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Original Article

PROCESS VALIDATION OF BETA-SITOSTEROL HAIR GEL FORMULATION AND EVALUATION OF 5 ALPHA REDUCTASE INHIBITION *IN VITRO* FOR THE TREATMENT OF ANDROGENETIC ALOPECIA

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ABSTRACT

Objective: The present study was aimed to develop topical gel containing β-sitosterol using carbopol 940 as a gelling agent and to investigate 5 alpha reductase (5α-reductase) inhibitory activity of suitable gel formulation and compare it with a commercial product used topically for alopecia.

Methods: Three different batches of β-sitosterol hair gel formulation were manufactured and evaluated. Additionally, the 5α-reductase inhibitory activity of the prepared formulation, finasteride as a positive control, was evaluated and compared to the commercial herbal formulation used.

Results: According to the analytical findings of three different batches, the gel formulation is good in appearance, homogeneous, and easily spreadable. Based on findings from HPLC and HPTLC, the amount of β-sitosterol in those formulations complies with the label claim. By checking different critical parameters of those batches, we established the manufacturing process method validation and the process reproducibility. *In vitro* results showed the good 5α-reductase inhibitory potential of prepared gel formulation and then commercial product. The IC_{50} value of the prepared formulation was 118.960±0.634 (µg/ml) and standard beta-sitosterol 88.854±0.70 (µg/ml), whereas Finasteride (positive control) 224.372±3.103 (ng/ml).

Conclusion: Thus, β-sitosterol formulation utilises a straightforward, low-cost production, less time-consuming process with minimal facility and equipment requirements. The formulation may be a promising candidate for future investigation into their antiandrogenic activities.

Keywords: 5α-reductase, Hair loss, Beta-sitosterol, NADPH, HPTLC, Hair gel, Alopecia

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INTRODUCTION

Since more than 2,000 y ago, alopecia has been recognised as a dermatological condition. It is a synonym for baldness, which refers to a lack of hair or hair loss, particularly on the head. It is widespread and is thought to afflict between 0.2% and 2% of the world's population [1]. Androgenic alopecia, often known as AGA, is the most prevalent alopecia that affects both men and women equally and is the cause of hair loss in many [2]. Testosterone (T) is converted into dihydrotestosterone (DHT) by the nuclear membrane-bound enzyme steroid 5α-reductase (5αR), which has two subtypes called type 1 (5αR1) and type 2 (5αR2) [3]. The NADPH-dependent conversion of T to DHT is catalysed by 5αR [4]. Numerous human diseases, including male pattern baldness in both sexes, alopecia, benign prostatic hyperplasia (BPH), prostate cancer, acne and hirsutism, are impacted by the 5αR and its metabolite DHT [5]. Finasteride [6, 7] and epristeride are two common 5αR inhibitors on the market [8]; however, their use is limited due to several side effects. This problem might be solved by using a herbal alternative for 5αR inhibition. In order to find potential 5αR inhibitors, we evaluated the alternative resource for antiandrogenic activity and their potential to block the 5αR.

Because β-sitosterol can inhibit 5αR, it can stimulate hair growth [9, 10]. There is some topical commercial formulations use 0.25 % β-sitosterol named Riaheal, Mebo ointment for burn, wounds, mouth ulcers etc [11, 12].

Nigella sativa, *Serenoa repens* (Saw palmetto), *Cucurbita pepo* (pumpkin seed), *Pygeum africanum*, black cumin seed, pecans, cashew fruit, avocados, rice bran, wheat germ, corn oils and soybeans are only a few of the plants that contain β-sitosterol [13].

Poor water and oil solubility is the major issue with the oral administration of beta-sitosterol. Another significant problem is the substance's crystalline structure at the body and ambient temperatures and large daily dosages (up to 3 g/day). It has been investigated to increase the oral absorption of β-sitosterol by complexation with cyclodextrin, liposomes, electrospun nanofibers, solid lipid nanoparticles, self-emulsion drug delivery system, and nanostructured lipid carriers [14]. However, the effective incorporation of β-sitosterol in pharmaceutical formulations is a complicated phenomenon.

For treating AGA, topical β-sitosterol therapy has drawn much interest. Upadhyay *et al.* (2012) developed phytovesicles containing β-sitosterol by complexing it with phosphatidylcholine, and they discovered that β-sitosterol had better activity and more significant absorption in the treatment of alopecia [15]. However, the drawbacks of phospholipid vesicles include drug leakage and fusion, phospholipid oxidation, high cost, poor stability, and limited solubility.

Prabahar *et al.* (2022) designed a transdermal drug delivery system of β-sitosterol that has the potential to treat androgenic alopecia by utilising a nanostructured lipid carrier incorporated with polymeric microneedles [16]. However, the negative aspects of microneedles are dose precision; a continual injection may cause vein collapse, dermal tissue that is compressed can obstruct hollow microneedles etc [17].

To overcome the drawback, our goal of the current study was to develop a topical gel formulation containing β-sitosterol utilising a straightforward, low-cost production, less time-consuming process with minimal facility and equipment requirements. Additionally, process validation using three batches and several analytical parameters (ex., viscosity, HPLC, HPTLC) was done to ensure consistent product quality. Additionally, an *in vitro* 5 alpha-reductase inhibition experiment was performed and compared to a commercial product to assess the therapeutic effect of the gel formulation.

MATERIALS AND METHODS

Materials

β-sitosterol (from Sisco research laboratories) was used as an active medicinal component. In addition, sodium methylparaben (SMP), sodium propylparaben (spp), carbopol 940, propylene glycol, glycerine, cremophor RH 40, kollidon 30, triethanolamine, EDTA disodium, tartrazine yellow, brilliant blue was gifted by Drakt International. The excipients are pharmaceutical grade (Indian Pharmacopoeia: IP). Analytical grade methanol, toluene, ethyl acetate, and acetonitrile were acquired from Merck (Mumbai, India). Whatman's syringe filter (0.45 µm) was employed for the sample and standard filtering.

Methods

Preparation of β-sitosterol gel formulation

Every ingredient in the composition falls under the category of "Generally Recognized as Safe (GRAS)" as per the FDA and is used only by the permitted limits. Our label claims that the final concentration of β-sitosterol in the formulation was 0.20% as the active ingredient. The gel was prepared using pure β-sitosterol. The gel was prepared using carbopol 940 (1.2%), propylene glycol, cremophor RH 40 (PEG-40 hydrogenated castor oil), SMP, spp, EDTA disodium, triethanolamine and purified water in quantity to prepare 100 g gel. The water required for the formulations was divided into three parts. In one part, the exact amount of β-sitosterol was mixed. To this calculated quantity of cremophor RH 40, propylene glycol, SMP, spp, polyvinyl pyrrolidine, glycerine was added step by step, and in another part, EDTA disodium, carbopol 940 was added in water. Both of these solutions were mixed in a beaker. Tartrazine yellow and brilliant blue dissolved in water and mixed in the above mixture to make a green colour. Finally, dropwise, triethanolamine was added to the final mixture to obtain gel consistency. Three batches were manufactured using the same composition and procedure to ensure reproducibility. The composition of β-sitosterol hair growth gel is given below in table 1.

Table 1: Manufacturing of gel formulation

Steps	Ingredients	Function in formulation	Specification	Quantity (g) used on 100 g basis
1	β-sitosterol (pure)	Active ingredient	IH	0.20
	Sodium methyl paraben	Antimicrobial preservative	IP	0.03
	Sodium propyl paraben		IP	0.01
	Glycerin	Humectant, emollient and	IP	5
	Propylene glycol	preservative	IP	11
	Cremophor RH 40	Emulsify and solubilizer	IP	0.60
	Kollidon 30	Sustained release polymer	IP	0.10
	Purified water	Solvent	IP	38.20
2	Carbopol 940	Gelling agent	IP	1.20
	EDTA Disodium	Chelating agent and stabilizer	IP	0.05
	Purified water	Solvent	IP	39
3	Purified water	Solvent	IP	5
	Tartrazine yellow	Colouring agent	IP	0.0035
	Brilliant blue		IP	0.0015
4	Triethanolamine	pH adjuster	IP	0.77

Manufacturing process

The solution I: 11 g of propylene glycol should be placed in a 50 ml beaker and heated for 4-5 min at 40–50 °C on a heating mantel. Then, while constantly stirring, cremophor RH 40 was added. Then, measured amounts of β-sitosterol are added to the solution mentioned above with steady stirring.

In a beaker with 38.20 g of purified water, polyvinyl pyrrolidine (Kollidon 30), SMP, and spp were progressively added and stirred to dissolve. Next, 5 g of glycerine was added, and then it was blended. The combination containing β-sitosterol was then gently added to the solution while stirring in a 50 ml beaker.

Solution II: 39 ml of water and EDTA disodium stirred until it dissolved. Then specified quantity of carbopol 940 was added, stirred for 30 min, and allowed to swell for an hour.

Solution III: Tartrazine yellow and brilliant blue were dissolved in water and then added to solution I, which resulted in a green colour.

Solution IV: With continuous mixing, Solution III was added to Solution I. Triethanolamine was gradually added to the mixture while constantly stirring for ten min to achieve the desired gel consistency.

Evaluation of gel formulation

Net content

The net content was calculated by subtracting the container weight from the total weight of the goods inside the container.

pH

Using a digital pH metre, the pH of gel formulations was assessed [18]. Each formulation's pH was measured three times, with the average readings being computed.

Viscosity

The viscosity of the produced gel is measured using a Brookfield viscometer [19]. Spindle no. 64 rotates the Brookfield viscometer at a speed of 50 rpm. Each reading was obtained after the sample's equilibrium at the end of two min. The samples were repeated three times.

Spreadability

Spreadability was determined by wooden block and glass slide apparatus [20]. Weights measuring about 20 g were placed on the pan, and the amount of time it took for the upper slide (movable) to fully separate from the fixed slides was recorded. Spreadability was then calculated by using the formula

$$S = \frac{ML}{T}$$

Where S = Spreadibility, M = Weight tide to upper slide, L = Length of a glass slide, and T = Time taken to separate the slide from each other. The therapeutic efficacy of a formulation also depends upon its value.

Physical stability

The physical characteristics of the gel formulations, such as phase separation and colour change, odour, and rheological parameters, were assessed [21].

Homogeneity

After being put in the container, all produced gels were visually inspected to determine their homogeneity. Then, they underwent testing for the appearance and the existence of any aggregates [22].

Skin irritation test

A test for irritation was conducted on human test subjects [21]. Five participants were chosen for each gel, and 1.0 g of the prepared gel

was applied to a 2 square-inch region on the back of the hand. Onlookers looked for lesions and irritation in the participants.

Percentage of drug content

This procedure combines 10 ml of methanol with 1 g of produced gel. Vortex the mixture for 15 min, then filtered the stock solution. Appropriate dilutions were used to make aliquots or varying concentrations, and the absorbance was then measured. The equation derived from a calibration or linear curve was used to determine drug content. HPLC and HPTLC were used to determine the percentage of drug content.

Standardization and quantification of β-sitosterol, in hair gel by HPTLC

HPTLC method was used to quantify the β-sitosterol reported by Bhattacharjee *et al.* (2017) with minor modifications [23]. The CAMAG HPTLC system consisting of WINCATS software, LINOMAT V automatic sample applicator, an automatic development chamber, scanning densitometer CAMAG scanner 3, and photo documentation apparatus CAMAG reprostar 3 were used. The stationary phase used was aluminum-based silica gel plate 60 F_{254} (Merck, Mumbai) with 10 cm x 10 cm in particle size of 5-10 µm. All the solvents were used for an analytical grade. A 100 µlsyringe (HAMILTON, Switzerland) was used for sample application on HPTLC plates. About 1 mg of the β-sitosterol standard was weighed and dissolved in 1 ml of methanol, used as a standard solution. The sample solution was prepared by taking 2 g of three β-sitosterol gel formulations (Batch-A, Batch-B, Batch-C) in three separate centrifuge tubes. 20 ml methanol was added to each centrifuge tube. Mixed in vortex mixture for 15 min and put in ultrasonication bath for 30 min. It was then filtered through a 0.45 µ syringe filter and kept in a 1 ml Eppendorf tube for further study. HPTLC analysis was performed using an isocratic technique. The mobile phase was optimized with toluene: ethyl acetate: methanol in a ratio of 5: 2: 3 v/v. The temperature was kept at 25 °C, and the mobile phase was developed in a twin trough glass chamber. A standard stock solution of three biomarkers was applied consequently in the range of 1.5-6 µl with a 1.5 µl gradual increment. The sample solution was applied 20, 19, and 18 µl of Batch-A, Batch-B and Batch-C, respectively. All seven tracks in the HPTLC plate were used for standardization, including standard and sample solution, respectively, in a band-wise fashion. After development, plates were dried with a hand dryer. The dry plate was treated with sulphuric acid anisaldehyde spraying reagent. The plate was kept at 110 °C for 5 min in a hot air oven, and evaluation was carried out at 530 nm. Coloured bands were observed at 366 to 530 nm.

Standardization and quantification of β-sitosterol in hair gel by HPLC

HPLC method was performed to quantify the β-sitosterol reported by Bhattacharjee *et al.* (2017) with some modifications [23]. RP-HPLC system (Shimadzu Prominence, Kyoto, Japan) equipped with two Shimadzu LC-20 UFLC reciprocating pumps, a variable Shimadzu SPD-M20A Prominence PDA detector and a rheodyne manual injector with a loop size of 20 µl was used. The peak area was calculated with LC solution software. The analysis was carried out using a C_{18} reverse phase column having a dimension of 250 mm (length) × 4.6 mm (width) with a particle size of 5 µm (Phenomenex-Luna C_{18}, Torrance, CA, USA). A standard stock solution of β-sitosterol was prepared with methanol (1000 µg/ml). Further dilutions were made as required with methanol for the calibration curve. Working standard solution was done using fresh methanol in the tube to make 20 µg/ml. The gel formulation sample solution was prepared using the same method performed in HPTLC. It was then filtered through a 0.45 µ syringe filter.

Further dilution was carried out by fresh methanol to obtain a concentration of 20 µg/ml of β-sitosterol in a sample solution. HPLC analysis was performed using an isocratic technique. The mobile phase was optimized with acetonitrile and methanol at 70:30 v/v, respectively. The temperature was kept at 25°C, and the standard and sample were injected 20 µl. The standard stock solution of biomarker was applied in the range of 2.5 to 15 µl with 2.5 µl gradual increment for the calibration curve. The sample solution was applied 20 µl. Detection was carried out at 210 nm wavelength. The flow rate was adjusted to 1 mg/ml.

Enzyme inhibition assay preparation for 5α-reductase

A few changes were made to the approach described by Nahata and Dixit, 2013 [24]. The procedure's details are shown below.

Methods of preparation 5α-reductase solution

Enzyme preparation was carried out by the following process [24], adult male goat prostate was collected from the local slaughterhouse. The equivalent of 320 mg was taken from it, minced into small pieces and then mixed with a 20 ml medium (20 mmol sodium phosphate, pH 6.5, containing 0.32 mol sucrose and 1 mmol EDTA), followed by centrifugation for 15 min at 4000 rpm (716 g), and the supernatant obtained was utilized as an enzyme source. Using the Bradford technique of protein quantification, the protein content in the supernatant was calculated. Bradford's technique of protein quantification was used to estimate the amount of enzyme in the supernatant. This method produced a stock bovine serum albumin (BSA) solution of 1 mg/ml in deionized water. From stock, serial dilutions were made with concentrations of 0.5, 0.25, 0.125 and 0.625 mg/ml. Next, 5 µl of the produced BSA solution were put into a 96-well microplate at various concentrations. Then, 200 µl of Bradford reagents were added to the BSA solution. At 592 nm, absorbance was observed. A standard curve was created by plotting the standard concentrations against the absorbance at 592 nm. 5 µl of enzyme homogenate solution was incubated for 5 min with 200 µl of Bradford reagent. The protein concentration was then calculated by using the BSA standard curve. The isolated prostate protein content was 0.44 mg/ml. The solution was further diluted to 100 µg/ml for the enzyme assay using tissue homogenization media.

Preparation of standard curve of NADPH

At 340 nm, a standard NADPH curve was created in methanol using concentrations of 1, 3, 5, 8, 10, 12, 15, and 20 µg/ml. The equation of a straight line revealed a good linear connection between absorbance and concentration, the correlation value (r^2), which was equal to 0.991, and the equation y = 0.098x-0.015.

Preparation of test solutions

Testosterone solution (75 µmol) in methanol, NADPH solution prepared in methanol (22 µmol) and 0.5 mol of Tris-HCl buffer in distilled water.

Preparation of Finasteride solution

The 5 alpha reductase enzyme inhibition test was utilized as a positive control using finasteride. Finasteride stock solution was prepared by dissolving 0.0037 g of powder in 10 ml methanol (1000 µmol). Sonication was carried out for an hour and vortexed for 15 min. Then filtration was carried out by 0.45 µ syringe filter, and the filtrate was collected in two separate eppendorf tubes. 2 µl of stock finasteride solution was diluted with 1998 µl methanol to obtain 1 µmol working solutions. Further dilution was done: 0.1, 0.2, 0.4, 0.6, 0.8 µmol, respectively, for determining the IC_{50} value.

Preparation of test samples for enzyme inhibition

Six groups of test samples were created. The following was done to the various groups:

Group A Finasteride (positive control)

Group B Beta-sitosterol pure solution

Group C Beta-sitosterol gel methanol (Me. OH) extract

Group D Gel vehicle without beta-sitosterol methanol extract

Group E and Group F Commercial gel formulation methanol and petroleum ether (Pet. ether) extract

The prepared beta-sitosterol gel formulation was extracted using methanol (1 mg/ml). Beta-sitosterol-free gel vehicle extract in methanol at a concentration of 1 mg/ml was used to observe the inherent potential of inhibitory enzyme potential. Two solvents, one methanol and the other petroleum ether extraction were carried out to create 1 mg/ml concentration as their parent solvent to determine the polarity of extracts utilized in commercial

formulation. All the samples were preserved for later research after being filtered via a 0.45 µ syringe filter. Additionally, dilutions of 25, 50, 75, 100, 150, 200, 300 and 350 µg/ml were carried out for all those formulations. Beta-sitosterol pure standard solution was prepared in methanol (1 mg/ml). Further dilution was done at 25, 50, 75, 100, 150, and 200 µg/ml.

In vitro assay procedure of 5α-reductase inhibition

Based on the procedure outlined by Nahata and Dixit (2013), 5α-reductase inhibition was carried out [24]. The enzyme homogenate solution, T, and NADPH test samples were mixed. Table 2 describes the specific reaction mixtures. All reaction mixtures underwent a 30 min incubation period at 37 °C. At 340 nm, absorbance was determined spectrophotometrically. From the NADPH standard curve, the test samples corresponding NADPH concentrations were determined. NADPH concentrations that remain in the reaction medium were calculated. The NADPH concentration was calculated as a percentage of NADPH scavenging. To calculate the net absorbance of NADPH, blank absorbance was subtracted from the test samples. The 5α-reductase inhibition was calculated for each test substance, showing the test substance's original efficacy against the enzyme. The percentage of NADPH scavenging potential was used to calculate the percent inhibition of 5α-reductase.

All of the extracts were subjected to a blank test to determine their intrinsic *in vitro* antioxidant activity or their capacity to convert NADPH to NADP, which would prevent them from initially being able to inhibit the 5αR present in the reaction medium. Therefore, it was considered that 2 ml of 75 µmol of T was transformed into DHT by 3 ml of 22 µmol NADPH. The 5αR inhibition was then calculated for each extract to assess the actual activity against the enzyme.

Table 2: Enzyme, substrate and coenzyme mixture

Sample ID	Methanol (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		Vortex and	12
Negative control	2	4	3	1			incubate at 37 °C for 10	2	incubate at 37 °C for	12
Finasteride		4	3	1	2		min	2	30 min	12
Test samples		4	3	1		2		2		12

Absorbance at 340 nm,

$$\text{Net absorbance of test} = (\text{Test Absorbance} - \text{Blank Absorbance})$$

Calculate the NADPH concentration in each sample from NADPH standard curve prepared previously.

$$\%\ \text{inhibition} = 100 - [(\frac{54.78 - \text{concentration of NADPH obtained from net absorbance of the test solution}}{54.78}) \times 100]$$

To measure the percentage inhibition of various test sample concentrations required to establish the IC_{50} value of the test extracts.

RESULTS AND DISCUSSION

A clear gel of light green colour is formed and has good adhesion. The three batches of the formulation are good in appearance, homogeneity and easily spreadable shown in fig. 1. Table 3 displays the outcomes of the three batches.

HPTLC data revealed that the percentage content of β-sitosterol in a gel formulation was 97.50, 97.11 and 96.66 % w/w, respectively, in three different batches Batch-A, Batch-B and Batch-C. This was determined by a calibration curve with y = 3813.444x+435.851 (correlation coefficient = 0.9469), as shown in fig. 1, where x represents the amount of β-sitosterol and y represents the area under the curve. The R_f value of standard β-sitosterol was found to be 0.78. Specificity was confirmed by comparing the R_f of the standard and sample shown in fig. 1.

HPLC data showed that the percentage content of β-sitosterol in gel formulations Batch-A, Batch-B, and Batch-C was found to be 98.62, 97.59, and 98.35 % w/w, respectively. A calibration curve determined this with y = 2649.8x+228397 (correlation coefficient = 0.9923), where x represents the amount of β-sitosterol and y represents the area under the curve. The retention time (R_t) value of standard β-sitosterol was 2.3 min. Specificity was confirmed by comparing the R_t of standard and sample fig. 2.

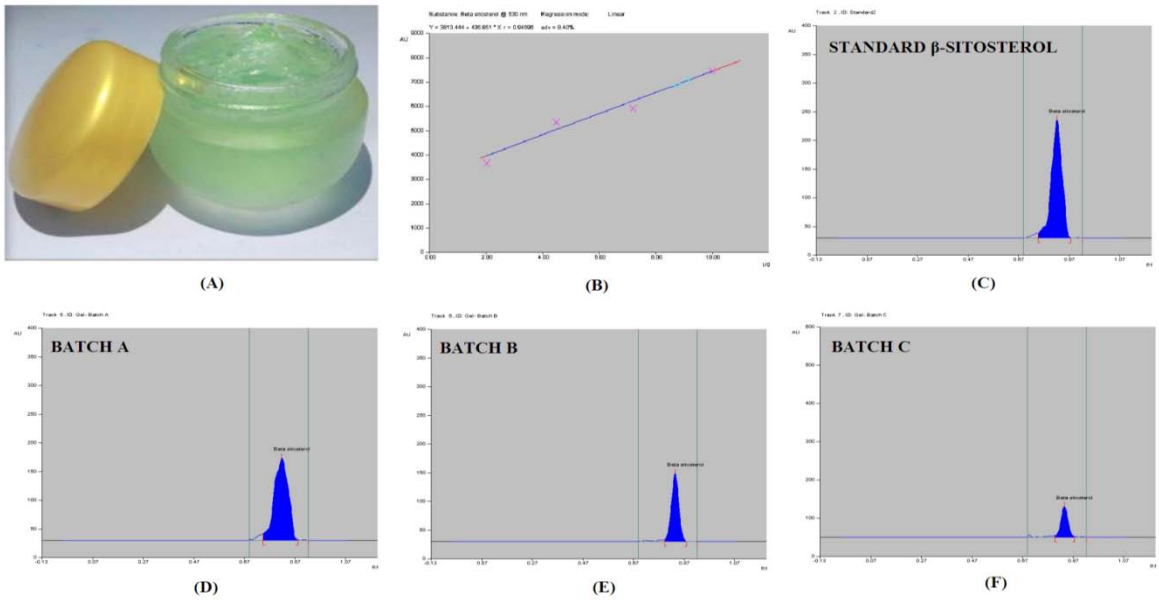

Fig. 1: (a) β-sitosterol hair gel formulation; (b) Calibration curve of β-sitosterol in HPTLC; (c) HPTLC chromatogram of standard β-sitosterol; (d) HPTLC Chromatogram of Batch A; (e) HPTLC chromatogram of Batch B; (f) HPTLC chromatogram of Batch C

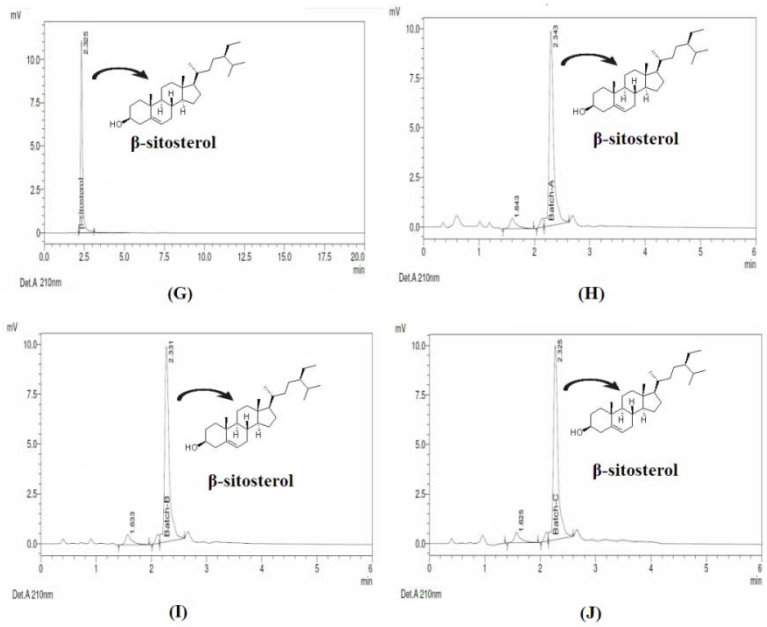

Fig. 2: (g) HPLC chromatogram of standard β-sitosterol; (h) HPLC chromatogram of Batch A; (i) HPLC chromatogram of Batch B; (j) HPLC chromatogram of batch C

Table 3: Evaluation parameters of β-sitosterol hair gel formulation

S. No.	Parameters	Results			(mean±SD)#
		Batch A	Batch B	Batch C	
1.	Net content (g)	99.52	99.82	99.61	99.65±0.12
2.	pH	6.65	6.67	6.61	6.64±0.03
3.	Viscosity	4736	4735	4735	4735.33±0.57
4.	% of drug HPTLC	97.50	97.11	96.66	97.09±0.34
5.	content HPLC	98.62	97.59	98.35	98.18±0.43
6.	Physical appearance	Light green, odourless, have a good consistency.	Light green, odourless, have a good consistency.	Light green, odourless, have a good consistency.	-
7.	Homogeneity	Homogenous, no aggregates found.	Homogenous, no aggregates found.	Homogenous, no aggregates found.	-
8.	Skin irritation test	Skin compatible, no irritation found.	Skin compatible, no irritation found.	Skin compatible, no irritation found.	-
9.	Spreadability (g. cm/s)	10.56	10.47	10.52	10.51±0.04

#SD: Standard deviation, where n (number of batches manufactured): 3

Statistical analysis is utilized to calculate IC_{50} values. Plotting the curve with the percentage of inhibition vs. the concentrations of the several investigations was used to get the IC_{50} values, which were then represented as mean±standard deviation. Each test was performed three times (n= 3) replication. The statistical analysis was performed utilizing Graph pad software version 6.0, and one-way ANOVA and the Bonferroni post-hoc test was performed. P was deemed to have a significant difference of less than 0.05. The IC_{50} value of the prepared gel formulation methanolic extract (group c) was 118.960±0.634 µg/ml. At the same time, 5α-reductase inhibition of beta-sitosterol (group b) and finasteride (positive control: group a) was shown to be 88.854±0.705 µg/ml and 224.372±3.103 ng/ml, respectively. A produced gel formulation of methanolic extract without β-sitosterol was used as a negative control (group d) test, and the result obtained 248.608±1.583 µg/ml. Further commercial formulation extracts using methanol (group e) and petroleum ether (group f) were employed, and IC_{50} values of 217.608±1.89 and 141.426±1.578 µg/ml were determined. Fig. 3 displays the 5α-reductase inhibition of test samples. Table 3 and fig. 4 compare the samples' comparative 5αR inhibitory potential.

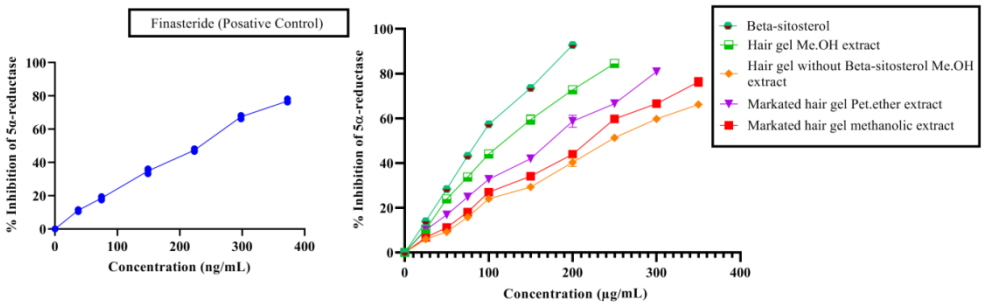

Fig. 3:5α-reductase inhibition of finasteride (positive control), β-sitosterol and different gel formulations

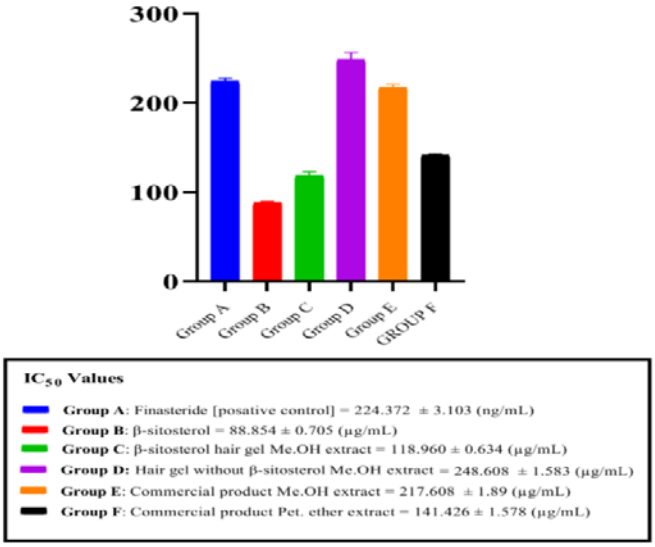

Fig. 4: Comparison study of 5αR inhibitory potential (Comparison study of 5α-reductase inhibition IC_{50} values of finasteride as positive control, β-sitosterol, prepared gel formulation in methanol extract and commercial gel formulation with methanol and petroleum ether extract)

Delivering an adequate amount of the active chemicals to the target location is essential for any delivery mechanism to be successful. The concentration of the drug at the target site depends on the solubility of the delivery system. When taken orally in solid dosage forms, β-sitosterol has a very low bioavailability. There are often several contributing variables, but a prevalent problem is poor absorption due to slow and incomplete drug dissolution in the lumen of the gastrointestinal tract. Increasing the rate and degree of drug solubilizing in intestinal fluids can promote increased bioavailability.

Topical β-sitosterol therapy has drawn much attention to overcome this restriction in managing AGA. In the present experiment, β-sitosterol transparent hair gel is prepared by using cremophor RH 40 as an emulsifier and solubilizer. The combination of cremophor RH 40 and propylene glycol increases the solubilizing property of β-sitosterol in gel formulation. Furthermore, the formulated gel was characterized using different physical and chemical parameters.

On application, the odourless gel with a light green colour was observed. Table 3 provides reports on the pH, viscosity, spreadability, physical appearance, homogeneity, and skin irritation tests. All the data is represented as the mean±standard deviation of three batches. According to a literature review, the average pH of male and female hair is 5.604±0.93 and 6.784±0.16, respectively [25]. The pH of the prepared gel, which is 6.64±0.03, is comparable to the values mentioned above and the skin's pH. A suitable viscosity is necessary for an appropriate formulation; a too-viscous gel may cause pourability issues, while a viscosity that is too low may result in the dispersed contents settling during storage. The gel had a viscosity of 4735.33±0.57 cps and seemed moderately viscous. Using a gel is most pleasant if the base spreads readily and exhibits the most slip and drag. The gel produced has a decent spreadability of 10.51±0.04 (g cm/sec). Gels with a high consistency index are often less spreadable. A good correlation was found between HPLC and HPTLC data of assay percentage. The percentage concentration of β-sitosterol in the gel formulation was 97.09±0.34 and 98.18±0.43%, respectively, according to HPLC and HPTLC results. The percentage concentration was found as per the label claim. An *in vitro* 5 alpha-reductase inhibition experiment was performed and compared to a commercial product to assess the therapeutic effect of the gel formulation. The research discovered that β-sitosterol gel has more significant 5αR inhibitory action than the commercially available commercial hair care product. Except for finasteride, which was utilized as a positive control, the β-sitosterol standard was shown to have the most robust 5αR inhibitor action. Cabeza1 *et al.* (2003) suggested that β-sitosterol inhibits 5α-reductase activity. IC50 data indicated that β-sitosterol is a less effective 5αR inhibitor than finasteride [26]. Zamani *et al.* (2021) suggest β-sitosterol and stigmasterol, two naturally occurring substances derived from *Serenoa repens*, are suggested to be safe and new AGA medications for hair regrowth since they may impede the normal function of 5αR type 1 [27]. According to Roy *et al.* (2008), *E. alba* has a large amount of β-sitosterol, which has been proven to be a potent 5αR inhibitor. *E. alba* can be used for the purpose above since 5α-reductase inhibition aids in treating androgenic alopecia [28]. It is widely known that β-sitosterol inhibits 5α-reductase in saw palmetto petroleum ether extract; this suggests that it may also be a potential mechanism of action for *E. alba* [29]. Thus β-sitosterol is useful for AGA by inhibiting 5αR enzyme activity.

Limitations of the protocol

We concluded that all reaction mixtures in our experiment contained similar reagents as the blank and the investigated test samples. When NADPH (22 µmol) was introduced to the blank solution (3 ml), the absorbance visible at time zero in the blank control group was detected. In order to make an accurate comparison at this time, a new NADPH-only group without any test samples was recommended. It would have shown the inherent absorption of NADPH. Therefore, it is advised that the experiment include a new group as the NADPH control group in the future for a relevant comparison.

Another factor to consider is the concentration of NADPH in the enzyme solution. This concentration may be measured earlier to increase precision and rule out any potential NADPH-related influence. Since the same amount of enzyme was used for all measurements in this experiment, the results were consistent, and the probability that the absorbance would change was almost negligible. However, future studies should still prioritize this aspect to allow for better group comparisons.

Standardization of β-sitosterol dosage in the formulation and time-dependent investigation, which would have given additional details regarding use, are potential future directions for this work. In addition, finding out how precisely the substances affect the enzyme, how well they interact with the androgen receptor that NADPH binds to, and how they block NADPH, whether by competitive inhibition or site-specific inhibition, more research on the potent substances of the study is necessary.

Whatever the reason, the manufacturing process of β-sitosterol gel and screening effectively evaluated the potential drug used to treat AGA.

CONCLUSION

Topical gels containing β-sitosterol can be successfully prepared using a simple, affordable production technique that required little facility. Gels prepared using carbopol 940 as a gelling agent found good in appearance, homogeneous, and easily spreadable. By comparing different critical parameters of those 3 batches, we established the manufacturing process method validation and process reproducibility. As a result of *in vitro* 5α-reductase inhibition, it was shown that β-sitosterol standard significantly inhibits 5αR enzyme and that β-sitosterol gel has greater 5αR inhibitory efficacy compared to currently available commercial products used for hair care. Thus, by preventing the over-expression of the 5αR enzyme, β-sitosterol gel may be effective in the treatment of androgenic alopecia.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors claim to have no conflicts of interest.

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