International Journal of Pharma and Biosciences
Content Available at www.lapinjournals.com ISSN: 0975-6299

A SCIENTIFIC APPROACH TO HERBAL ANTI-DANDRUFF SERUM DEVELOPMENT AND EVALUATION

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Article History: Received: 16.01.2026 Revised: 03.02.2026 Accepted: 07.Mar.2026

Abstract
Dandruff is a prevalent scalp condition characterized by white or yellowish flakes of dead skin, frequently accompanied by itching and irritation. It is primarily caused by overgrowth of the opportunistic fungus *Malassezia furfur*, which, while not a serious medical condition, can substantially impair self-confidence and, in chronic cases, may contribute to alopecia. This study describes the formulation and evaluation of a polyherbal anti-dandruff hair serum comprising *Abutilon indicum* leaf extract, neem (*Azadirachta indica*) bark extract, eucalyptus (*Eucalyptus globulus*) essential oil, menthol, and *Aloe barbadensis* (aloe vera) gel. Each ingredient was selected for its well-documented antifungal, antibacterial, and scalp-conditioning properties. The serum was evaluated for organoleptic and physicochemical parameters including color, fragrance, pH, density, viscosity, skin irritation (patch test), and anti-dandruff efficacy (agar well diffusion method). The formulation displayed a uniform yellowish-green appearance, a pH of 6.0 (within the optimal scalp-compatible range of 4.5–6.5), a density of 1.027 g/mL, and a viscosity of 1.1 cP. Skin patch testing on 10 volunteers demonstrated no irritation, and microbiological assessment confirmed significant antifungal activity against *Malassezia furfur*. The serum exhibited excellent stability, nourishing properties, and anti-dandruff efficacy, indicating strong potential as a safe and effective natural alternative to synthetic anti-dandruff formulations.
Keywords: *Dandruff; herbal anti-dandruff serum; Malassezia furfur; Abutilon indicum; Eucalyptus globulus; Azadirachta indica; agar well diffusion; antifungal activity.*

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DOI: https://doi.org/10.22376/ijpbs.v17i1.142

I. INTRODUCTION

Dandruff is a chronic, relapsing dermatological condition of the scalp resulting from excessive desquamation of corneocytes. It affects approximately 50% of the global adult population and is often associated with seborrheic dermatitis, *Malassezia* yeast overgrowth, and dysregulation of sebaceous gland activity [1,2,4].

Factors implicated in dandruff pathogenesis include altered scalp microbiota, epidermal barrier dysfunction, and environmental stressors. Conventional therapeutic approaches employ keratolytic agents (salicylic acid), antifungal compounds (ketoconazole, zinc pyrithione), and anti-inflammatory agents [3,5].

The genus *Malassezia* encompasses numerous species including *M. dermatis, M. furfur, M. globosa, M. japonica, M. nana, M. obtusa, M. pachydermatis, M. restricta, M. slooffiae,* and *M. sympodialis.* Colonies mature within 5 days at 30–37°C. The lipase enzyme produced by *M. furfur* degrades long-chain fatty acids on the scalp surface, triggering the inflammatory cascade responsible for dandruff formation and related skin disorders such as pityriasis versicolor and atopic dermatitis [6,22,23]. A growing body of evidence supports the antimicrobial potential of plant-derived phytoconstituents-including flavonoids, tannins, saponins, terpenoids, and alkaloids-against a wide spectrum of pathogenic fungi and bacteria [7,33]. Herbal anti-dandruff serums represent an advanced botanical formulation strategy that integrates plant-derived bioactives with demonstrated antifungal, anti-inflammatory, and keratolytic properties. *Abutilon indicum* (Malvaceae), *Azadirachta indica* (Meliaceae), *Eucalyptus globulus* (Myrtaceae), and *Aloe barbadensis* (Liliaceae) collectively inhibit the growth of *Malassezia*

species, a key contributor to dandruff pathogenesis, while the inclusion of aloe vera provides additional soothing and moisturizing benefits that reduce scalp irritation and prevent dandruff recurrence [7,8,26,27].

Phytochemical investigations of *A. indicum* leaves have identified amino acids, glucose, fructose, and galactose. Root extracts yield non-drying oils comprising linoleic, oleic, stearic, palmitic, lauric, myristic, caprylic, capric, and an unusual C17 fatty acid, as well as sitosterol and amyrin from the unsaponifiable fraction [9,37].

The present investigation aims to develop and evaluate a polyherbal anti-dandruff serum containing optimized concentrations of these botanical actives, providing a scientifically validated, safe, and effective alternative to conventional antifungal treatments.

1.1 Herbal Ingredients and Their Pharmacological Significance

The botanical ingredients used in the present formulation are summarized in Table 1 along with their taxonomic classification, vernacular nomenclature, and documented therapeutic applications. Figures 1–3 depict representative images of the key plant materials.

Table 1: Taxonomic classification, vernacular names, and pharmacological activities of herbal ingredients used in the anti-dandruff serum formulation.

S.No.	Herbal Drug	Family / Scientific Name	Common Names (Multilingual)	Medicinal Uses
1	Abutilon indicum (Fig. 1)	Family: Malvaceae Sci: Abutilon indicum (L.) Sweet	Hindi: Kanghi, Kakahi Tamil: Petari, Jhapi, thuthi English: Country mallow Bengali: Petari, Jhapi Marathi: Mudra Gujarati: Khapat, Dabali, Kamsaki	Demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, anti-convulsant [10], anti-inflammatory [11], anthelmintic, analgesic, antimicrobial. Used in treatment of leprosy, ulcers, headaches, gonorrhea, and bladder infections [12,13,14].
2	Azadirachta indica (Neem Bark) (Fig. 2)	Family: Meliaceae Sci: Azadirachta indica A. Juss	Hindi/Sanskrit: Nimba English: Neem, Margosa, Indian Lilac	Broad-spectrum anti-inflammatory, anti-arthritic, antipyretic, antifungal, antibacterial, antioxidant, and anti-tumor activities. Used in skin care, hair conditioning, and treatment of seborrheic dermatitis [15,27,28].
3	Eucalyptus globulus (Fig. 3)	Family: Myrtaceae Sci: Eucalyptus globulus Labill.	English: Blue Gum, Fever Tree Hindi: Safeda	Essential oil rich in 1,8-cineole exhibits broad antimicrobial, antifungal, insecticidal, acaricidal, and herbicidal properties. Disrupts fungal cell membranes and inhibits key metabolic processes relevant to Malassezia growth [16,30,31,32].
4	Aloe barbadensis (Aloe Vera) (Fig. 4)	Family: Liliaceae Sci: Aloe barbadensis Miller	English: Aloe Vera, Healing Plant Hindi: Ghritkumari Sanskrit: Ghrita-kumari	Moisturizing, soothing, anti-inflammatory, wound-healing, and antifungal properties. Stimulates immune function via macrophage activation; promotes cell proliferation and wound healing in normal and diabetic subjects [17,18,34,35,36].

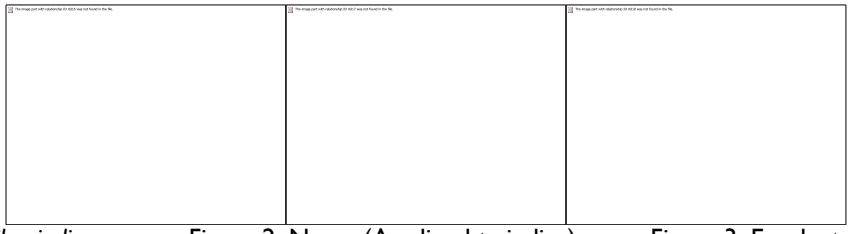

Figure 1. *Abutilon indicum* Figure 2. Neem (Azadirachta indica) Figure 3. Eucalyptus (E. globulus)

1.2 Aloe Vera-The Healing Plant

Aloe barbadensis (aloe vera), belonging to the family Liliaceae, is a perennial succulent plant widely recognized as 'the healing plant'. Its gel is rich in polysaccharides, amino acids, and bioactive glycoproteins [17,18]. Aloe vera gel is incorporated as the aqueous base in the present serum formulation, offering deep scalp moisturization, soothing effects, and a well-documented antifungal action against *Malassezia*species [18,35,36].

In vitro studies have demonstrated that aloe vera extracts stimulate cell proliferation, accelerate wound healing, and exert immunostimulatory effects via macrophage activation [18,36].

2. MATERIALS AND METHODS

2.1 Plant Materials-Collection and Authentication

The selection and collection of plant materials was guided by their established pharmacological profiles [38]. *Abutilon indicum* leaves were collected from the village of Amkotwa. The plant material was authenticated by a recognized botanist. The leaves exhibit antibiotic, antifungal, and antiviral activities, qualifying the extract as an active pharmaceutical ingredient (API) [9,10,37] Bark of *Azadirachta indica* (neem) was collected from trees on the Hygia College campus and authenticated by an expert botanist. Neem bark extract is employed as API due to its broad-spectrum medicinal properties including anti-inflammatory, antifungal, and antibacterial activities, largely attributable to its rich antioxidant content [27,28]. *Eucalyptus globulus* leaves were also collected from the Hygia College campus and authenticated by a qualified botanist. The essential oil derived from eucalyptus leaves has well-documented efficacy in inhibiting the growth of various fungal pathogens, including those implicated in scalp conditions [16,30].

2.2 Collection Time and Season

Plant material was harvested at optimal phenological stages to maximize bioactive compound concentration, consistent with established phytochemical practices [38].

A. indicum leaves were collected during the pre-flowering stage (July–September), the period of highest beneficial compound concentration. Neem bark was harvested from mature trees (4–5 years old) during the dry season to ensure peak alkaloid content. Eucalyptus leaves were collected in the early morning hours when volatile oil concentrations peak [38].

2.3 Selection of Excipients

Excipients were selected for their specific functional roles within the serum matrix. *Aloe vera* gel served as the aqueous base and primary moisturizing vehicle. Glycerine was incorporated as a humectant to maintain scalp hydration [17,35].

Gum acacia (acacia gum) was used as a natural emulsifier and thickening agent to ensure uniform dispersion of oils in the aqueous base. Citric acid (2% w/v) was added for its antioxidant and pH-stabilizing properties. Sodium benzoate (0.5% w/v) was incorporated as a preservative to prevent microbial contamination and extend shelf life.[38]

2.4 Extraction Methods

2.4.1 Maceration (Neem Bark and A. indicum Leaf Extract)

Extraction of neem bark and *A. indicum* leaves was carried out using cold maceration. Five grams of each dried, powdered plant material was separately dissolved in 50 mL of a hydroalcoholic solvent (ethanol:water, 30:70 v/v) and macerated for 48 hours with intermittent stirring. After filtration to remove undissolved particles, the filtrates were evaporated to dryness using a water bath. The dried extracts were weighed and stored in airtight amber vials at 4°C until use. These extraction conditions were based on validated protocols for maximizing yield of flavonoids and alkaloids [38,6].

2.4.2 Hydro-Distillation (Eucalyptus Essential Oil)

Eucalyptus essential oil was extracted by hydro-distillation using a Clevenger apparatus (Figure 4). Twenty grams of dried, finely powdered *E. globulus* leaves were placed in a round-bottomed flask containing 250 mL of distilled water. The mixture was heated to boiling; steam carried volatile oil components through a water-cooled condenser and into the graduated receiver. Oil and water phases separated spontaneously due to immiscibility. The oil layer was collected, dried over anhydrous sodium sulphate to remove residual moisture, and stored in sealed amber vials under refrigeration (4°C) [16,30].

Figure 4. *Maceration extraction of neem bark and A. indicum and* Figure 5. *Hydro-distillation of Eucalyptus oil using Clevenger apparatus*

2.5 Optimization of Herbal Ingredients

Systematic concentration optimization was performed for the three primary bioactive ingredients to determine the optimal concentration that balances antifungal efficacy, sensory acceptability, and formulation stability. Three concentrations were screened for each ingredient, as presented in Tables 2–4 [6,16,26].

2.5.1 Abutilon indicum Leaf Extract

Table 2. Optimization of Abutilon indicum leaf extract concentration in the anti-dandruff serum. (*Optimized concentration selected for final formulation.)

Trial No.	Concentration (%w/v)	Observation
1	2%	No detectable antifungal activity observed; insufficient to inhibit Malassezia growth.
2	5%	Weak antifungal activity observed; partial inhibition only.
3*	10% (Optimized)	Best balance of dandruff reduction, scalp spreadability, and sensory acceptability.

2.5.2 Neem Bark Extract (Azadirachta indica)

Table 3. Optimization of Azadirachta indica (neem) bark extract concentration in the anti-dandruff serum. (*Optimized concentration selected for final formulation.)

Trial No.	Concentration (%w/v)	Observation
1	5%	No significant antifungal activity observed.
2*	8% (Optimized)	Good antibacterial and antifungal activity; notable soothing effect without skin irritation.
3	10%	Formulation showed slight irritation and an overpowering odor; not acceptable.

2.5.3 Eucalyptus Essential Oil (Eucalyptus globulus)

Table 4. Optimization of Eucalyptus globulus essential oil concentration in the anti-dandruff serum. (*Optimized concentration selected for final formulation.)

Trial No.	Concentration (%v/v)	Observation
1	1%	Minimal cooling sensation; insufficient antimicrobial effect.
2	3%	Slight antimicrobial activity; moderate cooling effect.
3*	5% (Optimized)	Optimal cooling effect, antimicrobial benefit, and acceptable fragrance balance.

2.6 Formulation Development

Based on optimization data (Tables 2–4), the final serum was prepared as follows:

Step 1 – Emulsifier Preparation: 0.6 g of gum acacia was dissolved in 5 mL of warm distilled water with continuous stirring until complete solubilization. The solution was cooled to room temperature.

Step 2 – Oil Incorporation: 0.5 mL eucalyptus oil and 0.2 mL menthol were gradually added to the cooled acacia solution under continuous magnetic stirring, facilitating homogeneous emulsification.

Step 3 – Aloe Vera Gel Preparation: Fresh aloe vera leaves were washed, the epidermal layer was carefully removed, and the inner parenchymatous gel was blended to uniform consistency, then filtered through cheesecloth to remove fibrous matter. Citric acid (2% w/v) was added to the gel filtrate as an antioxidant stabilizer.

Step 4 – Assembly: The prepared aloe vera gel was transferred into a beaker on a magnetic stirrer. Under gentle agitation, 0.5 mL glycerine was added, followed by the dropwise addition of 0.8–1.0 mL of the combined *A. indicum* and neem bark extract, and the emulsified oil mixture.

Step 5 – Preservation: 2 mL of 0.5% (w/v) sodium benzoate solution was incorporated with continuous stirring to ensure uniform distribution.

The final formulation was filled into airtight amber containers and stored at room temperature (25 ± 2°C) protected from direct light for subsequent evaluation.

3. RESULTS AND EVALUATION

The formulated herbal anti-dandruff serum was subjected to comprehensive physicochemical and microbiological evaluation. All results are summarized in Table 5. Figures 6 and 7 illustrate the density measurement and antifungal efficacy assessment, respectively.

3.1 Organoleptic and Physical Properties

Visual and sensory evaluation revealed a uniform yellowish-green coloration and smooth, non-sticky texture, indicating homogeneity and stability without phase separation. Fragrance-primarily from eucalyptus essential oil and menthol-was assessed as pleasant and stable. Spreadability on the scalp was excellent, consistent with the targeted serum viscosity profile.[19,25]

3.2 pH Determination

The pH of the formulation was measured using calibrated pH paper and confirmed to be 6.0, which lies within the optimal scalp-compatible range of 4.5–6.5 [19,20].

This value ensures dermatological compatibility, minimizing scalp irritation and maintaining the integrity of the hair shaft and follicular microenvironment [2,21].

3.3 Density Determination

Density was determined using a pycnometer (specific gravity bottle) according to the formula:

$$\rho_{serum} = [(W3 - W1) / (W2 - W1)] \times \rho_{water}$$

where W1 (empty pycnometer) = 13.53 g, W2 (pycnometer + water) = 24.11 g, W3 (pycnometer + serum) = 23.74 g, and ρwater = 0.998 g/mL. The calculated density was 1.027 g/mL, confirming the water-based nature of the formulation and within the standard range for aqueous serums (1.000–1.030 g/mL) [19,20].

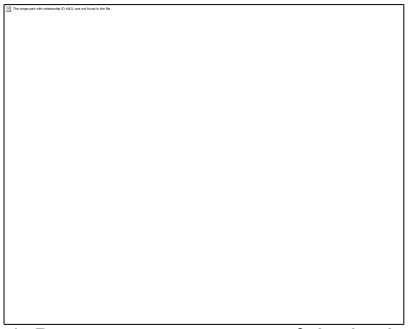

Figure 6. Density measurement of the herbal anti-dandruff serum using a specific gravity bottle

(pycnometer). The calculated density was 1.027 g/mL, consistent with a water-based serum formulation.

3.4 Viscosity Determination

Viscosity was measured using an Ostwald viscometer and calculated using the following relationship:

$$\eta 1 = \eta 2 \times (\rho 1 / \rho 2) \times (t1 / t2)$$

where: η1 = viscosity of serum (unknown); η2 = viscosity of water (1.0 cP); ρ1 = 1.027 g/mL (serum density); ρ2 = 0.988 g/mL (water density); t1 = 51.6 sec (serum flow time); t2 = 42.76 sec (water flow time).

The calculated viscosity was 1.1 cP (centipoise), confirming a light, non-sticky, easily spreadable consistency ideal for scalp application, analogous to previously reported water-based hair serums [19,25].

3.5 Skin Irritation Test

Dermatological safety was assessed using a standard patch test protocol. 0.5 mL of serum was applied to a 2 cm^2 area on the inner forearm of 10 healthy volunteers, occluded with a hypoallergenic patch, and evaluated at 24 and 48 hours. No volunteer exhibited signs of severe erythema, oedema, or sensitization, indicating excellent dermatological compatibility [19,20,21].

Preclinical Draize test results further confirmed the non-irritating and non-sensitizing character of the formulation. These findings are consistent with published safety profiles of herbal scalp formulations.[8,19]

3.6 Anti-Dandruff Efficacy-Agar Well Diffusion Method

Dandruff-associated fungal samples were collected from a donor scalp and initially cultured on modified Leeming–Notman agar (malt extract 3%, peptone 0.6%, beef extract 2%, glycerine 0.2%, Tween 80 1%, agar 4%, in 100 mL distilled water) at 32°C for 3 days. The culture was sub-cultivated onto a second selective agar supplemented with chloramphenicol (50 µg/mL) to inhibit bacterial contamination and selectively propagate *Malassezia*species [6,24].

Antifungal evaluation was conducted by the agar well diffusion method. The fungal inoculum was spread uniformly over agar plates using an inoculation loop. Wells (6 mm diameter) were bored with a sterile cork borer, and 0.1 mL of the test serum, individual API solutions (eucalyptus oil, neem extract, *A. indicum* extract), and a vehicle control were applied separately into designated wells. Plates were incubated at 32°C for 72 hours. Zone of inhibition (ZOI) diameters were measured and compared against controls [24,26,33].

Clear inhibition zones were observed around the wells containing the polyherbal serum, as well as individual API solutions, confirming significant antifungal activity against *Malassezia furfur* (Figure 7). No fungal growth was detected in the zone surrounding the test serum, indicating potent antifungal efficacy [6,16,26]. The antimicrobial activity of the serum may be attributed to synergistic interactions between eucalyptus 1,8-cineole (membrane disruption), neem azadirachtins (enzyme inhibition), and *A. indicum* alkaloids (cell wall interference), consistent with the established mechanisms reported in the literature [6,16,27,29,30].

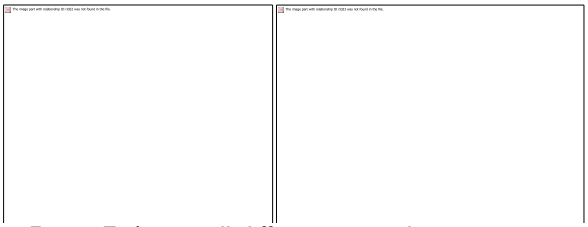

Figure 7. Agar well diffusion assay demonstrating antifungal activity of the herbal serum and individual API components (S = serum, E = Eucalyptus oil, N = Neem extract, A = Abutilon indicum extract) against Malassezia furfur. Clear inhibition zones confirm antifungal efficacy. pH paper result (pH 6.0) also shown.

3.7 Summary of Physicochemical and Microbiological Evaluation

The complete evaluation profile of the formulated herbal anti-dandruff serum is summarized in Table 5.

Table 5: Summary of physicochemical and microbiological evaluation parameters of the formulated polyherbal anti-dandruff serum.

S.No.	Parameter	Observation / Result	Interpretation
1	Colour	Yellowish-green, uniform	Homogeneous formulation; no phase separation[19]
2	Fragrance	Pleasant, stable	Acceptable sensory profile; attributed to eucalyptus oil[25]
3	pH	6.0	Within optimal scalp range (4.5–6.5); non-irritating[19,20,21]
4	Density	1.027 g/mL	Confirms water-based nature; standard range 1.000–1.030 g/mL[19,25]
5	Viscosity	1.1 cP (Ostwald viscometer)	Light, spreadable consistency; suitable for scalp application[25]
6	Skin Irritation	No reactions (10 volunteers, 48 h patch	Safe for dermatological use; confirmed by Draize test[19,20]

S.No.	Parameter	Observation / Result	Interpretation
		test)	
7	Antifungal Efficacy	Clear inhibition zones against M. furfur (agar well diffusion)	Significant antifungal activity; effective dandruff control[6,24,26]

4. DISCUSSION

The herbal anti-dandruff serum exhibited favorable stability, a uniform yellowish-green texture, and a pleasant fragrance derived from eucalyptus oil and menthol, ensuring ease and comfort of application. The pH of 6.0 aligns with the natural scalp pH range (4.5–6.5), minimizing irritation risk and supporting the scalp's natural acidic mantle that protects against microbial colonization [1,2,21].

The measured density of 1.027 g/mL falls within the established range for water-based cosmetic serums and confirms the formulation's aqueous base. The low viscosity of 1.1 cP, determined by Ostwald viscometry, is consistent with a serum-type product intended for direct scalp application-light enough to penetrate the hair and scalp while avoiding excessive runoff [19,25]. Skin irritation testing (patch test on 10 volunteers; 48 h observation) yielded no adverse reactions, indicating dermatological safety. These findings corroborate the known tolerability of *Aloe vera*-based formulations and the use of optimized herbal concentrations that avoid irritant thresholds [8,18,19]. The agar well diffusion assay confirmed potent antifungal activity against *Malassezia furfur*. The observed zones of inhibition may be attributed to the synergistic action of: (i) eucalyptus 1,8-cineole, which disrupts fungal cell membranes and inhibits respiratory enzymes;[16,30].

(ii) neem azadirachtins and nimbidin, which inhibit fungal enzyme systems;[27,28]

(iii) *A. indicum* phytoconstituents including alkaloids and terpenoids that disrupt cell wall synthesis [9,37].

Compared to synthetic antifungal agents (ketoconazole, zinc pyrithione), polyherbal formulations offer multi-target mechanisms of action, reducing the likelihood of resistance development [3,7,33].

Taken together, the formulation demonstrated a well-balanced composition, effective antifungal action, and skin-friendly properties, supporting its candidacy as a safe and efficient alternative to conventional synthetic anti-dandruff treatments [7,19,26].

5. CONCLUSION

The polyherbal anti-dandruff serum formulated with optimized concentrations of *Abutilon indicum* (10% w/v), neem bark extract (8% w/v), eucalyptus essential oil (5% v/v), menthol, and aloe vera gel demonstrated desirable physicochemical characteristics and significant antifungal efficacy against *Malassezia furfur*. The serum's pH (6.0), density (1.027 g/mL), and viscosity (1.1 cP) were within acceptable ranges for scalp application. Dermatological safety was confirmed by patch testing. Microbiological assessment by agar well diffusion validated clear antifungal activity against the primary dandruff-causing organism [6,19,22,24].

The holistic botanical approach, combining multiple bioactives with complementary mechanisms of action, ensures broad-spectrum antifungal efficacy while minimizing the adverse effects inherent to synthetic formulations. The formulation represents a promising candidate for further development as a safe, effective, and patient-friendly natural anti-dandruff treatment [7,25,33].

Future studies should assess long-term stability under ICH Q1A guidelines, extended clinical trials in larger patient cohorts, consumer acceptability surveys, and in vivo comparison with standard anti-dandruff preparations to establish clinical equivalence.[20,21]

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the research, authorship, and/or publication of this article.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the faculty and staff of Hygia College of Pharmacy, particularly Mr. Shadab, for their invaluable support during the laboratory studies. Special thanks are extended to A. Baghel, A. Mishra, A. Yadav, A. Kumar, and D. Gupta for their assistance throughout this work.

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