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Research Article

Cream for Alopecia

Formulation and Evaluation of Herbal Cream for the Management of Androgenetic Alopecia

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Abstract: The current study aimed to develop a herbal hair cream formulation including plant extracts traditionally used for hair growth, and further research was conducted to understand the hair growth capability better using the 5 alpha reductase inhibitory model. 5 alpha reductases, an enzyme confined to the nuclear membrane, converts testosterone into dihydrotestosterone. The 5 alpha-reductase and its metabolite dihydrotestosterone influence various human disorders, including male pattern baldness, benign prostatic hyperplasia (BPH), and prostate cancer. Finasteride and alfatradiol are examples of synthetic medications; however, because of their negative impacts, researchers are currently focused on creating safer 5α-reductase inhibitors from plants. The hair cream formulation contained 0.5% extracts of *Hibiscus rosa sinensis* flower, *Eclipta alba* whole plant, and *Solanum nigrum* plant berries in a ratio of 1:1:1. Plant extracts used in the formulation were characterized by chemical biomarkers. The cream formulation was developed, and the quality control parameters were examined. Furthermore, the produced formulation's 5α-reductase inhibitory activity was tested using finasteride as a positive control. *H. rosa sinensis*, *E. alba*, and *S. nigrum* extracts, which comprise 0.35% oleanolic acid, 4.65% β-sitosterol, and 31.18% linoleic acid, were used in the formulation. HPTLC data revealed that each 10 gm of cream formulation contains 16.68 ± 0.21 mg of linoleic acid, 3.61 ± 0.18 mg of β-sitosterol and 0.380 ± 0.11 mg oleanolic acid. *In-vitro* results showed a good 5α-reductase inhibitory potential. The IC_{50} value of the developed formulation was 85.254 ± 0.888 µg/ml, whereas finasteride (positive control) was 223.039 ± 1.367 ng/ml. Thus, the herbal hair cream composition benefits alopecia therapy by inhibiting the 5α-reductase enzyme. In addition, the formulation might be a potential option for further research into its antiandrogenic properties.

Keywords: Alopecia, 5α-reductase, HPTLC, herbal cream, *Hibiscus rosa sinensis*, *Solanum nigrum*, *Eclipta alba*.

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1. INTRODUCTION

A person's self-esteem, mental well-being, and overall quality of life can all be significantly impacted by hair loss, even though it is not life-threatening. Androgenic alopecia (androgenetic alopecia or AGA) is the most frequent hair loss in the condition, affecting both men and women in large numbers.[1,2] Steroid 5 alpha-reductase (5α-reductase or 5αR), an enzyme confined to the nuclear membrane, which has two subtypes named type 1 (5αR1) and type 2 (5αR2), converts testosterone (T) into dihydrotestosterone (DHT).[3] The NADPH-dependent conversion of T to DHT is catalyzed by 5αR.[4] The 5αR and its metabolite DHT influence various human disorders, including male pattern baldness in both sexes, alopecia, benign prostatic hyperplasia (BPH), prostate cancer, acne, and hirsutism.[5] Finasteride, dutasteride, and alfatradiol are synthetic medications; however, researchers are currently focused on creating safer 5α-reductase inhibitors from plants because of their negative impacts.A member of the Solanaceae family, *Solanum nigrum* (Black nightshade) is a plant used for medicinal treatments. *S. nigrum* has traditionally been used to cure a variety of diseases. *Solanum nigrum* berries treat alopecia.[6]Linoleic acid is an omega-6 polyunsaturated fatty acid. It is known to have various physiological properties such as 5α-reductase inhibitor, anti-anaphylactic, anticancer, antieczemic, and antifibrinolytic. So on.[7,] Linoleic acid is said to be the most prevalent unsaturated fatty acid in *Solanum nigrum* oil.[8,9] Asteraceae family member *Eclipta alba* (Bhringaraj) is a small branching perennial herbaceous plant. *E. alba* oil is a well-known tonic used to maintain black hair and reverse balding. It is frequently termed as "king of the hair".[10] β-sitosterol is one of the numerous phytosterols identified in high concentration in the petroleum ether extract of *E. alba*, which is a powerful 5αR inhibitor.[11]*Hibiscus rosa sinens*is a medicinal plant from the Malvaceae family. *H. rosa sinens*is used to cure various ailments, including alopecia. [12-14] The pentacyclic triterpenoid molecule oleanolic acid has been demonstrated to enhance hair development.[15] The flower of *Hibiscus rosa sinens* is reported to contain oleanolic acid as a phytoconstituent.[16]The current study aimed to develop a herbal hair cream formulation combining plant extracts of *H. rosa sinensis* flower, *E. alba* whole plant, and *S. nigrum* berries and assess the potential of 5α-reductase as a therapeutic model for androgenic alopecia. Standardizaton of the plant extracts was done using an appropriate biomarker. Oleanolic acid, β-sitosterol, and linoleic acid can be used to quantify *H. rosa sinensis*, *E. alba*, and *S. nigrum* plant extracts, respectively. After developing the formulation, it has to be assessed for the physical and chemical parameters (assay of biomarkers) to correlate with the therapeutic effectiveness of 5α-reductase potentials. HPTLC simultaneously quantified the three biomarkers above in the formulation of the polyherbal cream, which has not been reported earlier.

2. MATERIALS AND METHODS

2.1. Materials

Linoleic acid and β-sitosterol from Sisco research laboratories were used as a biomarker. Oleanolic acid (biomarker) was purchased from Sigma Aldrich. In addition, sodium methylparaben (SMP), sodium propylparaben (SPP), butylated hydroxyl toluene (BHT), igsurf 1540, propylene glycol, glycerine, kollidon 30, ethylenediaminetetraacetic acid disodium salt (EDTA disodium), potassium hydroxide, sodium carbonate, stearic acid ware gifted by Drakt International. The excipients are pharmaceutical grade (Indian Pharmacopoeia: I.P) except igsurf 1540 (Cosmetic grade: C.G). Analytical grade methanol, toluene, ethyl acetate, and n-hexane were acquired from Merck (Mumbai, India) for extraction and testing purpose. Whatman's syringe filter (0.45 µm) was employed for the sample and standard filtering.

2.2. Methods

2.2.1. Collection and Identification of Plant Materials

In February, *Hibiscus rosa sinensis* flowers, *Solanum nigrm* barriers, and *Eclipta alba* entire plant were collected from Kolkata (W.B.). School of Natural Product Studies, Jadavpur University, Kolkata, identified and authenticated the plant parts. A voucher specimen of *H. rosa sinensis*, *E. alba*, and *S. nigrum*(Voucher number are SNPS/JU/2015/1088, SNPS/JU/2015/1089, and SNPS/JU/2015/1088, respectively). The plant components were finely pulverized, air-dried, and then passed through filter number ten.

2.2.2. Preparation of Extracts

Three 500 ml conical flasks, each containing 100 g of crushed *H. rosa sinensis* flower, *S. nigrm* barriers, and *E. alba* entire plant parts. *H. rosa sinensis* was extracted with 250 ml of methanol. *S. nigrm* and *E. alba* were extracted with 250 ml of petroleum ether, respectively. It was maintained in an airtight container at 25 °C to 30 °C for three days. It was then filtered using regular filter paper. In a 500 ml beaker, the filtrate was kept. The filtrates were concentrated using a rotary vacuum evaporator at 40 to 45 °C.

2.2.3. Standardization and Quantification of Plant Extracts by HPTLC

Hibiscus rosa sinensis flower methanol extract (HRME), *Solanum nigrm* barriers petroleum ether extract (SNPE), and *Eclipta alba* whole plant petroleum ether extract (EAPE) were standardized by the biomarker oleanolic, linoleic acid and β-sitosterol respectively through HPTLC. With slight modifications, various extracts used the method to quantify oleanolic[17], linoleic acid[18], and β-sitosterol[19]. The following details are provided for the measurement of phytoconstituents using an HPTLC system with an automated development chamber:
Application software program: WINCATS
Automated sample dispenser: Linomat V
Scanning densitometer: CAMAG scanner 3
Photo documentation equipment: CAMAG Reprostar 3
Stationary phase: Aluminum-based silica gel plate 60 F254 (Merck, Mumbai) with a particle size of 5-10 µm measuring 10 cm × 10 cm. A 100 µl syringe (HAMILTON, Switzerland) was used to apply samples to HPTLC plates.

Table I: Details of Parameters Used in the HPTLC System

		HRME[17]	EAPE[18]	SNPE[19]
Standard preparation		Oleanolic acid in methanol (0.25 mg/ml)	β-sitosterol in petroleum ether (0.5 mg/ml)	Linoleic acid petroleum ether (0.5 mg/ml)
Sample preparation		20 mg/ml extract in methanol	5 mg/ml extract in petroleum ether	2.5 mg/ml extract in petroleum ether
Application volume	Standard	2, 4, 6, 8 µl	2, 4, 6, 8 µl	2, 4, 6, 8 µl
	Sample	5, 8, 15 µl	5, 10 µl	3, 8 µl
Mobile phase		Toluene: methanol (9:1)	Toluene : methanol 9:1 v/v	n-hexane: ethyl acetate 5:4 v/v
Distance travel		8 cm	8 cm	8 cm
Post-chromatographic derivatization reagent		Dipping in sulfuric acid-anisaldehyde	Dipping in sulfuric acid - anisaldehyde	Dipping in sulfuric acid - anisaldehyde
Heating		110 °C for 5 mins	110 °C for 2 mins	110 °C for 2 mins
Scanning		525 nm, a speed of 20 µl/s was used.	530 nm, a speed of 20 µl/s was used.	540 nm, a speed of 20 µl/s was used.

2.2.4. Preparation Method of Cream Formulation

As active components, the cream was made with dried HRME, SNPE, and EAPE. All of the extracts utilized in the formulation were 0.5% w/w. The cream was prepared using stearic acid (17%), propylene glycol, glycerin, sodium methyl paraben, sodium propyl paraben, butylated hydroxyl toluene (BHT), ethylene diamine tetra acetic acid disodium salt (EDTA disodium), potassium hydroxide, sodium carbonate, igsurf 1540 and purified water in quantity to prepare 100 g cream. The formulation was divided into aqueous phase and the oil phase. In the aqueous phase, the exact amount of extracts was mixed in glycerin. To this measured quantity of igsurf 1540, sodium methyl paraben, sodium propyl paraben, EDTA disodium, purified water, and in oil part, stearic acid was dissolved in propylene glycol, and potassium hydroxide, sodium carbonate, BHT, were added sequentially. Both solutions were blended in a beaker with steady stirring while heating to achieve the desired cream. Table 2 shows the composition of polyherbal hair growth cream.

Table II: Composition of Herbal Hair Cream

Steps	Ingredients	Specification	Quantity Used On 100 g basis
I.	Stearic acid	I.P	17 g
	Potassium hydroxide	I.P	0.5 g
	Sodium carbonate	I.P	0.5 g
	Propylene glycol	I.P	5.5 g
	BHT	I.P	0.01 g
II.	Glycerin	I.P	7 g
	Igsurf 1540	C.G	0.5 g
	Hibiscus rosasinensis methanol extract	I.H	0.5 g
	Eclipta alba petroleum ether extract	I.H	0.5 g
	Solanumnigrum petroleum ether extract	I.H	0.5 g
III.	EDTA Disodium	I.P	0.1 g
	Sodium methylparaben	I.P	0.3 g
	Sodium propyl paraben	I.P	0.1 g
	Purified water	I.P	67 g

2.2.4.1. The manufacturing method of Cream Formulation

Step I: In a heating mantle, propylene glycol was heated to 60-70 °C. Propylene glycol was then combined with stearic acid until it was dissolved. Then the appropriate amount of potassium hydroxide, sodium carbonate, and BHT were added and stirred one after the other until they dissolved.

Step II:7 g of glycerin was taken and heated in a 50 ml beaker at 40 °C to 50 °C for two to three minutes. Then, while constantly stirring, Igsurf 1540 was taken and added to it. The solution above is mixed with measured amounts of HRME, SNPE, and EAPE while continuously stirred until well mixed.

Step III: In a beaker with 67 g of purified water, SMP, SPP, and EDTA disodium were added one after the other. The solution was then gradually added to solution II and continually stirred with a glass rod until it was mixed properly.

Finally, solution III was added to the solution I with constant mixing at 40-50 °C.

2.2.5. Evaluation of Hair Cream Formulation

2.2.5.1. Determination of Organoleptic Properties

The cream's color, consistency, and grade were used to evaluate its appearance.[20]

2.2.5.2. pH

5 g of the cream formulation was weighed precisely and dispersed into 45 ml of water. At 27 °C, the pH of the suspension was measured using a digital pH meter.[21]

2.2.5.3. Homogeneity

The uniformity of the formulation was evaluated by touch and visual appearance.[22]

2.2.5.4. Smear test

A cream was applied to measure the results on a human volunteer's skin surface. The kind of film or smear developed on the skin after the cream application was examined.[21]

2.2.5.5. Emolliency

Emolliency, slipperiness, and the quantity of residue left behind after applying predetermined amounts of cream were evaluated.[21]

2.2.5.6. Viscosity

The Brookfield Viscometer was used to measure the viscosity at a temperature of 25 °C and a spindle speed of 20 rpm. The calculations were made three times, and the average of the data was recorded.[21]

2.2.5.7. Dilution Test

The emulsion is diluted in this test, either with water or oil. The emulsion will remain stable if it is of the o/w type and is diluted with water since water serves as the dispersion medium; but, if it is diluted with oil because oil and water are not miscible with one another, the emulsion will break. Unlike water in oil emulsion, which may be diluted with an oily liquid, oil in water emulsion is readily diluted with an aqueous solvent.[21]

2.2.5.8. Percentage of Active Content in Formulation by HPTLC

In this process, 10 g of cream was mixed with 50 ml of petroleum ether. After 15 mins of vortexing, the sample stock solution was filtered. Three biomarkers, namelyβ-sitosterol, oleanolic, and linoleic acid, were quantified using the same HPTLC configuration system. The stationary phase was used 20 cm x 10 cm. About 1 mg of the linoleic acid standard was dissolved in 1 ml of petroleum ether and applied in the range of 2, 4, 6, and 8 µl with gradual increments. Besides, 0.25 mg of oleanolic acid and β-sitosterol standard were taken in 2 separate Eppendorf tubes containing 1 ml of petroleum ether and dissolved. Both were applied in the 2, 3.5, 5, and 7 µl. The sample stock solution was prepared previously and applied 5, 10, 15, and 20 µl in an HPTLC plate. 16 bands were applied in the HPTLC plate including standards and samples. The mobile phase for resolving the extracts was n-hexane: ethyl acetate (6: 5 v/v). After development, plates were dried with a hand dryer. The dry plate was treated with sulphuric acid-anisaldehyde dipping reagent. The plate was held in a hot air oven at 110 °C for a short while, and the evaluation was 540 nm. At 366 and 540 nm, colored bands were visible.

2.2.6. 5α-Reductase Enzyme Inhibition Assay Preparation

A few modifications were made to the strategy outlined by Nahata& Dixit, 2013.[23] Details of the 5α-reductase enzyme inhibition method are displayed below,

2.2.6.1. Techniques for Preparing Solution for 5α-Reductase Enzyme

The following method produced enzyme preparation[23], where adult male goat prostate obtained from a nearby slaughterhouse. It was cut into small pieces corresponding to 300 mg and combined with a 20 ml medium (20 mmol sodium phosphate, pH 6.5, containing 0.32 mol sucrose and 1 mmol EDTA). Centrifugation at 4000 rpm for 15 minutes (716 g) and the resulting supernatant was used as an enzyme source. The protein content of the supernatant was determined using the Bradford method of protein measurement. A stock bovine serum albumin (BSA) solution of 1 mg/ml in deionized water was produced using this technique. Serial dilutions of 0.5, 0.25, 0.125, and 0.625 mg/ml were created from the stock. Then, 5 µl of the prepared BSA solution was added to a 96-well microplate at various concentrations. To the BSA solution, 200 µl of Bradford reagents were then added. Absorbance was observed at 592 nm. Plotting the standard concentrations against the absorbance at 592 nm resulted in the formation of a standard curve. 200 µl of Bradford reagent and 5 µl of enzyme homogenate solution were then incubated for 5 min. The BSA standard curve was then used to calculate the protein concentration. 0.320 mg/ml of isolated prostate protein was found. The solution was further diluted to 100 µg/ml for the enzyme test using a tissue homogenization medium.

2.2.6.2. Preparation of Standard Curve of NADPH

A standard curve of NADPH was prepared in methanol using concentrations of 1-20 µg/ml at 340 nm. There is a substantially linear relationship between absorbance and concentration, as shown by the straight line equation, the correlation value (r^2), which was equal to 0.991, and the equation y = 0.098x - 0.015.

2.2.6.3. Preparation of Test Solutions

75 µmol testosterone solution in methanol, 22 µmol NADPH solution in methanol, and 0.5 mol of Tris-HCl buffer in distilled water.

2.2.6.4. Preparation of Finasteride Solution

Finasteride was employed in the 5α-reductase enzyme inhibition assay as a positive control. To make a stock solution of finasteride (1000 µmol), 0.0037 g of powder was dissolved in 10 ml of methanol. Then, further dilution was done from 0.1 to 0.8 µmol, respectively, to determine the IC_{50} value.

2.2.6.5. Preparation of Test Samples for Enzyme Inhibition

Five groups of test samples were prepared. The following was done to the various groups:
Group A Finasteride (positive control)
Group B Herbal hair cream methanol (Me. OH) extract
Group C Herbal hair cream petroleum ether (Pet. ether) extract
Group D Methanol extract of the cream vehicle without plant extract
Group E Petroleum ether extract of the cream vehicle without plant extract

Separate extractions of the obtained cream formulation were performed using petroleum ether and methanol (1 mg/ml). Furthermore, plant extracts free cream vehicles were extracted in methanol and petroleum ether at 1 mg/ml concentration to examine the inherent potential of enzyme inhibitory capability as a negative control. To evaluate the polarity of extracts used in formulation, two solvent extractions, one with methanol and the other with petroleum ether, were performed to provide 1 mg/ml concentrations as their parent solvent. After being filtered via a 0.45 µ syringe filter, all samples were stored for further investigation. Additionally, 25, 50, 75, 100, 150, 200, 300, and 350 µg/ml dilutions were performed for each group to calculate the IC_{50} value.

2.2.6.6. Quantitative Measurement of 5α-Reductase Enzyme Inhibition In-Vitro

5α-reductase inhibition was carried out according to the technique Nahata & Dixit (2013) report.[23] Enzyme homogenate solution and the T, NADPH, and test samples were mixed. The specific reaction mixtures are described in Table 3. All reaction mixtures were incubated for 30 minutes at 37 °C. Absorbance was measured spectrophotometrically at 340 nm. The NADPH concentrations in the test samples were calculated using the NADPH standard curve. The concentrations of NADPH that remained in the reaction medium were determined. The percentage of NADPH scavenging was used to compute the NADPH concentration. Blank absorbance was subtracted from the test samples to get the net absorbance of NADPH. The inhibition of 5α-reductase was determined for each test substance, indicating the test substance's initial effectiveness against the enzyme. The percentage inhibition of 5α-reductase was calculated using the percentage of NADPH scavenging potential.

Sample ID	Methanol / Petroleum ether (ml)	Tris HCL (ml)	NADPH (ml)	Enzyme (ml)	Finasteride (ml)	Test sample (ml)		Testosterone (ml)		Total volume (ml)
Blank Control	4	4	3	1			Vortex and incubate at 37°C for 10 min		Vortex and incubate at 37°C for 30 min	12
Negative control	2	4	3	1				2		12
Finasteride		4	3	1	2			2		12
Test samples		4	3	1		2		2		12

A blank test was performed on all of the test samples to assess their intrinsic *in-vitro* antioxidant activity or capacity to convert NADPH to NADP, which would prevent them from initially inhibiting the 5αR present in the reaction medium. As a result, it was assumed that 3 ml of 22 µmol NADPH transformed 2 ml of 75 µmol T into DHT. The 5αR inhibition was then calculated for each extract to assess the actual activity against the enzyme. Finally, the IC_{50} value was derived by calculating the percentage inhibition of several test samples at various concentrations.

3. RESULTS

3.1. Percentage of Extraction Yield of Plant Materials

H. rosasinensis, *E. alba*, and *S. nigrum* extraction yields were 2.1%, 0.85%, and 1.25% w/w, respectively.

3.2. Quantification of Plant Extracts by HPTLC System

The raw materials was standardized and quantified based on their assay percentage to standardize the end formulation. For this purpose, HPTLC was utilized to standardize and quantify the extracts included in the formulation based on their active biomarkers. The calibration curve equation represents the data, where x indicates the number of biomarkers and y represents the height. *Hibiscus rosa sinensis* flower methanol extract contained 0.35% oleanolic acid (calibration curve y = 0.084 x - 3, correlation coefficient = 0.997) was found. The amount of β-sitosterol in the petroleum ether extract of *E. alba* was 4.64% (calibration curve y = 65.6 x + 13, correlation coefficient = 0.997). Linoleic acid was detected in 31.18 % concentration in *Solanum nigrum* berries petroleum ether extract (calibration curve obtained y = 127.8 x - 18, correlation coefficient = 0.992). In this particular, y shows the area under the curve for linoleic acid measurement—the R_f values of standard β-sitosterol, linoleic and oleanolic acid 0.81, 0.62, 0.78, respectively. Specificity was confirmed by comparing the R_f of the standard and sample shown in Figure 1. Figure 2 shows the image of the HPTLC plates at 540 nm.

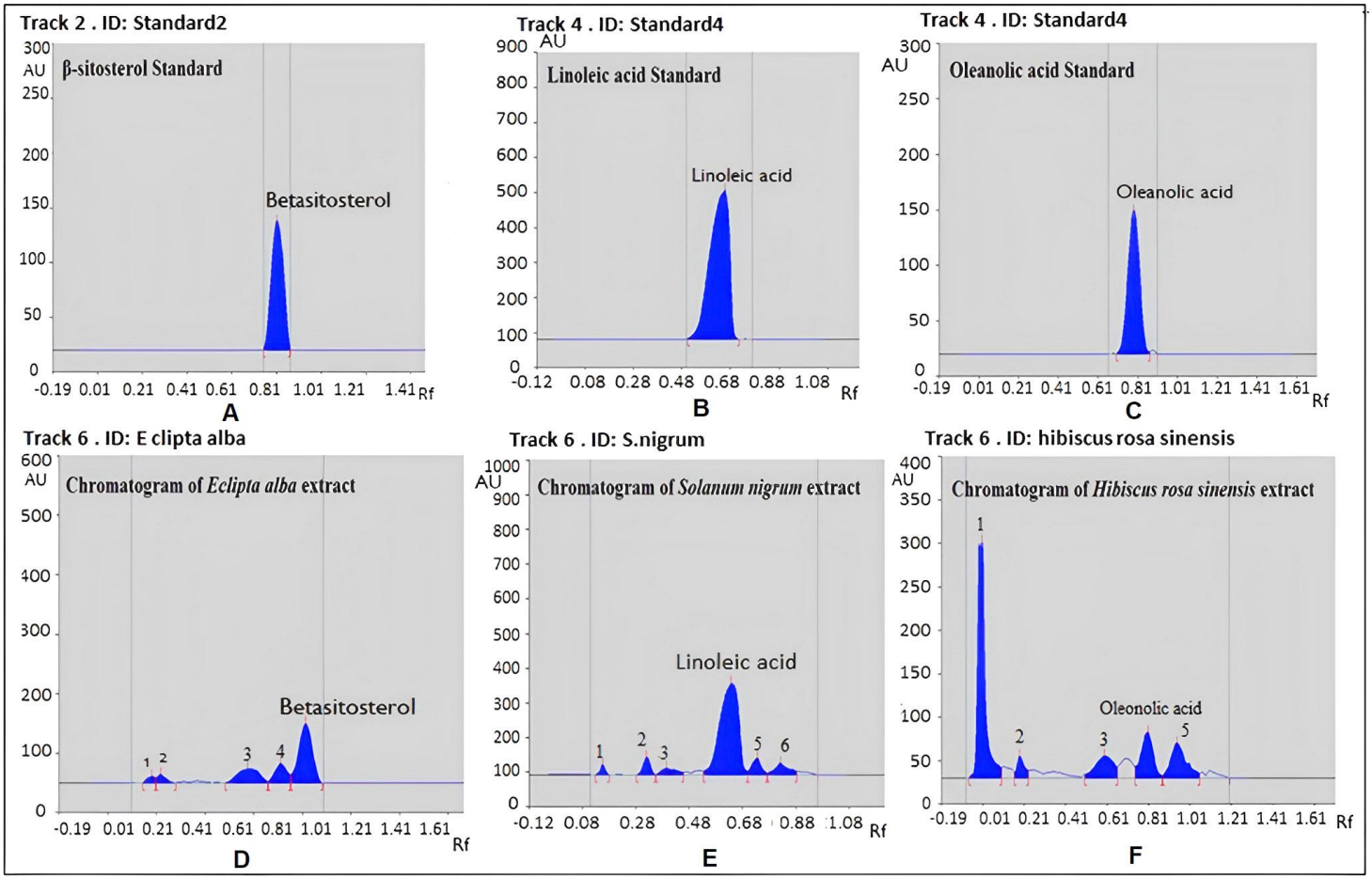

Fig I: (A) HPTLC Chromatogram of standard β -sitosterol; (B) HPTLC Chromatogram of standard linoleic acid; (C) HPTLC Chromatogram of standard oleanolic acid; (D) HPTLC Chromatogram of *Eclipta alba* extract; (E) HPTLC Chromatogram of *Solanumnigrum*extract; (F) HPTLC Chromatogram of *Hibiscus rosasinensis* extract.

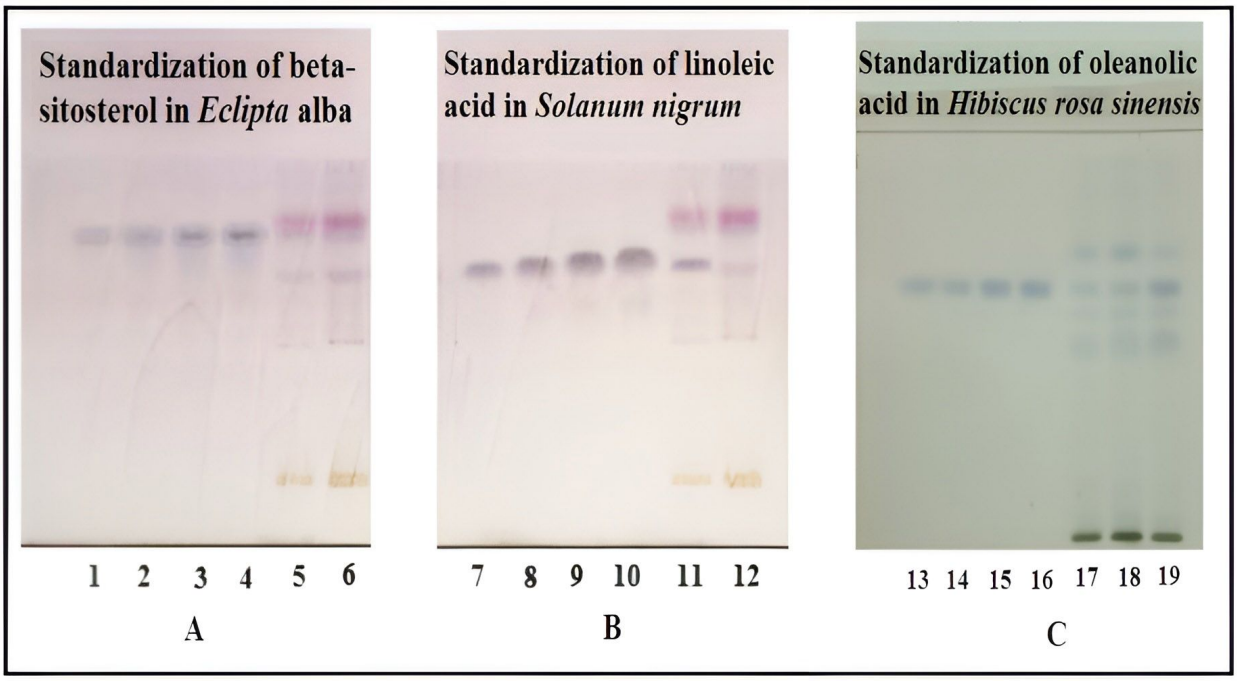

Fig II: (A) Standardization of β -sitosterol in *Eclipta* alba extract in TLC plate; (B) Standardization of linoleic acid in *Solanumnigrum*extract in TLC plate; (C) Standardization of oleanolic Acid in *Hibiscus rosasinensis* extract in TLC plate. Track 1-4: Standard β -sitosterol, track 5-6: *Ecliptaalba* extract, track 7-10: standard linoleic acid, track 11-12: *Solanumnigrum*extract, track 13-16: standard oleanolic acid, track 17-19: *Hibiscus rosasinensis* extract.

3.3. *Evaluation of Cream Formulation*

A light yellow color, odorless cream found with good adhesion. The formulation was good in appearance, homogeneity, and nongreasy (figure 3). Table 4 displays the outcomes of polyherbal cream.

Fig III: Herbal hair cream formulation.

3.4. *Quantification of Active Biomarkers in Cream Formulation by HPTLC*

HPTLC data revealed that each 10 gm of cream formulation contains 16.68 ± 0.21 mg of linoleic acid, 3.61 ± 0.18 mg of β-sitosterol and 0.380 ± 0.11 mg oleanolic acid. The calibration curve equation represents the data, where x indicates the number of biomarkers and y represents the height. The calibration curve of linoleic acid, β-sitosterol, and oleanolic acid was y = 10.804 x + 84.038 (correlation coefficient = 0.9764), y = 28.33 + 0.056 x (correlation coefficient = 0.9915) and y = 0.64 x + 31.93 (correlation coefficient = 0.9926). The R_f value of standard β-sitosterol, linoleic and oleanolic acids is 0.92, 0.76, and 0.85, respectively, shown in Figure 4. Figure 5 shows an image of the HPTLC plate at 366 and 540 nm.

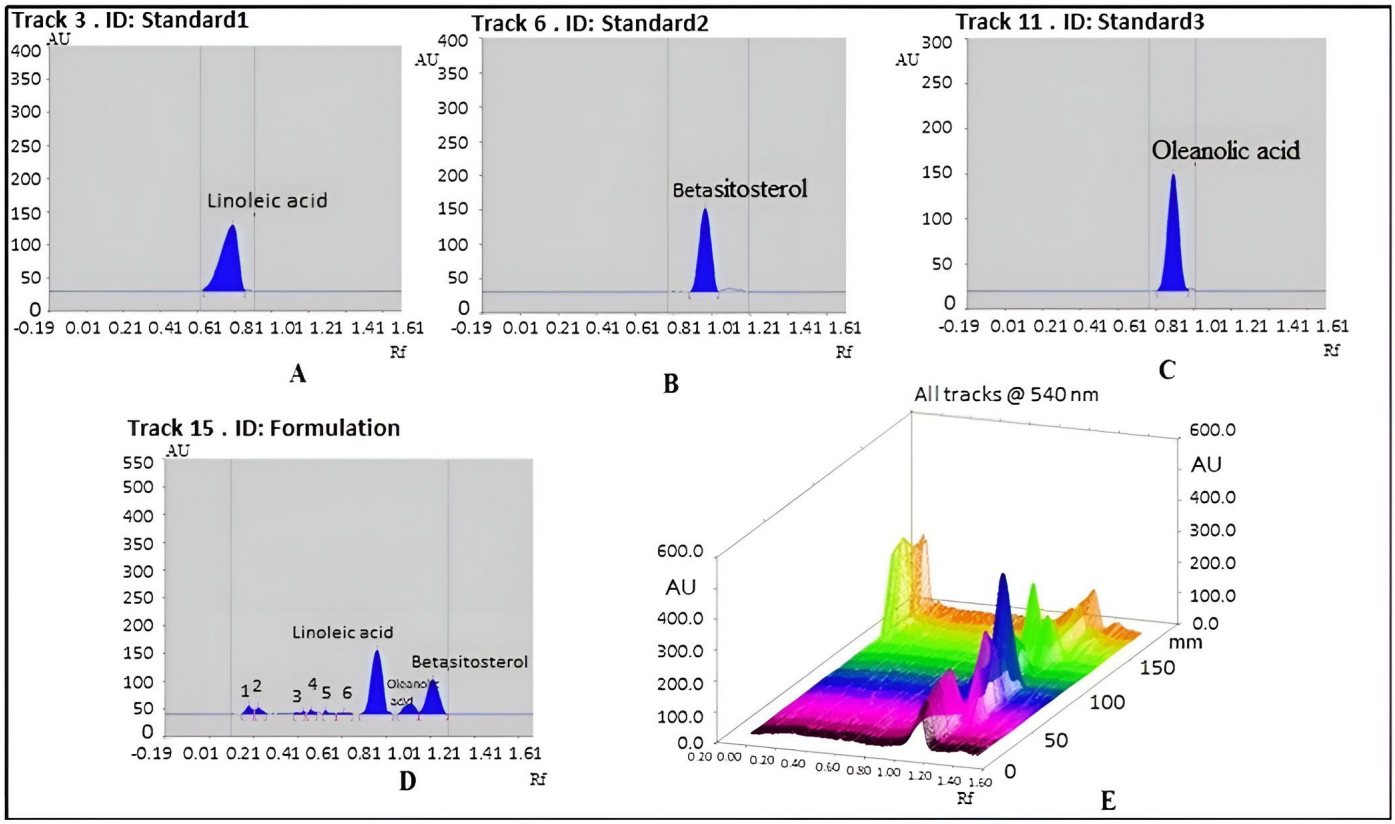

Fig IV: (A) HPTLC Chromatogram of standard linoleic acid; (B) HPTLC chromatogram of standard β - sitosterol; (C) HPTLC Chromatogram of standard oleanolic acid; (D) HPTLC Chromatogram of herbal hair cream formulation; (E) HPTLC 3D Chromatogram of cream formulation and standard bio-markers in UV 540 nm.

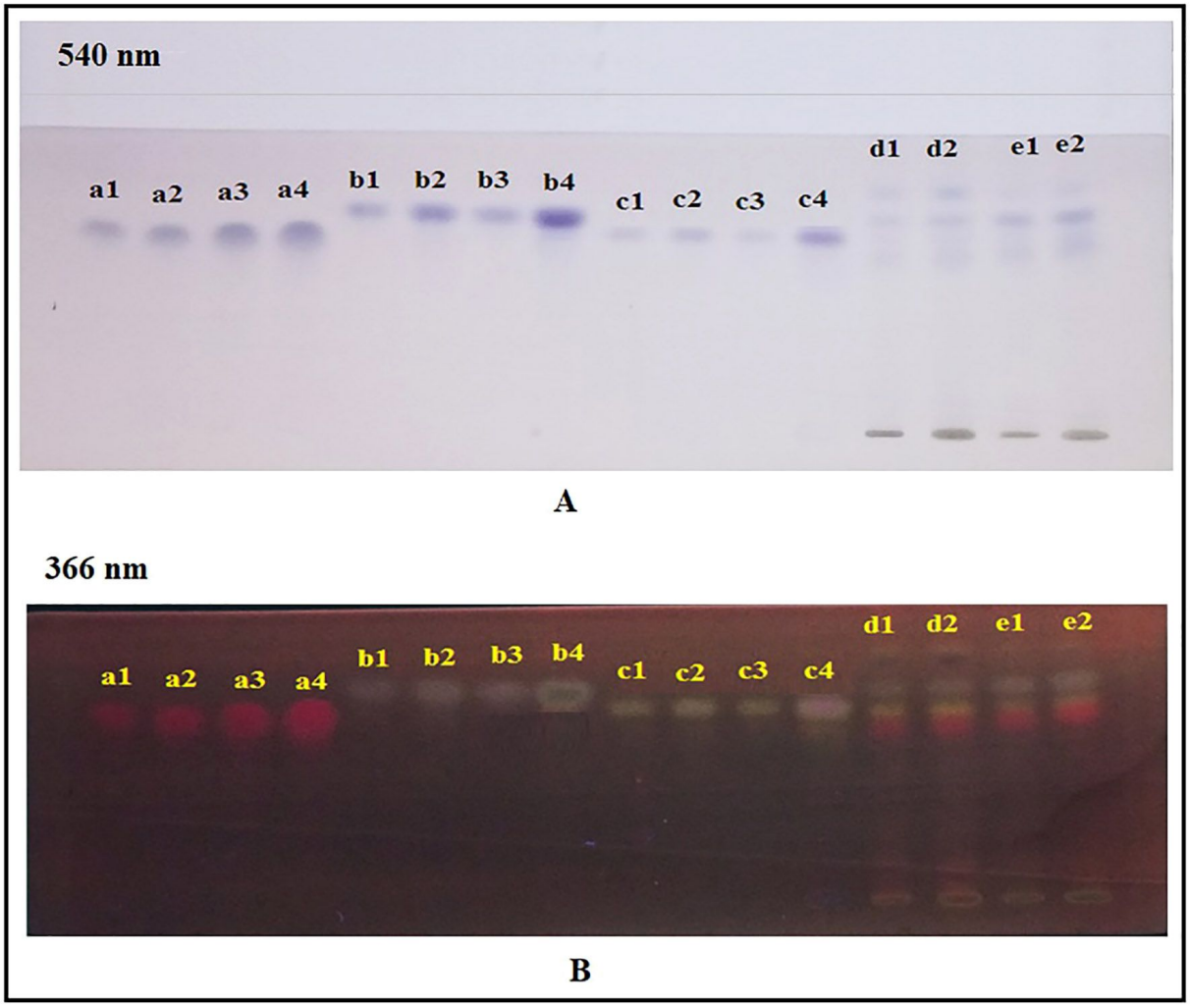

Fig V: (A) HPTLC fingerprint at 540 nm; (B) HPTLC fingerprint at 366 nm. Track a1-a4: standard linoleic acid, track b1-b4: standard β -sitosterol, track c1-c4: standard oleanolic acid, Track d1-d4: Sample solution of herbal cream.

Table IV: Evaluation Parameters of Hair Cream Formulation

Sl. no.	Parameters	Results
1.	Organoleptic properties	Light yellow color, odorless cream found.
2.	pH	6.36 ± 0.02
3.	Homogeneity	Homogeneous, smooth, and consistent.
4.	Smear test	The cream produced a non-greasy film on the skin's surface.
5.	Emolliency	No residue left.
6.	Viscosity (cps)	27015 ± 0.21
7.	Dilution test	O/W type emulsion.
8.	Percentage of active content by HPTLC	Each 10 gm of cream formulation contains 16.68 ± 0.21 mg of linoleic acid, 3.61 ± 0.18 mg of β -sitosterol and 0.380 ± 0.11 mg of oleanolic acid.

3.5. Effect of Formulation on 5α-Reductase Enzyme Inhibition

Statistical analysis was used to get IC_{50} values. The IC_{50} values were obtained by plotting the curve with the percentage of inhibition versus the concentrations of the various experiments, which were then expressed as mean ± standard deviation. Each test was replicated three times (n = 3). The statistical analysis used Graph pad software version 6.0, with one-way ANOVA and the Bonferroni post - hoc test. Compared to the reference standard, the P was determined to have a significant difference of less than 0.05. The IC_{50} value of the developed cream formulation in methanol and petroleum ether extracts was 155.209 ± 1.537 and 85.254 ± 0.888 µg/ml, respectively. At the same time, the IC_{50} value of finasteride (positive control) is 223.039 ± 1.367 ng/ml, which is the most potent. Furthermore, the IC_{50} value for a blank formulation in two distinct extraction mediums was obtained. The IC_{50} value of the blank formulation in methanol and petroleum ether extracts was determined to be 283.376 ± 2.82 and 141.426 ± 1.578 µg/ml, respectively, indicating the inherent ability of enzyme inhibition. Figure 6 displays the 5α-reductase inhibition of test samples. Figure 7 and Table 5 compare the samples' comparative 5αR IC_{50} potential.

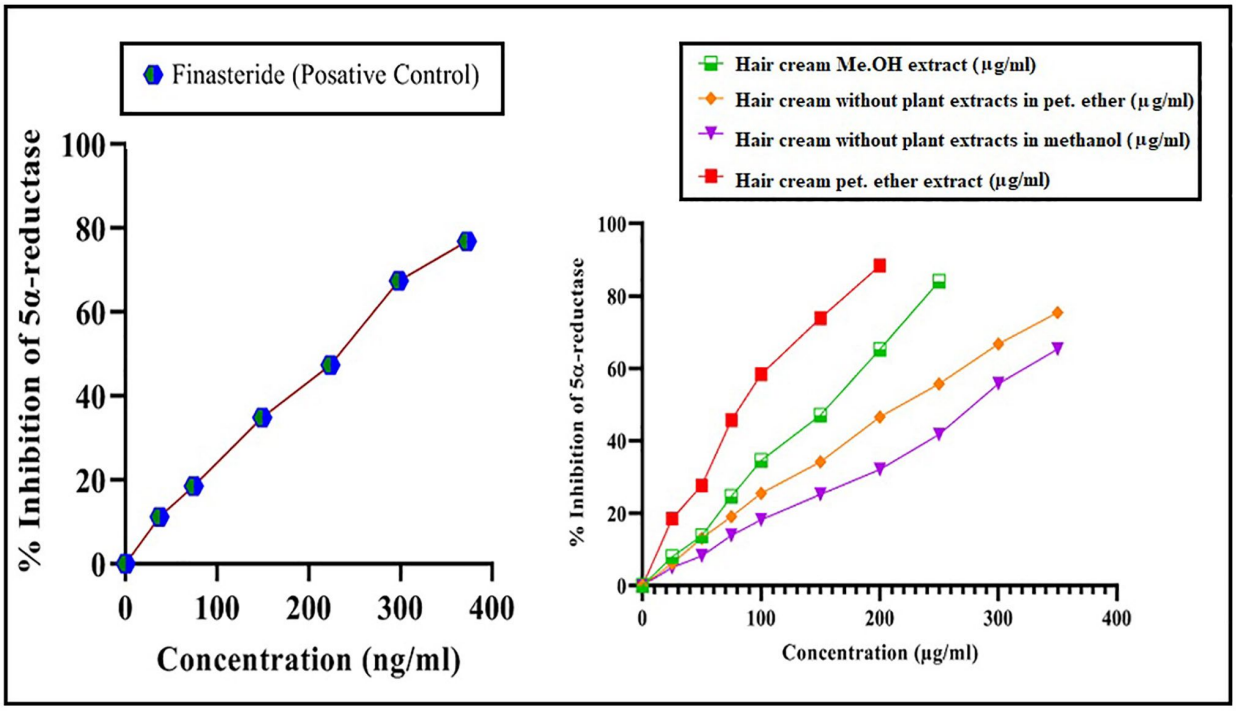

Fig VI: 5α-reductase inhibition of finasteride (positive control) and cream formulation.

Table V: Effect of Formulation on IC_{50} Values of 5α-Reductase

Group	Treatments	IC_{50} value
Group A	Finasteride (positive control)	223.039 ± 1.367 ng/ml
Group B	Herbal hair cream methanol extract	155.209 ± 1.537 µg/ml
Group C	Herbal hair cream petroleum ether extract	85.254 ± 0.888 µg/ml
Group D	Methanol extract of a cream vehicle without plant extract (negative control of methanol extract)	283.376 ± 2.82 µg/ml
Group E	Petroleum ether extract of a cream vehicle without plant extract (negative control of petroleum ether extract)	141.426 ± 1.578 µg/ml

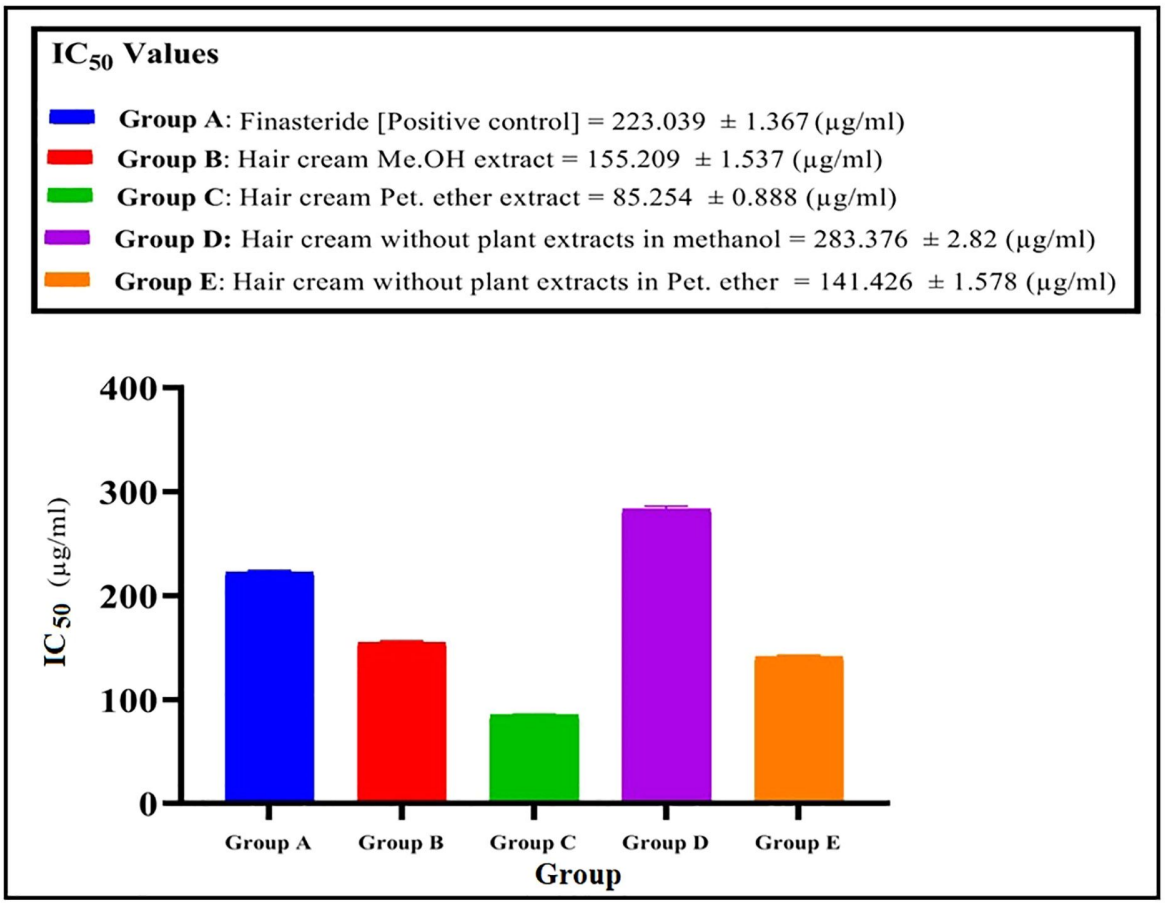

Fig VII: A comparison study of 5α R inhibitory potential. For each sample, the number of experiments (n) is 3. The mean ± SD was used to depict the data.

4. DISCUSSION

Androgenic alopecia is prevalent in men and women.[1,2]Dihydrotestosterone (DHT) is produced when the nuclear membrane-bound enzyme 5α-reductase converts testosterone (T).[3] 5αR catalyzes the NADPH-dependent conversion of T to DHT.[4] The 5αR and its metabolite DHT influence a variety of human disorders, including male pattern baldness in both sexes, alopecia, benign prostatic hyperplasia (BPH), etc.[5]*Eclipta alba* used traditionally for retaining black hair and reverse balding. It is frequently termed as "king of the hair".[10] Petroleum ether extract of *E. alba* contains 4.64 % of β-sitosterol, used in the formulation. β-sitosterol is one of the numerous phytosterols identified in high concentration in the petroleum ether extract of *E. alba*, which is a powerful 5αR inhibitor.[11] Alopecia is one condition that the medicinal plant *Hibiscus rosa sinens* has historically been used to treat. [12-14] The flower part of *H. rosa sinens* is reported to contain oleanolic acid as a phytoconstituent.[16] Methanol extract of the flower appears to contain 0.35 % of oleanolic acid, used in the composition. The pentacyclic triterpenoid molecule oleanolic acid has been demonstrated to enhance hair development.[15] A member of the Solanaceae family, *Solanum nigrum* barriers has used in the treatment of alopecia.[6]Linoleic acid is an omega-6 polyunsaturated fatty acid have a variety of physiological properties such as 5α-reductase inhibitor, anti anaphylactic, etc.[7]Oleanolic acid appears to be present in 31.18 % of the petroleum ether extract of the barries, which was employed in the composition. Linoleic acid is said to be the most prevalent unsaturated fatty acid in *Solanum nigrum* oil.[8,9]The current study used plant extracts of *Hibiscus rosa sinensis* flower (containing 0.35% oleanolic acid), *Eclipta alba* entire plant (carrying 4.65% β-sitosterol), and *Solanum nigrum* berries (containing 31.18% linoleic acid) to develop a herbal hair treatment formulation. Quality control parameters were evaluated, and an investigation was carried out to understand better hair development's ability to employ the 5α-reductase inhibitory model for treating AGA. The odorless cream with a light yellow color was detected on the skin after application, with no residue left. The pH, viscosity, dilution test, emollience, homogeneity, smear, and percentage content tests are all reported in Table 4. The mean ± standard deviation of three consecutive test results represents all data. According to a literature survey, the average pH of male and female hair is 5.604 ± 0.93 and 6.784 ± 0.16, respectively.[24] The pH of the formulated cream, 6.36 ± 0.02, is equivalent to the values indicated above and the skin's pH. A suitable viscosity is required for an acceptable formulation; a cream that is too thick may cause pourability concerns, while a cream that is too low may result in the dispersed contents settling during storage. The cream seemed thick, with a viscosity of 27015 ± 0.21 cps. The smear tests indicated the cream left a non-greasy film on the skin's surface. The dilution test showed that the emulsion was o/w. According to HPTLC data, each 10 gm of cream formulation includes 16.68 ± 0.21 mg linoleic acid, 3.61 ± 0.18 mg β-sitosterol, and 0.380 ± 0.11 mg oleanolic acid. An *in-vitro* 5α-reductase inhibition experiment was performed to assess the therapeutic effect of the cream formulation. The IC_{50} value of the petroleum ether extract of the formulation was more potent than that of methanol, which might be due to non-polar components contained in the petroleum ether extraction medium being more potently active to inhibit the enzyme. Two non-polar extracts were used in the

formulation: *Eclipta alba* and *Solanum niagrum*. In our present findings, β-sitosterol, a non-polar compound, was abundant in the petroleum ether extract of *Eclipta alba*, which has been previously proven to be a potent 5αR inhibitor.[11] Furthermore, the petroleum ether extract of *Solanum niagrum* contains a high concentration of unsaturated fatty acids (linoleic acid, linolenic acid, and so on)[8,9], and it has been demonstrated that inhibition of 5αR by lipophilic extracts of *Sabal serrulata* fruits is entirely due to free fatty acid content.[25]Oleonolic acid, a component of the methanol extract from the flowers of *H. rosa sinensis* utilized in formulation, suggested a potential inhibitor of 5α-reductase *in-vitro* that is useful in AGA.[26]Furthermore, the IC_{50} value for a blank formulation in two distinct extraction mediums was obtained. The IC_{50} value of the blank formulation (negative control) indicates the least inherent ability of enzyme inhibition. Moreover, the results demonstrate the efficacy of the extracts employed in the formulation compared to a blank formulation in an enzyme inhibition model for treating alopecia. Thus, the researchers discovered that using those standardized plant extracts to treat AGA, the developed cream formulation has more significant 5αR inhibitory action. Future goals for this study include a time-dependent investigation and an *in-vivo* effectiveness study, which would have offered more information about the plants. This methodology provides a simplified, sensitive, and precise way to determine the amount of NADPH, indirectly revealing the testosterone level in the reaction mixture. The technique, therefore, suggests that the extracts 5α-reductase inhibitory action.

5. CONCLUSION

In conclusion, a polyherbal hair cream formulation was developed which contains standardized extracts of *Hibiscus rosa sinensis* flower (containing 0.35% oleanolic acid), *Eclipta alba* entire plant (carrying 4.65% β-sitosterol), and *Solanum nigrum* berries (containing 31.18% linoleic acid): a light yellow color, odorless cream found with good adhesion. The formulation was good in appearance, homogeneity, and non-greasy. HPTLC data revealed that each 10 gm of cream formulation contains 16.68 ± 0.21 mg of linoleic acid, 3.61 ± 0.18 mg of β-sitosterol and 0.380 ± 0.11 mg oleanolic acid. *In-vitro* results showed a good 5α-reductase inhibitory potential. The IC_{50} value of the developed formulation was 85.254 ± 0.888 µg/ml, whereas finasteride (positive control) was 223.039 ± 1.367 ng/ml, which is a good 5α-reductase inhibitory potential. Thus, the developed hair cream composition benefits alopecia therapy by inhibiting the 5α-reductase enzyme. In addition, the formulation might be a potential option for further research into its antiandrogenic properties.

6. ABBREVIATIONS

5αR: 5 alpha reductase; **AGA**: Androgenetic alopecia; **T**: Testosterone; **DHT**: Dihydrotestosterone; **BPH**: Benign prostatic hyperplasia.

7. AUTHORS CONTRIBUTION STATEMENT

Concept, design, and definition of intellectual content: Arpan Chakraborty, Arka Bhattacharjee, Manas Chakraborty, Goutam Mukhopadhyay. Literature search and experimental studies: Arpan Chakraborty, Arka Bhattacharjee, Baishakhi Mondal, Alpana Majumder. Data acquisition, data analysis, statistical analysis: Arpan Chakraborty, Manas Chakraborty, Goutam Mukhopadhyay. Manuscript preparation and editing: Arpan Chakraborty, Arka Bhattacharjee, Alpana Majumder, Baishakhi Mondal. Manuscript review: Arpan Chakraborty, Manas Chakraborty, Goutam Mukhopadhyay, Arka Bhattacharjee, Alpana Majumder.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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