RESEARCH ARTICLE

Exploring the Potential of *Eclipta alba*: A Promising Approach for Hair Treatment Management through 5-Alpha Reductase Inhibition

Arpan Chakraborty[1*], Arka Bhattacharjee[1], Baishakhi Mondal[1], Manas Chakraborty[2], Goutam Mukhopadhyay[3], Maitrish Ghosh[4], Alpana Majumder[5]

[1]Department of Pharmaceutical Technology, Maulana Abul Kalam Azad University of Technology, Nadia, West Bengal, India.
[2]Department of Pharmaceutical Technology, Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences, Howrah, West Bengal, India
[3]Department of Pharmaceutical Technology, BCDA College of Pharmacy and Technology, Kolkata, West Bengal, India.
[4]Department of Pharmaceutical Technology, NSHM College of Pharmaceutical Technology, Kolkata, West Bengal, India.
[5]Department of Kayachikitsa, Institute of Post Graduate Ayurvedic Education and Research, Kolkata, West Bengal, India.

Received: 16th February, 2023; Revised: 22th May, 2023; Accepted: 10th June, 2023; Available Online: 25th June, 2023

ABSTRACT

A class of medications known as 5 alpha reductase (5α-reductase or 5αR) inhibitors is used to treat male pattern hair loss and benign prostatic hyperplasia. This study shows that *Eclipta alba* has 5αR inhibitory action that is helpful in the treatment of androgenic diseases. For 5αR enzyme inhibition evaluation, *E. alba* was extracted using methanol and petroleum ether. Further phytochemical screening can be done. Phytosterols test negatively found in methanol extract during phytochemical screening but positive in petroleum ether extract of *E. alba*. HPTLC data of different extracts was performed based on the phytochemical screening found. According to the HPTLC analysis, petroleum ether extract of *E. alba* contained 0.11% of β-sitosterol, while the methanolic extract had a higher concentration of 4.75%. The inhibitory activity of these plant extracts against 5αR was examined in comparison to the commonly used 5αR inhibitor, finasteride. IC_{50} measurements for petroleum ether extract of *E. alba* and β-sitosterol (a chemical biomarker derived from the plant material) were established as 150.76 ± 4.56 and 77.09 ± 3.07 µg/mL, correspondingly. These results indicate their potential as compelling contenders worthy of deeper exploration regarding their anti-androgenic properties. The notable abundance of β-sitosterol in the petroleum ether extract of *E. alba* enhances its potential for significant biological activity, particularly in terms of inhibiting the 5αR enzyme.

Keywords: 5α-reductase, Hair loss, NADPH, *Eclipta alba*, β-sitosterol, HPTLC.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.2.07

How to cite this article: Chakraborty A, Bhattacharjee A, Mondal B, Chakraborty M, Mukhopadhyay G, Ghosh M, Majumder A. Exploring the Potential of *Eclipta alba*: A Promising Approach for Hair Treatment Management through 5-Alpha Reductase Inhibition. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):283-288.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Androgens, through their interaction with the androgen receptor, exert cellular effects that regulate crucial processes related to the prostate's natural growth, organizational structure, and normal functioning. Moreover, substantial evidence supports the claim that androgens play a contributory role in the development of prostate cancer and androgenic alopecia.[1] Located within the nuclear membrane, the steroid 5α-reductase or 5αR enzyme acts as a vital catalyst for the conversion of testosterone (T) to dihydrotestosterone (DHT). Two different types of enzymes, designated type 1 (5αR1) and type 2 (5αR2), are responsible for this mechanism.[2] The coenzyme NADPH helps turn into DHT.[3] The presence of the 5αR and its potent byproduct DHT exerts a significant influence on a variety of human diseases, including male and female pattern baldness, benign prostatic hyperplasia (BPH), prostate cancer, acne, hair loss, and hirsutism.[4] Recognizing the vital role of DHT in prostate development and androgenic alopecia, therapeutic inhibitors targeting the 5αR enzyme have been developed.[5] The study's aim was to address the limitations of the market's current leading 5α-reductase inhibitors, finasteride and epristeride, which are known to have undesirable side effects. To achieve this, we investigated the anti-androgenic potential of *Eclipta alba* with the goal of discovering a natural alternative for inhibiting 5αR. Our

**Author for Correspondence: arpan.soleria@gmail.com*

research focused on identifying potential 5αR inhibitors that could potentially be beneficial in the field.

Within the traditional Indian medical system of Ayurveda, various herbs have been listed for their purported benefits in treating hair loss. Among these is *E. alba*, a plant belonging to the Asteraceae family. *E. alba* has gained recognition for its potential to promote hair development. This herb is a small, highly branched annual plant with white flower heads, typically found in humid regions of India. Interestingly, it has the remarkable ability to grow up to a height of 600 feet shortly after the onset of the rainy season. *E. alba* has a long-standing reputation as a natural aid in enhancing hair growth and improving hair luster.[6] *E. alba* finds application in various activities, such as the treatment of jaundice and its role as an anti-hepatotoxic. In enlarged liver and spleen cases, plant juice is employed as a tonic and deobstruent. Extracts derived from the roots are utilized topically as antiseptics for ulcers and lesions in cattle. Additionally, they serve as emetics and purgatives.[7]

Studies have employed a variety of models to assess the ability to promote hair growth of EA for the treatment of alopecia. Methanol (MeoH) and petroleum ether (PE or Pet. Ether) extracts of EA has been tested for its capacity to promote hair growth in albino rats. The extracts significantly reduced the time required for hair development, cutting it in half compared to untreated control mice.[8] Begum *et al.* (2015) used naked mice to determine the effectiveness of EA in increasing hair development. PE extract and various solvent fractions of EA were topically administered on the backs of naked mice. Therapy with petroleum ether (PE) extract resulted in remarkable follicular hypertrophy and keratinocytes in basal epidermal and matrix cells. To investigate hair loss caused by abnormal keratinization, Begum *et al.* 2014 performed a study on nude mouse models. The study provided additional evidence supporting the effectiveness of EA in promoting hair growth. The study demonstrated that topical application of the MeoH extract of EA significantly impacted hair development in mouse models. Post-therapy, an increase in hair follicles was observed, indicating EA as effective hair growth stimulator.[10] Limited scientific research has been conducted to explore the folklore belief that EA promotes hair growth as a 5αR inhibitor for treating alopecia.

The Ayurvedic book Bhavaprakash mentions the use of medication to treat "Indralupta," which refers to hair loss. The Sanskrit name for the plant EA, "Bhringraj" or "Keshraja," refers to using the herb to improve hair condition.[11]

This research aims to elucidate the potential mechanism of EA, a plant known for its utilization of herbal remedies and cosmetics. The plant has been traditionally employed to address hair loss, stimulate hair growth, nourish hair, and as an ingredient in natural cosmetic products. Using a biochemical approach this study will help to explain the 5αR inhibitory activity of these plant extracts. The comparison of different extracts of EA with finasteride (potent 5αR inhibitor) in 5αR inhibition helps to understand the most biologically active extract among all. Further chemical standardization of phytochemicals leads to more potential biologically active extracts. Therefore, the identification of the most promising extract from EA holds potential for its application in cosmetics and pharmaceuticals aimed at treating alopecia. This research may have significant clinical implications for addressing the issue of hair loss. Based on our current understanding, there is a lack of scientific research investigating the folklore belief that EA promotes hair development through its role as a 5αR inhibitor. However, it is worth noting that the historical utilization of the whole plant has long been associated with the enhancement of hair growth.

MATERIAL AND METHODS

Plant Material Collection and Identification

In the months of January, the whole EA plant was collected in Kolkata (WB). The entire plant, EA, was characterized and authenticated. The plant material was finely pulverized, air dried, and passed through filter number 10.

Preparation of Extracts

In two conical flask of 1000 mL, each containing 100 g of crushed EA (whole plant) material, were treated with 500 mL of MeoH and 500 mL of PE. The mixture was kept in an airtight container at 25 to 30°C for 72 hours. After filtration using regular filter paper, the filtrate was stored in a 1000 mL beaker. Subsequently, the filtrate was concentrated using a rotary vacuum evaporator under ambient conditions with a temperature range of 40 to 45°C.

Drugs and Chemicals

NADPH tetra-sodium salt was purchased from Sisco Research Laboratory (SRL). Sigma-Aldrich was used to purchase finasteride, beta-sitosterol, tris-HCl buffer, and testosterone. Sucrose, sodium phosphate, and ethylenediaminetetraacetic acid (EDTA) were bought from Merck in Mumbai. Methanol, ethanol (95%), toluene, ethyl acetate, and petroleum ether (analytical grade) were bought from Merck in Mumbai. Analytical-grade compounds were employed for all other substances in the investigation.

Qualitative Evaluation of *E. alba* Extracts

The extracts underwent various qualitative analyses to identify the presence of plant components, including saponins, phytosterols, alkaloids, glycosides, carbohydrates, flavonoids, proteins, tannins, and phenolic compounds. The testing protocol used in this study was in accordance with the methodology established by Singh R. and Kori ML in 2022.[12]

Standardization of β-sitosterol in *E. alba* Extracts using HPTLC

The approach described by Bhattacharjee *et al.* (2017) has been modified in some respects.[13] CAMAG HPTLC system includes the scanning densitometer, LINOMAT V automated sample applicator, and automatic development chamber, with WINCATS software. The image documentation tools CAMAG Reprostar 3 and CAMAG Scanner 3 were utilized. The experimental approach was detailed in Table 1 below.

Table 1: HPTLC experimental approach

Experimental procedure	*Details*
Sample preparation	10 mg/mL of the EA-lyophilized MeoH extract and 5 mg/mL of the PE extract dissolve in the parent solvent
Standard preparation	β-sitosterol (0.5 mg/mL) dissolved in MeoH
Application of sample	Applied 8 and 10 µL for both MeoH and PE extracts
Application of standard	2–10 µL
Sample application	100 µL syringe from Hamilton (Switzerland)
Stationary phase	Silica gel GF_{254} TLC plate
Mobile phase	Toluene:methanol (80:20 v/v)
Development	Drying with a hand dryer after 20 minutes of mobile phase development
Spraying reagent	Anisaldehyde-sulfuric acid
Heating	Hot air oven at 110°C for 5 minutes.
Observation	Discernible colored bands were observed at 530 nm

Enzyme Inhibition Assay

The approach provided by Nahata & Dixit (2013) underwent a number of modifications.[14] The procedure's specifics are mentioned below:

Methods of preparation 5αR solution

A local abattoir provided the prostate of an adult male goat. Prostate tissue, weighing 420 mg, was minced into small pieces. The minced tissue was then mixed with a medium containing 20 mM sodium phosphate (pH 6.5), 0.32 M sucrose, and 1-mM EDTA. Subsequently, it was centrifuged for 15 minutes at 4000 rpm (716 g) to separate the homogenate. The resulting supernatant, which contained enzymes, was collected for further experiments. The Bradford technique was employed to measure the protein content of the supernatant. A stock solution of bovine serum albumin (BSA) with a concentration of 1-mg/mL was diluted to create solutions with concentrations of 0.5, 0.25, and 0.125 mg/mL. Each BSA solution (5 µL) was dispensed into separate wells on a microplate. Each well, 200 µL of Bradford reagent was added and thoroughly mixed. The absorbance of the BSA solution was measured at 592 nm using a spectrophotometer. A standard curve was constructed by utilizing absorbance values and known concentrations, facilitating the quantification of protein concentration in unknown samples. In a microwell plate, a solution of enzyme homogenate (5 µL) was added for the enzyme assay. Next, 200 µL of bradford reagent was included in the test solution. The absorbance at 592 nm was measured, and the protein concentration was determined using the BSA standard curve. The isolated protein exhibited a concentration of 0.685 mg/mL. To perform the enzyme assay, the solution was diluted to a final 100 µg/mL concentration using tissue homogenization media.

Preparation of NADPH standard curve

With concentrations ranging from 1 to 20 µg/mL, a calibration curve for NADPH in methanol was created at 340 nm using the formula $y = 0.0244x + 0.00056$ ($r^2 = 0.9978$).

Preparation of test solutions

Testosterone solution (75 µM) in MeOH, NADPH solution (22 µM) prepared in MeOH, extract solution (1-mg/mL) in the parent solvent, and Tris-HCl buffer (0.5 M) in distilled water.

Preparation of finasteride solution

A stock solution of finasteride was prepared at a concentration of 1-µM. Subsequent dilutions were carried out to determine IC_{50} value.

Preparation of plant extracts and β-sitosterol

A stock solution of *E. alba*'s methanol and petroleum ether extract was prepared with a 5 mg/mL concentration in the parent solvent. Additionally, a 1-mg/mL beta-sitosterol solution was prepared in methanol. After vortexing, sonication, and passing through a 0.45 µ syringe filter, the substance was diluted within the range of 25 to 300 µg/mL.

Assay procedure for the inhibition of 5αR in E. alba and β-sitosterol

Following the procedure outlined by Nahata & Dixit (2013),[14] experiments were conducted to assess the inhibition of 5αR. Information regarding the experimental procedure is provided in Figure 1. The reaction mixtures employed are described completely in Table 2. Extracts underwent a blank test to evaluate their antioxidant activity. The conversion of NADPH to NADP was measured, impacting their initial ability to inhibit the 5αR enzyme. The degree of 5αR inhibition determined the specific inhibitory activity of each extract. In the experiment, it was assumed that 2 mL of with a concentration of 75 µM was converted to DHT with the help of 3 mL of NADPH having a concentration of 22 µM.

The absorbance of the blank is subtracted to determine the test's net absorbance at 340 nm. The NADPH level was determined using the NADPH calibration curve.

Table 2: Enzyme, substrate and coenzyme mixture

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

Table 3: Phytochemical screening of *Eclipta alba* extracts

Test		*Methanol extract*	*Petroleum ether extract*
Phytosterols	Libermann-Burchard Test	+	+
	Liebermann's reaction	-	+
Glycosides	Keller-Killiani Test	+	-
	Bontragger's Test	+	-
Flavonoids	Shinoda test	+	-
	Ferric chloride test	+	-
Alkaloids	Mayer's test	+	-
	Dragendroff's test	+	-
Protein	Millon's reaction	+	-
	Xanthoproteic reaction	+	-
Carbohydrates	Molisch's test	+	-
	Fehling's test	+	-
	Barfoed reagent test	+	-
Tannins and Phenolic Compounds	Ferric chloride test	+	+
	Lead acetate solution	+	+
Saponins	Foam test with water	+	-
	Foam test with sodium carbonate	+	-

%inhibition = (100 – [(54.78- NADPH content determined by test solution's net absorbance)/54.78) × 100]

The percentage inhibition was measured at different sample concentrations to determine the IC_{50} value of the test extracts.

RESULTS

The percentage yield of extraction was 2.6 and 1.10% w/w in MeoH and PE extracts, respectively.

The MeoH extract gives positive tests for flavonoids, alkaloids, proteins, carbohydrates, tannins, glycosides, and saponins. It gives a negative result for phytosterols. PE extract gives positive tests for phytosterols and tannins, as shown in Table 3.

β-sitosterol content in *E. alba* MeoH and PE extracts was found to be 0.11 and 4.75%, respectively. This determination was made using a calibration curve (Y = 3.7646x + 101.12, r^2 = 0.9909). β-sitosterol (standard) R_f value was observed to be 0.66. The R_f values of the standard and sample were analysed to verify the specificity. Figure 2 shows the HPTLC chromatogram of different extract of *E. alba* and β-sitosterol at 530 nm.

The statistical methodology was used for determining the IC_{50} for an investigation into enzyme inhibition. In order to obtain IC_{50} values, a graph was created by comparing the inhibition percentage with the concentrations in various tests. The mean ± standard error mean of the IC_{50} data was then demonstrated. GraphPad Prism version 6.0 was used to conduct the statistical analysis. It involved the implementation of a one-way ANOVA followed by the Bonferroni post hoc test. A significance level of $p < 0.05$ was considered when comparing

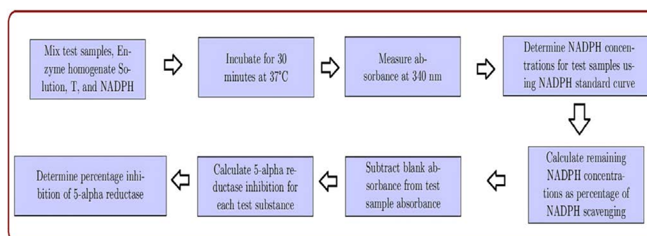

Figure 1: Schematic diagram of the assay procedure for 5αR inhibition

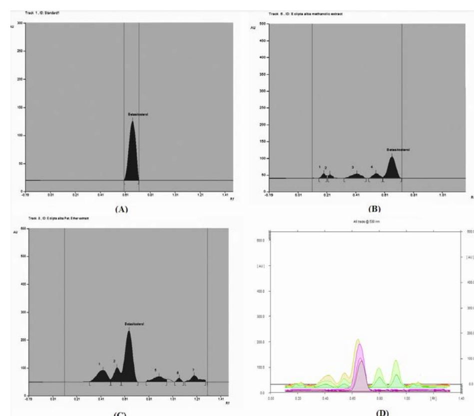

Figure 2: HPTLC chromatogram of (A) Standard β-sitosterol; (B) EA MeoH extract; (C) EA PE extract; (D) EA extracts and standard β-sitosterol at 530 nm.

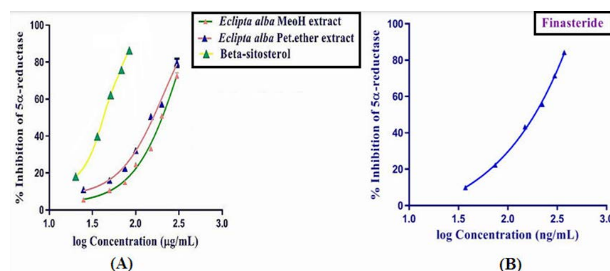

Figure 3: 5αR inhibition of (A) of β-sitosterol, EA methanol (MeoH) and petroleum ether (Pet. ether) extracts and (B) finasteride

the results to the reference standard, indicating a statistically significant difference. The IC_{50} values for the MeoH and PE extracts of *E. alba* were determined to be 215.87 ± 2.92 and 150.76 ± 4.56 µg/mL, respectively. In contrast, the inhibition of 5αR by β-sitosterol and finasteride was observed to be 77.09 ± 3.07 and 0.246 ± 0.02 µg/mL, respectively. The inhibition of 5αR by the EA extract, β-sitosterol, and finasteride is illustrated in Figure 3. Figure 4 presents a comparison of the IC_{50} values for different substances.

DISCUSSION

Androgenic alopecia affects both men and women.[15,16] The enzyme 5αR, located within the nuclear membrane, converts T into DHT.[3] This conversion process relies on NADPH.[17] The activity of 5αR and the effects of DHT influence various human diseases, such as BPH, alopecia, and male pattern hair loss in both males and females.[4]

EA has historically been used to prevent balding and maintain black hair. It is often referred to as the

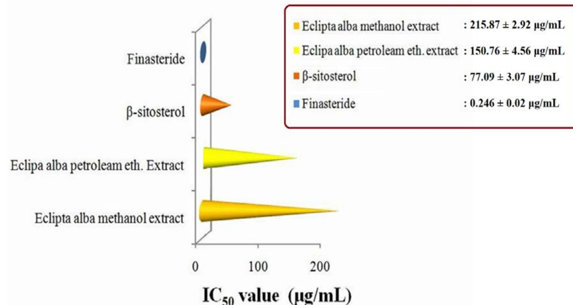

Figure 4: Comparison study of 5αR inhibitory potential

"king of the hair".[18] Phytosterols test negatively found in MeoH extract during phytochemical screening but positive in PE extract of *E. alba*. The HPTLC analysis revealed that the PE extract of EA contained approximately 0.11% of β-sitosterol, while the MeoH extract had a higher percentage content of about 4.75%. β-sitosterol is abundantly present in the PE extract of *E. alba* compared to the MeoH extract. Notably, β-sitosterol is one of the phytosterols found in high quantities within the PE extract of EA.[10]

To evaluate the therapeutic benefits of several EA extracts an *in-vitro* 5αR inhibitory experiment was conducted. The IC_{50} value of PE extract of EA was more potent than that of MeoH, which might be due to non-polar components contained in PE extraction medium being more potently active to inhibit 5αR. In the research presented here, a previously described non-polar molecule called β-sitosterol was identified and quantified in large amounts in the PE extract of the plant EA.[11] The β-sitosterol standard was found to have the most potent 5αR inhibitor activity, except for finasteride, was used as a positive control, as indicated in Figures 3, and 4. Due to the presence of the highest concentration of β-sitosterol, it may be able to associate the maximum activity of *E. alba*'s PE extract with its 5αR inhibitor activity. Cabeza *et al.* (2003) state that β-sitosterol inhibits 5αR enzyme activity. According to IC_{50} data, it has been observed that β-sitosterol exhibits lower potency as a 5αR inhibitor than finasteride.[19] Stigmasterol and β-sitosterol, two naturally occurring compounds derived from *Serenoa repens*, have emerged as safe and promising therapeutic options for hair regeneration in cases of androgenetic alopecia. These compounds have shown the potential to disrupt the normal functioning of 5αR type 1.[20] The inhibitory effect of β-sitosterol in saw palmetto PE extract on 5αR is widely recognized, suggesting that a similar mode of action may exist for EA extract. This raises the intriguing possibility that EA extract may also exert its effects through the inhibition of 5αR, like β-sitosterol.[10] The extract of EA shows promising potential in the treatment of alopecia by effectively inhibiting the activity of the 5αR enzyme. Considering the known benefits of 5αR inhibition in the management of androgenic alopecia, EA can be considered a valuable option for addressing this condition.

Research Limitations

The experiment involved adding extracts to reaction mixtures to conclude. It is suggested to include a separate NADPH-only group for accurate assessment. Testing NADPH concentration beforehand can enhance accuracy. The same amount of enzyme produced consistent results. Time-dependent investigation and understanding of drug-enzyme interaction mechanisms are recommended for future research.

Key elements are highlighted:

- NADPH-only group: Include a dedicated control group for NADPH to facilitate effective comparisons.
- NADPH concentration testing: Test NADPH concentration beforehand to enhance accuracy and eliminate potential interference.
- Consistent enzyme quantity: Using the same enzyme quantity yielded consistent results.
- Time-dependent investigation: Conduct investigations over time using various fractions for more information.

CONCLUSION

Medications called as 5αR inhibitors are employed to cure benign prostatic hyperplasia and male pattern hair loss. This study demonstrates that EA inhibits the activity of the enzyme 5αR, which is useful in the treatment of androgenic disorders. Two distinct polarity extraction mediums, MeoH and PE, were used to extract EA to assess the inhibition of the 5αR enzyme. Phytochemical analysis detected phytosterols in PE extract of EA but not in MeoH extract. Based on the results of the phytochemical screening, HPTLC data of two distinct extracts were done. HPTLC analysis revealed that the PE and MeoH extracts of EA used in the enzyme inhibition study contained 0.11 and 4.75% of β-sitosterol as a percentage. It was discovered that EA petroleum ether extract and β-sitosterol (a chemical marker of the plant material) had IC_{50} values of 150.76 ± 4.56 and 77.09 ± 3.07 g/mL, respectively, suggesting that they might be interesting candidates for further research into their anti-androgenic properties. The PE extract of EA has the highest concentration of β-sitosterol, which increases its potential biological activity as a 5αR enzyme inhibitor. Despite the fact that using the whole plant has historically been recommended for promoting hair development.

REFERENCES

1. Lieberman R. Evolving strategies for prostate cancer chemoprevention trials. World Journal of Urology. 2003;21(1):3–8. Available from: https://doi.org/10.1007/s00345-003-0317-4
2. Wright AS, Douglas RC, Thomas L, Lazier CB, Rittmaster RS. Androgen-induced regrowth in the castrated rat ventral prostate: role of 5α-reductase. Endocrinology. 1999;140(10):4509–4515. Available from: https://doi.org/10.1210/endo.140.10.7039
3. Levy ML, Brandt MS, Sheedy KM, Holt DA, Heaslip JI, Trill JJ, et al. Cloning, expression and functional characterization of type 1 and type 2 steroid 5α-reductases from Cynomolgus monkey: Comparisons with human and rat isoenzymes. The Journal of Steroid Biochemistry and Molecular Biology. 1995;52(4):307–319. Available from: https://doi.org/10.1016/0960-0760(94)00183-m
4. Guarna A, Poletti A, Catrambone F, Danza G, Marrucci A, Serio M, et al. Synthesis of a chemiluminescent probe useful for the purification of steroid 5α-reductase. Bioorganic & Medicinal Chemistry Letters. 1996;6(16):1997–2002. Available from:

https://doi.org/10.1016/0960-894x(96)00360-5
5. Russell DH, Wilson JD. Steroid 5α-reductase: two genes/two enzymes. Annual Review of Biochemistry. 1994;63(1):25–61. Available from: https://doi.org/10.1146/annurev.bi.63.070194.000325
6. The wealth of India. National Institute of Scientific Communication and Research, New Delhi 1992:612.
7. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. Publication and Information Directorate (CSIR), New Delhi. 1986:85.
8. Begum S, Lee M, Gu L, Hossain J, Sung CO. Exogenous stimulation with *Eclipta alba* promotes hair matrix keratinocyte proliferation and downregulates TGF-β1 expression in nude mice. International Journal of Molecular Medicine. 2014;35(2):496–502. Available from: https://doi.org/10.3892/ijmm.2014.2022
9. Begum S, Lee M, Gu L, Hossain J, Kim HJ, Sung CO. Comparative hair restorer efficacy of medicinal herb on nude (Foxn1nu) mice. BioMed Research International. 2014;2014:1–9. Available from: https://doi.org/10.1155/2014/319795
10. Roy RR, Thakur M, Dixit VK. Hair growth promoting activity of *Eclipta alba* in male albino rats. Archives of Dermatological Research. 2008;300(7):357–364. Available from: https://doi.org/10.1007/s00403-008-0860-3
11. Chunekar KC, Hota NP. Plants of Bhavprakash. Vol. 1. National Academy of Ayurveda, New Delhi. 1986:612.
12. Kori ML, Singh R. Phytochemical studies of *Tephrosia purpurea* Linn. and *Martynia annua* Linn. extracts for identification of chemical constituents. International Journal of Pharmaceutical Sciences and Drug Research. 2020;14(2):226–232. Available from: https://doi.org/10.25004/ijpsdr.2022.140211
13. Bhattacharjee A, Chakraborty AK, Dutta D, Sau A, Chakraborty S, Samanta N, Biswas PS, Mukhopadhyay G. Standardization of *Eclipta alba* by HPTLC, HPLC and AAS. Pharmaceutica Analytica Acta. 2017;8(4). Available from: https://doi.org/10.4172/2153-2435.1000541
14. Nahata A, Dixit VK. Evaluation of 5α-reductase inhibitory activity of certain herbs useful as antiandrogens. Andrologia. 2013;46(6):592–601. Available from: https://doi.org/10.1111/and.12115
15. Sperling LC. Hair and systematic disease. Dermatologic Clinics. 2001;19(4):711–726. Available from: https://doi.org/10.1016/s0733-8635(05)70310-7
16. Kumar N, Chaiyasut C. Hair growth promoting activity of *Carthamus tinctorius* florets extract-loaded nanostructured lipid carriers. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;7(2):252–257.
17. Nutbrown M, Hull SL, Cunliffe WJ, Randall VA. Abnormalities in the ultrastructure of melanocytes and the outer root sheath of clinically normal hair follicles from alopecia areata scalps. Journal of Investigative Dermatology. 1995;104:12S-13S. Available from: https://doi.org/10.1038/jid.1995.34
18. Caldecott T. Ayurveda: The Divine Science of Life. Elsevier Health Sciences. 2006.
19. Cabeza MA, Bratoeff E, Heuze I, Ramírez E, Sánchez M, Flores EF. Effect of β-sitosterol as inhibitor of 5α-reductase in hamster prostate. Proceedings of the Western Pharmacology Society. 2003;46:153–155.
20. Zamani P, Mokhtari O, Dehghanian F. Identification of beta-sitosterol and stigmasterol as possible inhibitors of 5 alpha-reductase 1: an in-silico study. Precision Medicine and Clinical Omics. 2022;1(1):1-8. Available from: https://doi.org/10.5812/pmco.121545