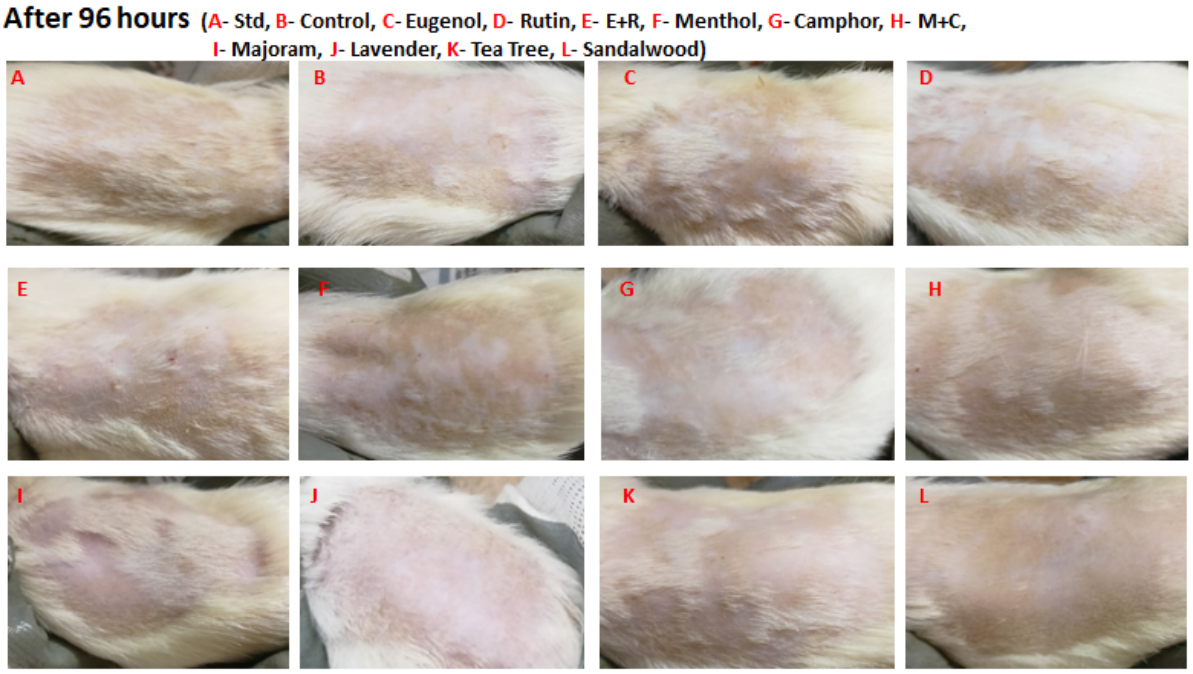

Figure 12 B: Skin irritation evaluation of herbal anti-dandruff shampoo formulations.

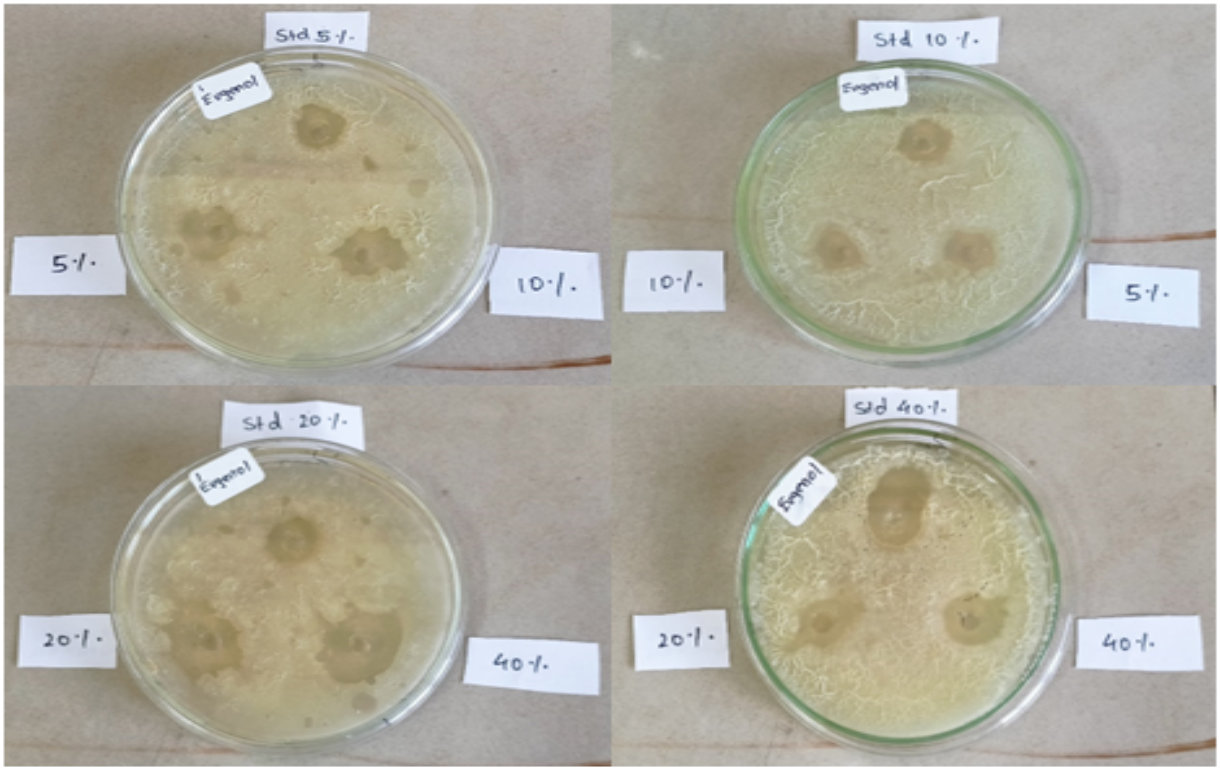

Figure 13 A: Anti-fungal evaluation of herbal anti-dandruff shampoo formulations (Eugenol and Std).

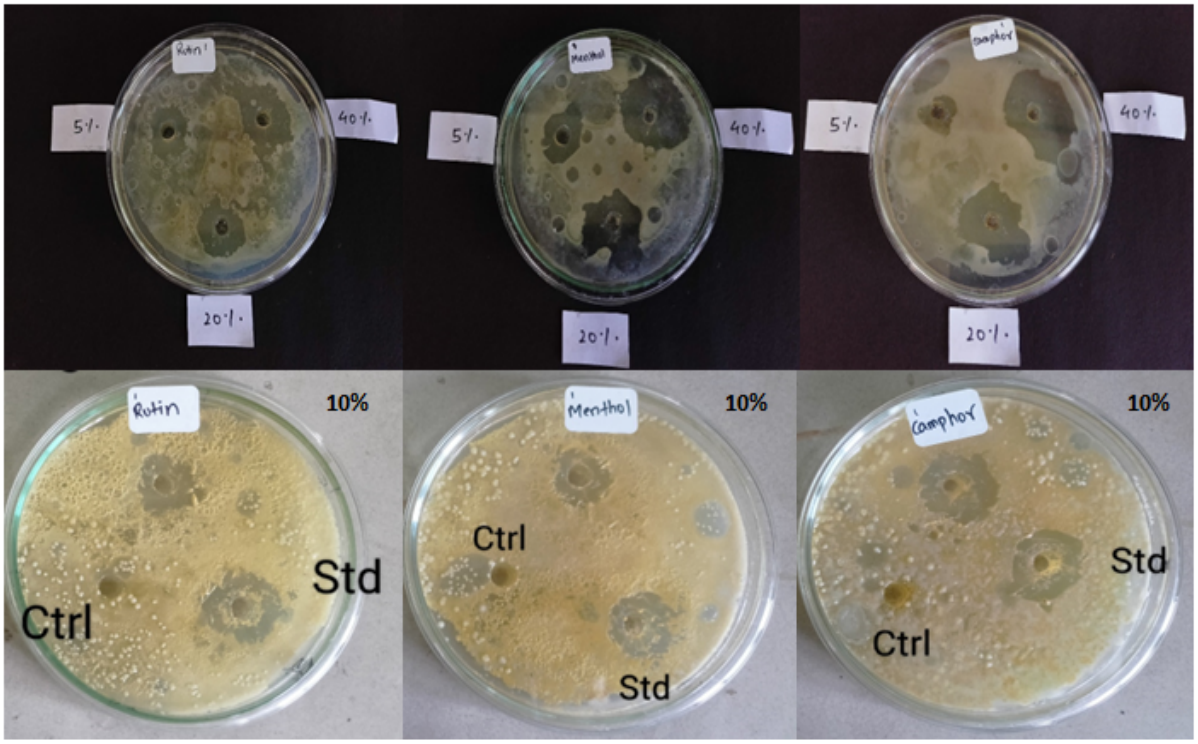

Figure 13 B: Anti-fungal evaluation of herbal anti-dandruff shampoo formulations (Rutin, Menthol, Camphor, Control and Std).

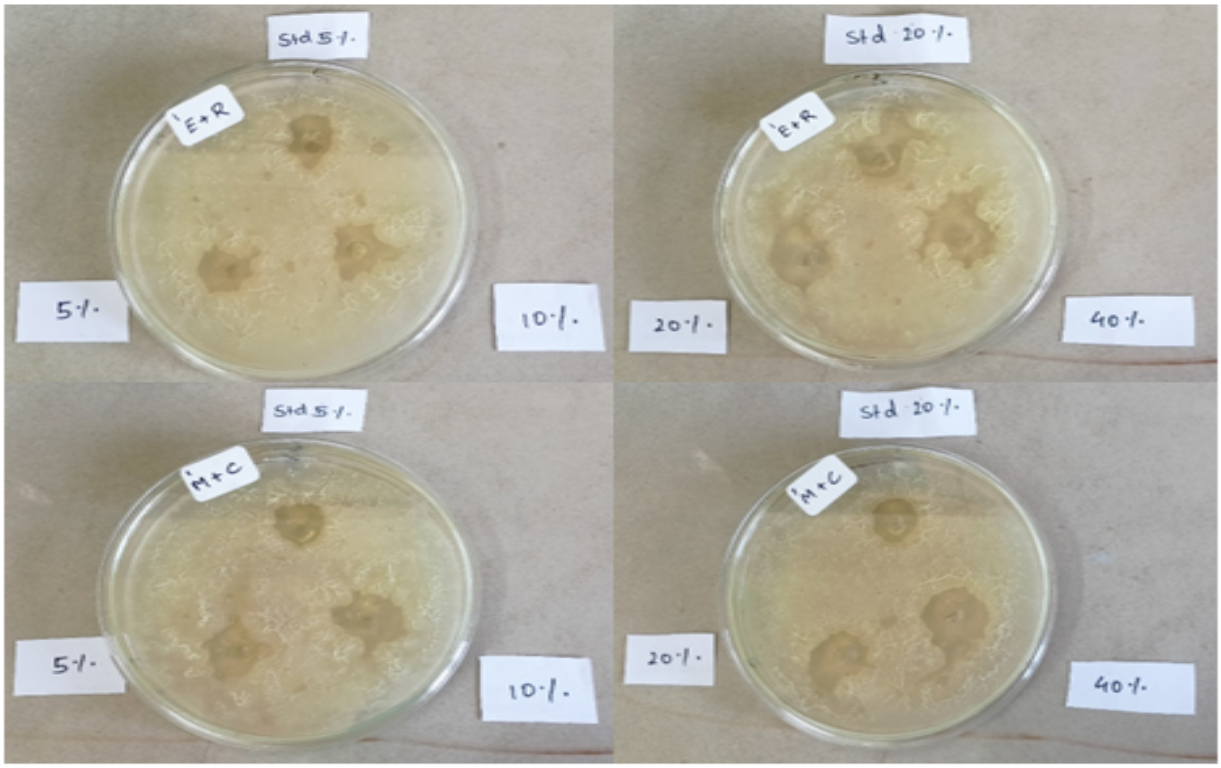

Figure 13 C: Anti-fungal evaluation of herbal anti-dandruff shampoo formulations (E+R: Eu, M+C and Std).