



FORMULATION AND EVALUATION OF HERBAL HAIR SERUM FOR ANTIMICROBIAL ACTIVITY AND HAIR GROWTH PROMOTION

^{1*}A. Haripriya, ²V. Prasath, ²G. Praveen Kumar, ²G. Priyadarshini, ²T. Priyadharshini
and ²S. Priyanka

¹Assistant Professor Department of Pharmaceutics, Aadhibhagawan College of Pharmacy,
Rantham, Vembakkam Tk, Tiruvannamalai, Tamil Nadu.

²B. Pharm Students, Aadhibhagawan College of Pharmacy, Rantham, Vembakkam Tk,
Tiruvannamalai, Tamil Nadu.

Received: 22 January 2026

Revised: 10 February 2026

Accepted: 02 March 2026

Corresponding Author: A. Haripriya

Address: Assistant Professor Department of Pharmaceutics, Aadhibhagawan College of Pharmacy, Rantham,
Vembakkam Tk, Tiruvannamalai, Tamil Nadu. DOI: <https://doi.org/10.5281/zenodo.18839419>

ABSTRACT

The present study focuses on the formulation and evaluation of a herbal hair serum and its preliminary phytochemical, physicochemical, spectral, antimicrobial, and hair regrowth activities. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, glycosides, saponins, and phytosterols, while proteins, terpenoids, and phenols were absent. Evaluation parameters indicated that the serum was dark brown in color, aromatic, smooth in texture, and had a pH of 8.2 with no irritation potential. The specific gravity (1.096), viscosity (0.91 cps), saponification value (291.72), and spreadability (9 cm) suggested suitable formulation characteristics. UV-Visible spectrophotometric analysis showed maximum absorbance (0.870) at 296 nm, identified as λ_{max} , with negligible interference in the visible region. IR spectral analysis confirmed the presence of functional groups corresponding to bioactive compounds. The formulation exhibited significant antifungal activity against *Trichophyton* spp. (10 mm) and *Candida albicans* (12 mm), and antibacterial activity against *Staphylococcus aureus* (10 mm) and *Escherichia coli* (22 mm). Hair regrowth studies demonstrated comparative effectiveness

with standard treatment, indicating the potential of the herbal hair serum as a natural therapeutic alternative.

KEYWORDS: Herbal hair serum, Phytochemical screening, UV analysis, IR spectrum, Antifungal activity, Antibacterial activity, Hair regrowth study, Physicochemical evaluation.

1. INTRODUCTION

Herbal Hair Serum is a natural hair care product designed to improve the overall health and appearance of hair. It is made using plant-based ingredients such as herbal extracts and natural oils that nourish the scalp and strengthen the hair from the roots to the tips. Unlike chemical-based products, herbal serums are gentle and suitable for regular use.

The serum works by providing essential nutrients that help reduce hair fall, dryness, and breakage. It deeply moisturizes the scalp, improves blood circulation, and supports healthy hair growth. In addition, it forms a protective layer over the hair strands, shielding them from pollution, heat, and other environmental damage.

Regular use of herbal hair serum makes hair smoother, shinier, and more manageable. It also helps control frizz and split ends, giving the hair a soft and healthy look. Overall, herbal hair serum is a safe and effective solution for maintaining strong, beautiful hair naturally.



Fig. 1: Herbal Hair Serum.

2. MATERIALS AND METHODS

2.1 PLANT

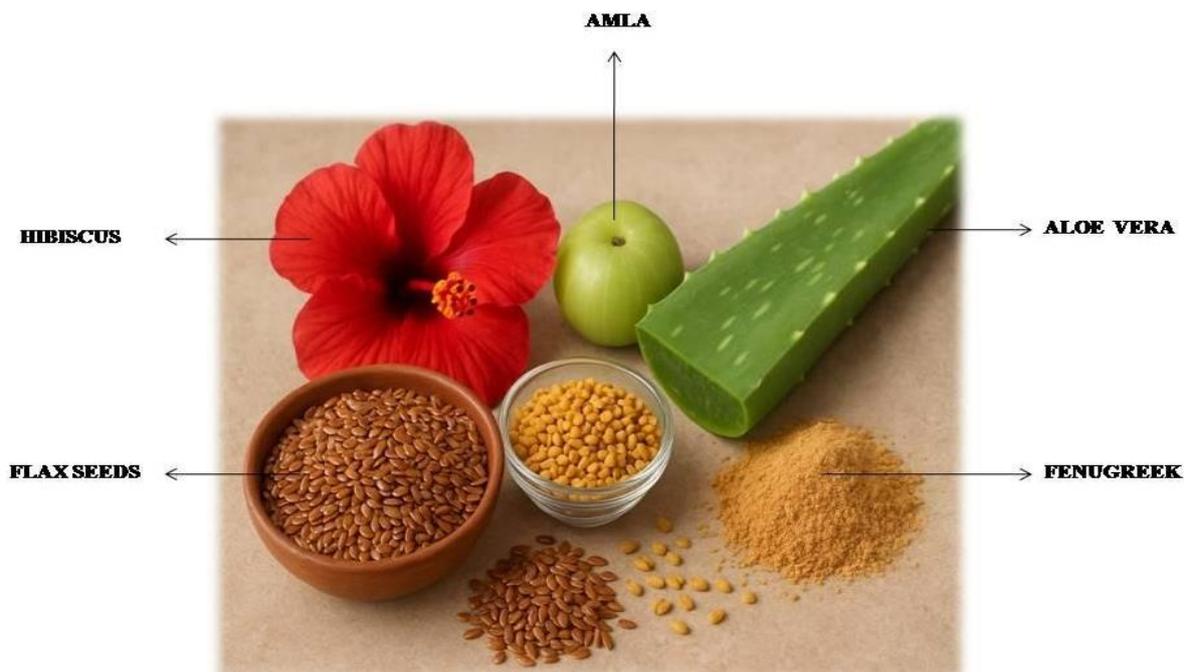


Fig. 2: Plant Image.

2.2 EXCIPIENT



Fig. 3: Excipients.

2.3 LIST OF INGREDIENTS:

- ❖ Purslane Plant Extract -rich in omega 3 fatty acid , minerals and vitamins
- ❖ Hibiscus Rosa-Sinensis (Hibiscus Flower) – promotes hair growth, conditions hair
- ❖ Trigonella Foenum-Graecum (Fenugreek Seeds) – reduces dandruff, strengthens hair
- ❖ Portulaca Oleracea (Purslane Leaves) – rich in omega-3, soothes scalp

- ❖ **Linum Usitatissimum (Flaxseeds)** – provides nourishment, reduces frizz
- ❖ **Aloe Vera Gel** – conditions scalp and provides moisture
- ❖ **Excipients** – Sodium benzoate, Coconut Oil, Rose Water, Almond Oil, Distilled Water.

2.4 PREPARATION OF HERBAL HAIR SERUM:

- ❖ **Collection and Cleaning:** All plant materials (purslane, fenugreek, flaxseeds, hibiscus) were cleaned thoroughly using distilled water and air-dried in shade.
- ❖ **Powdering:** The dried materials were coarsely ground separately using a blender.
- ❖ **Boiling:** A total of 200 g of plant powder was mixed with 1000 mL of distilled water in a stainless steel vessel and add coconut oil, Almond oil. The mixture was boiled on a low flame for 30–40 minutes, reducing the volume to approximately 500 mL.
- ❖ **Filtration:** After cooling, the decoction was filtered through a muslin cloth to remove plant residues and collect the clear extract.

2.5 FORMULATION HERBAL HAIR SERUM

Table 1: Formulation Of Herbal Hair Serum.

INGREDIENT	SCIENTIFIC NAME	QUANTITY (FOR 200 ml)	PURPOSE
Purslane Plant	<i>Portulaca Oleracea</i>	75 g	Anti-inflammatory, rich in omega-3
Fenugreek Seeds	<i>Trigonella Foenum - Graecum</i>	50 g	Strengthens roots, reduces dandruff
Flaxseeds	<i>Linum Usitatissimum</i>	25 g	Provides shine and reduces breakage
Hibiscus Petals	<i>Hibiscus Rosa-Sinensis</i>	50 g	Stimulates hair growth add adds softness
Aloe Vera Gel	<i>Aloe Vera Gel</i>	75 ml	Moisturizing and conditioning
Rose Water	<i>Rosa Damascena</i>	25 ml	Toning and fragrance
Almond Oil	<i>Prunus Dulcis</i>	125 ml	Adds shine and strengthens hair root
Coconut Oil	<i>Cocos Nucifera</i>	125 ml	Nourishment and scalp hydration
Preservative	<i>Sodium Benzoate</i>	0.5-1 g	Increasing shelf life
Distilled Water	<i>Liquid</i>	50 ml	Solvent for extraction
Amla	<i>Phyllanthus Emblica</i>	50g	Hair straightening

2.6 EVALUATION TEST FOR HERBAL HAIR SERUM

In physical evaluation, parameters like specific gravity, PH, Saponification value are conducted.

2.6.1 Organoleptic Property

- ❖ **Color:** Detected by naked eyes.
- ❖ **Sensitivity:** The prepared herbal hair serum was applied on 1 cm skin of hand and exposed to sunlight for 4-5 min.

❖ **PH:** The PH was determined by using digital PH meter. 20ml of herbal hair serum was taken in a beaker and the bulb of PH meter was dipped in hair tonic. The obtained PH values are noted down.

2.6.2 Specific gravity: Take the specific gravity bottle, rinsed it with distilled water, dry it in oven for 15 minutes, cool, closed it with cap and weigh it (a). Now fill the same specific gravity bottle with the sample and closed it with cap and again weigh it (b). Determine the weight of sample per milliliter by subtracting the weight (b-a).

Weight of empty specific gravity bottle = w_1 gms.

- Weight of specific gravity bottle with water = w_2 gms.
- Weight of specific gravity bottle with hair serum = w_3 gms.
- Specific gravity of hair serum ρ was calculated as $\rho = \frac{w_3 - w_1}{w_2 - w_1} \times \rho$

2.6.3 Viscosity Measurement: The viscosity of prepared herbal hair serum was estimated by Ostwald's Viscometer at a room temperature. The viscosity of prepared herbal hair serum was calculated by using the equation.

$$\text{Viscosity of liquid } (\eta_2) = \frac{\eta_1 \times \rho_2 \times t_2}{\rho_1 \times t_1}$$

- η_1 = Viscosity of water
- ρ_2 = Density of sample
- t_2 = Mean time of oil from A to B
- ρ_1 = Density of oil
- t_1 = Mean time of flow of water from A to B

2.6.4 Homogeneity Test: A clean and dry object glass was smeared with the hair serum, and a cover glass was sealed. The appearance under the light of some coarse particle/homogeneity was investigated. Herbal hair serum was tested by visual examination for homogeneity and tested for some lumps, flocculates, or aggregates.

2.6.5 Spreadability Test: Spreadability was measured by a parallel plate process typically used to assess and measure the spreadability of semisolid preparations. One gram hair serum was pressed between two horizontal plates of dimension 20× 20 cm, the upper of which

weighed. The spread diameter was measured after 1 min. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

2.6.6 FTIR Spectrum: An infrared spectrum of pure drug, mixture of drug with each retardant and physical mixture of optimized formulation was recorded using FTIR Spectrophotometer. The scanning range was 500–4000 cm^{-1} and the IR spectra of samples were obtained using KBr disc method. Any change in spectrum pattern of drug due to presence of polymers was investigated to identify any chemical interaction.

2.6.7 Saponification Value: 2ml of herbal hair oil was weighed and transferred into a 25ml of conical flask. To this 25ml alcoholic KOH solution was added. It was heated on a water bath for 30 minutes by frequently mixing the content of the flask phenolphthalein was added to cooled liquid and titrated against 0.5M HCL. Blank solution was performed and Saponification values were calculated.

Saponification value = (b-a) x 28.05/weight of substance

B = blank value

A = assay value.

$$= 28.05 (21.3 - 0.5) / 2$$

$$= 28.05 (20.8) / 2$$

$$= 583.44 / 2$$

$$= 291.72$$

2.6.8 Primary Skin Irritation Test: The prepared formulations were assessed for primary skin irritation test. Healthy human volunteers were selected for the study. The hair of each volunteer of 1cm^2 was shaved which could accommodate three test sites. It was cleaned with surgical spirit. The quantities of formulations were applied over the respective test sites were observed for erythema and edema for 48hrs after application.

2.6.9 Animal Study

Effects Of Hair Regeneration: Hair growth is controlled by a unique dynamic cycle comprising growth (anagen), regression (catagen) and rest (telogen) phases. Hair follicles remodel themselves repeatedly throughout adult life. It is generally known that the human hair cycle lasts several years; in contrast, the rabbit hair cycle is only three weeks. The hair

cycle in rabbit is similar to that of humans. The skin color of rabbit in the telogen phase was pink, which darkens along with anagen initiation and then becomes grey. In comparison with the application of the control, blackened skin areas, especially in the herbal hair serum group, were observed 7 d after shaving. the herbal hair serum group -treated group was covered with hair in the shaved skin, whereas other groups showed relatively less hair growth. Macroscopic assessment and alopecia score showed that herbal hair oil significantly stimulated hair growth.

2.6.10 Evaluation of Anti Fungal Activity

Preparation Of Agar Medium: Prepare MHA from the dehydrated medium according to the manufacturer's instructions. media should be prepared using distilled water or deionized water. Heat with frequent agitation and boil to dissolve the medium completely. Sterilized by autoclaving at 121°C for 15 minutes. Check the PH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature. This is done by macerating a small amount of medium in a little distilled water or by allowing a little amount of medium to gel around a PH meter electrode. Cool the agar medium to 40 to 50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4mm. Allow to solidified Prior to use, dry plates at 30-37°C in an incubator, with lids partly agar, for not more than 30minutes or until excess surface moisture have evaporated. media must be moist but free of water droplets on the surface. Presence of water droplets may result to swarming bacterial growth, which could give in accurate results. They are also easily contaminated.

Inoculum Preparation: From a fungal culture (not more than 48 hours, old except for slow growing organism) take 4 or 5 colonies with a wire loop. Transfer colonies to 5ml of trypticase soy broth or 0.9% saline .Incubate the broth at 30:c at an optimum growth temperature until it achieves or exceeds the turbidity of 0.5 macfarland standard (prepared by adding 0.5ml of 0.048m Bacl₂ to 99.5ml of 0.36 NH₂SO₄ ; commercially available). Compare the turbidity of the test bacterial suspension with that of 0.5 macfarland (vigorously shaken before use) against a white background with contrasting black line under adequate light. Arrow points to tube with correct turbidity. Reduce turbidity by adding sterile saline or broth.

Inoculation Of Plates: Dip a sterile cotton swab into the standardized fungal suspension. Remove excess inoculum by lightly pressing the swab against the tube wall at a level above

that of the liquid. Inoculate the agar by streaking with the swab containing the inoculum. Rotate the plate by 60° and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculum. Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

2.6.11 Evaluation Of Anti - Bacterial Activity: The bacterial strains were sub cultured to get fresh cultures of bacteria for this purpose, a single colony from bacterial strain was inoculated on nutrient broth. The broth was incubated for 24 h at 37 °C. 14 gm of nutrient agar media was dissolved in 1 L of distilled water at PH 7 and autoclaved for 20 min at 121 °C. The media were allowed to cool down to 45 °C and poured to petri plates for preparing 75 ml of solid media. using sterile cork borer 7 wells per plate were made in the solidified media. Agar diffusion method was used for antibacterial activity. Bacterial culture was inoculated on the surface of solid media. The synthesis product and fractions were dissolved in dimethyl sulfoxide (DMSO) at the same concentration of 2 mg/ml to prepare stock solutions. from the stock solution, 1000 µl was poured into respective wells. Ciprofloxacin was used as a positive control and DMSO was used as a negative control. The zone of inhibition of crude extract and fractions were measured in mm after 24 h of incubation at 37 °C and compared with the zone of inhibition of standard drug Ciprofloxacin.

3. RESULTS AND DISCUSSION:

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING:

Results of the Preliminary Phytochemical Constituents present in Herbal Hair Serum.

Table: 2 Preliminary Phytochemical Screening

S. NO	CONSTITUENTS	HERBAL HAIR OIL
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Protein	-
4.	Terpinoids	-
5.	Phenols	-
6.	Tannins	+
7.	Flavonoids	+
8.	Glycosides	+
9.	Saponins	
10.	Phytosterols	+

+ = Present

- = Absent

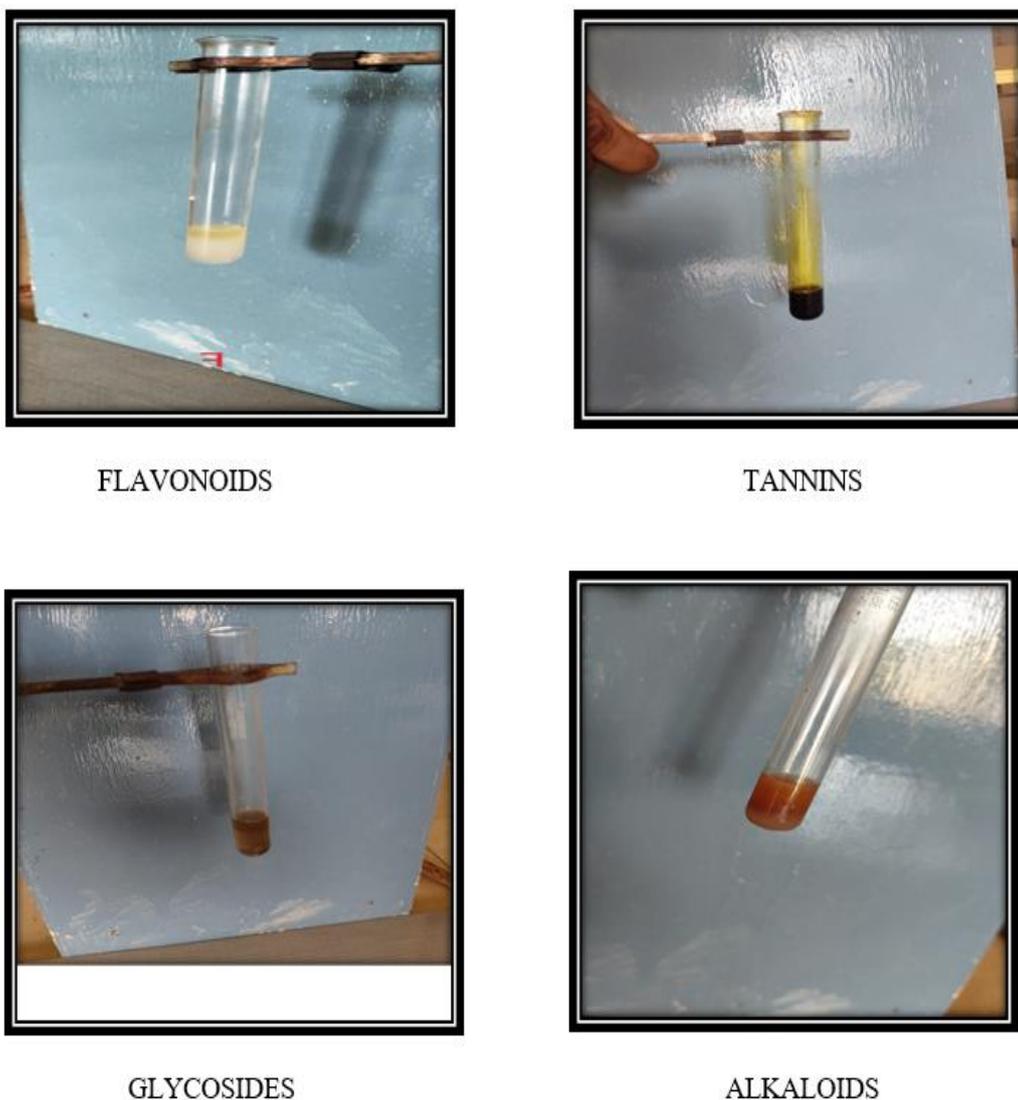


Fig. 4: Preliminary Phytochemical Screening.

3.2 EVALUATION OF HERBAL HAIR SERUM

Table 3: Evaluation Of Herbal Hair Serum.

S.NO	PARAMETERS	INFERENCES
1	State	Liquid
2	Color	Dark Brown Color
3	Odour	Aromatic Odour
4	p ^H	8.2
5	Grittiness	Smooth
6	Specific gravity	1.096
7	Viscosity (centipoise)	0.91
8	Irritation test	No Irritation
9	Saponification value	291.72
10	Spredability	9cm



Fig. 5: Herbal Hair Serum.

3.3 UV – ANALYSIS

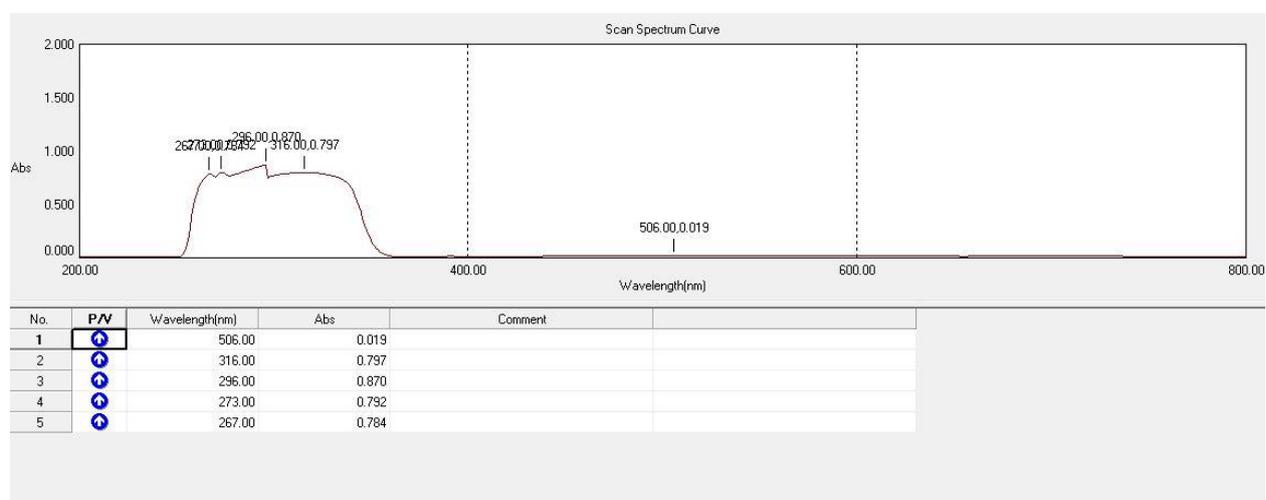


Fig. 6: UV-Spectrum of Herbal Hair Serum.

- The herbal hair serum formulation was subjected to UV–Visible spectrophotometric analysis by scanning in the wavelength range of 200–800 nm using a suitable blank.
- The UV scan spectrum of the herbal hair serum showed characteristic absorption peaks at 267 nm, 273 nm, 296 nm, and 316 nm.
- The maximum absorbance of 0.870 was observed at 296 nm, which was selected as the λ_{max} of the formulation.
- Negligible absorbance (0.019) was observed in the visible region at 506 nm, indicating the absence of interference from colored components in the formulation.

3.4 IR – SPECTRUM ANALYSIS

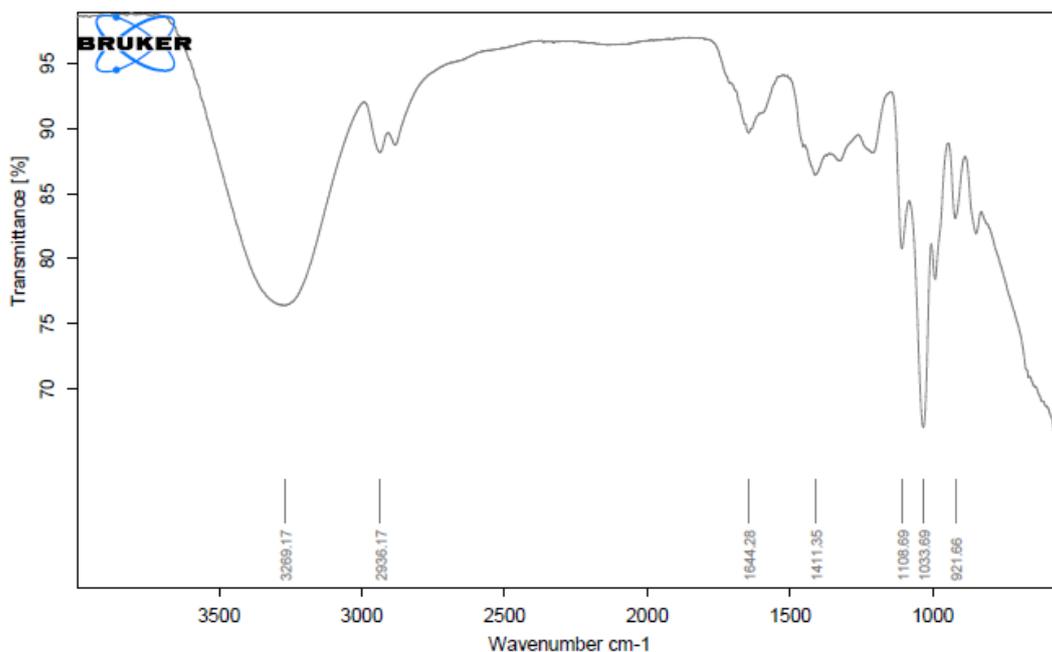


Fig. 7: IR-Spectrum of Herbal Hair Serum.

3.5 ANTI-FUNGAL & ANTI-BACTERIAL ACTIVITY

Table 4: Anti – Fungal Activity.

S. No.	Microorganisms	Control	HS	Amphotericin -B
		Zone of inhibition in mm		
1.	<i>Trichophyton sps</i>	-	10	13
2.	<i>Candida albicans</i>	-	12	15



Fig. 8: Anti – Fungal Activity.

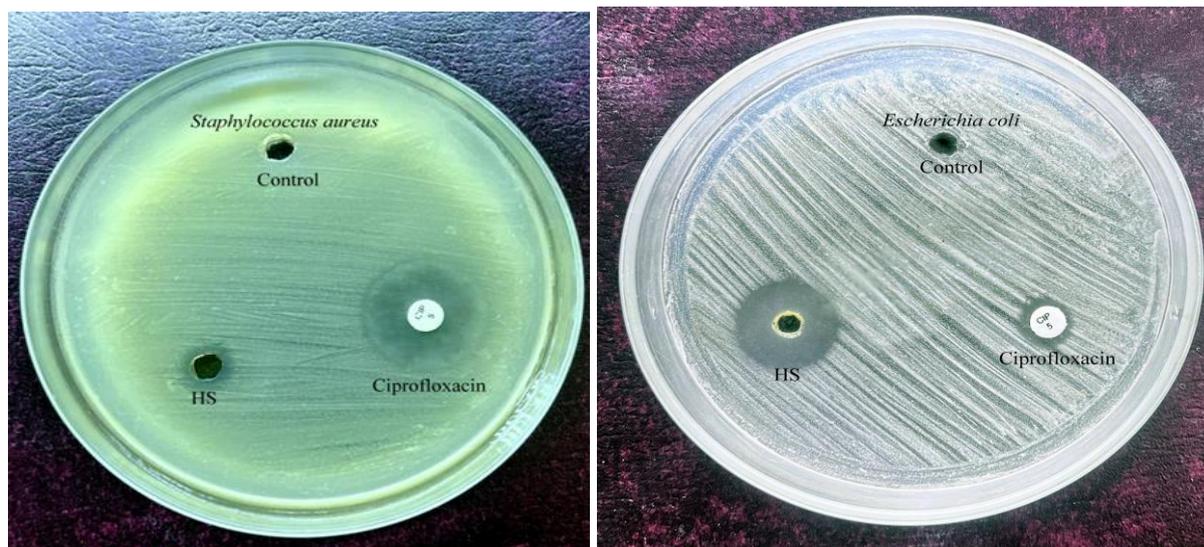


Fig. 9: Anti – Bacterial Activity.

Table 5: Anti – Bacterial Activity.

S. No.	Microorganisms	Control	HS	Ciprofloxacin
		Zone of inhibition in mm		
1.	<i>Staphylococcus aureus</i>	-	10	21
2.	<i>Escherichia coli</i>	-	22	08

3.6 Hair Regrowth Animal Study

Table 6: Hair Regrowth Study.

S.NO	GROUP	DAY 3	DAY 7	DAY 14	DAY 21
1	CONTROL Minoxidil Hair Serum				
2	Herbal Hair Serum				

DISCUSSION

Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, saponins, and phytosterols. These compounds are known for their antimicrobial, antioxidant, and scalp-nourishing properties, contributing to hair health and growth. The absence of proteins, phenols, and terpenoids suggests selective phytochemical composition based on the ingredients used in the formulation.

Physicochemical evaluation showed that the serum possessed desirable cosmetic and stability characteristics. The slightly alkaline pH (8.2) is within acceptable limits for topical applications. The smooth texture, good spreadability (9 cm), and appropriate viscosity (0.91 cps) indicate ease of application and uniform distribution on the scalp. The absence of irritation further confirms its safety for topical use.

UV–Visible spectroscopy revealed characteristic peaks at 267 nm, 273 nm, 296 nm, and 316 nm, with maximum absorbance at 296 nm (λ_{max}), indicating the presence of conjugated systems and bioactive phytoconstituents. Minimal absorbance in the visible region (506 nm) confirmed the absence of interfering colored impurities. IR spectral analysis further supported the presence of functional groups corresponding to phytochemicals detected in preliminary screening.

The antimicrobial studies demonstrated moderate antifungal and antibacterial activities. The herbal serum showed inhibition zones against *Trichophyton* spp. and *Candida albicans*, indicating its potential in managing fungal scalp infections such as dandruff. It also showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, supporting its role in maintaining scalp hygiene.

Hair regrowth studies indicated observable improvement compared to control and comparable effectiveness to standard formulations, suggesting the formulation's potential to stimulate hair growth naturally.

4. CONCLUSION

The formulated herbal hair serum demonstrated satisfactory physicochemical stability, significant phytochemical presence, and promising antimicrobial and hair growth-promoting activities. The absence of irritation and favorable evaluation parameters indicate its safety and suitability for topical application. Based on the findings, the herbal hair serum may serve as an effective natural alternative for promoting scalp health and hair growth. Further clinical studies are recommended to validate its long-term efficacy and safety.

5. REFERENCES

1. Anusha R., Akhila N., Nikhitha J., Harish K., Shaikh A. R., Sony Y., Inamul H. Formulation and Evaluation of Herbal Hair Serum – A Review. *Journal of Pharmacognosy and Phytochemistry*, 2023; 12(3): 128-133.

2. Pandey, A., Tripathi, S. Concept of Standardization, Extraction and Pre Phytochemical Screening Strategies for Herbal Drug. *Journal of Pharmacognosy and Phytochemistry*, 2014; 2(5): 115-119.
3. Rathi, P., & Jain, S. A Review on Herbal Cosmetics. *Journal of Drug Delivery and Therapeutics*, 2020; 10(6-s): 284-289.
4. Kokate, C. K., Purohit, A. P., & Gokhale, S. B. *Pharmacognosy*. 50th Edition, Pune: Nirali Prakashan, 2014.
5. Kapoor, V. P. Herbal cosmetics for skin and hair care. *Natural Product Radiance*, 2001; 1(1): 19-24.
6. Saha, P., & Paul, S. Herbal Formulations as Natural Cosmetics: A Review. *Research Journal of Topical and Cosmetic Sciences*, 2018; 9(1): 15-20.
7. Singh, R., & Kaur, H. Recent Trends in Herbal Hair Care Products: A Review. *Asian Journal of Pharmaceutical Research and Development*, 2021; 9(2): 70-76.
8. <https://www.ncbi.nlm.nih.gov/> (NCBI Database for Herbal Research Articles)
9. <https://pubmed.ncbi.nlm.nih.gov/> (PubMed Central for Peer-Reviewed Journals)
10. Sharma, P., & Patel, M. Formulation and Evaluation of Herbal Hair Serum using Hibiscus and Aloe Vera Extracts. *International Journal of Pharmaceutical Sciences and Research*, 2022; 13(7): 2825-2831.
11. Joshi, P., & Sharma, V. Hair Care and Herbal Oils. *International Journal of Pharmaceutical and Biological Archives*, 2012; 3(4): 615-620.
12. Mehta, R. M. (2015). *Pharmaceutics I*. 7th Edition. Vallabh Prakashan, New Delhi.
13. Gupta, A. K., & Sharma, M. Formulation and Evaluation of Herbal Hair Serum Containing Hibiscus and Aloe Vera Extracts. *Asian Journal of Pharmaceutical Education and Research*, 2020; 9(4): 30-35.
14. Dash, G. K. (2020). *Pharmacognosy and Phytochemistry-I*. CBS Publishers & Distributors Pvt. Ltd.
15. Kumar, S., & Mishra, A. A Review on Herbal Plants Used in Hair Disorders. *Journal of Medicinal Plants Studies*, 2015; 3(4): 74-78.
16. Bhowmik, D., Chiranjib, & Tripathi, K. K. Herbal Remedies for Hair Loss Treatment. *The Pharma Innovation Journal*, 2010; 1(2): 14-19.
17. Singh, A., & Navneet. Herbal Cosmeceuticals for Skin and Hair Care. *Journal of Pharmacognosy and Phytochemistry*, 2016; 5(3): 265-269.
18. Tiwari, S., & Bhatia, A. K. Recent Advances in Herbal Hair Care Products: An Overview. *International Journal of Green Pharmacy*, 2019; 13(2): 120-124.

19. Kokate, C.K., Gokhale, S.B. (2010). Textbook of Pharmacognosy. Nirali Prakashan, Pune.
20. <https://www.sciencedirect.com/> (ScienceDirect - Access to Scientific Journals & Articles)
21. Chauhan, A., & Sharma, P. Formulation and Evaluation of Herbal Hair Serum Using Natural Ingredients. *World Journal of Pharmaceutical Research*, 2018; 7(8): 1005-1013.
22. Kaur, M., & Kaur, H. A Review on Herbal Hair Formulations for Hair Care. *International Journal of Ayurveda and Pharmaceutical Chemistry*, 2021; 15(1): 251-258.
23. Patel, J., & Saini, T. R. Development and Evaluation of Herbal Hair Serum Using Aloe Vera and Fenugreek Extracts. *International Journal of Research in Pharmaceutical Sciences*, 2019; 10(2): 1525-1530.
24. Kokate, C.K., Purohit, A.P., & Gokhale, S.B. (2019). *Pharmacognosy*. 51st Edition, Pune: Nirali Prakashan.
25. Kumar, V., & Sahoo, L. Cosmetic Potential of Herbal Extracts for Hair Care Applications: A Review. *Asian Journal of Pharmaceutical and Clinical Research*, 2016; 9(4): 25-30.