

Original Research Article

Standardization of Amarantha hair oil with special reference to identification of β -sitosterol as major component having 5α -reductase inhibitory activity/hair growth promoting activity

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ABSTRACT

Background: Androgenetic alopecia is a common form of hair loss in both men and women. Synthetic drugs like minoxidil and finasteride are available treatment for androgenetic alopecia, however, use of these drugs on long term basis may cause many side effects. Hence there was a need to have an effective and safe alternative option. Ari Healthcare Pvt. Ltd. has developed Amarantha hair oil (herbal hair oil) to be used for dandruff, hair fall and premature greying of hair. The aim was to standardize Amarantha hair oil with special reference to identification of β -sitosterol as major component having 5α -reductase inhibitory activity/hair growth promoting activity.

Methods: Amarantha hair oil was prepared by using various herbal ingredients which contains β -sitosterol as active marker compound. Amarantha hair oil was evaluated for appearance, weight per ml, refractive index, acid value, saponification value, peroxide value, TLC Identification of β -sitosterol, assay of β -sitosterol content using high performance thin layer chromatography (HPTLC) and microbiological tests. Also accelerated stability study was performed.

Results: All the parameters tested were within normal limits. Amarantha hair oil was stable in accelerated stability study. We found that almost all the ingredients of formulation contain β -sitosterol when analysed using HPTLC. Amarantha hair oil contains 0.52% of β -sitosterol.

Conclusions: All the ingredients and Amarantha hair oil contain β -sitosterol that helps in promotion of hair growth.

Keywords: β -sitosterol, Amarantha hair oil, 5α -reductase inhibitory activity, Hair growth promoting, DHT, HPTLC

INTRODUCTION

Millions of men and women are affected by hair loss due to androgenic alopecia or common male pattern baldness. Male pattern baldness is hereditary and may be associated with the male sex hormones. Around two thirds of men are affected at the age of 35 and around 85% men by the age of 50.¹

Various causes of hair loss include high levels of dihydrotestosterone (DHT, derivative of testosterone, a

male hormone), poor blood flow, sebum emotional strains, stresses and nervous disorders, aging, infections, hormonal imbalance, polluted environment, toxic substances, injury and impairment, radiation.² But DHT is considered to be the main cause of hair loss in men and women.

Two drugs are mainly available for the treatment of androgenic alopecia. One is a potassium channel opener called minoxidil and the other drug that inhibits DHT synthesis is finasteride. Several side effects are associated

with these drugs when consumed daily for longer periods.^{3,4} Hence there is an immense need to have safe and effective treatment option for hair loss.

In Ayurveda, oil prepared using herbs such as *Trigonella foenum-graecum*, *Emblica officinalis*, *Cuscuta reflexa*, *Eclipta alba*, *Glycyrrhiza glabra*, *Ocimum sanctum*, *Terminalia bellerica*, *Hibiscus rosasinesis*, *Lawsonia inermis*, *Ficus bengalensis*, *Sesamum indicum*, *Cocos nucifera*, *Vitex nigrundo* have been used to treat hair loss, dandruff and premature greying. It has been found in scientific studies that many such herbs contain a chemical marker compound known as β -sitosterol. It is known to inhibit 5-alpha-reductase activity thereby implying hair growth promoting capacity.⁵ A randomized, double-blind, placebo-controlled trial to determine effectiveness of botanically derived inhibitor (β -sitosterol) of 5-alpha-reductase in the treatment of androgenic alopecia showed highly positive response to the treatment.^{6,7}

One study results of pumpkin seed oil which contains β -sitosterol when used as topical application showed hair growth promotion in the treatment of male pattern alopecia.⁸ In another study it is demonstrated that topical application of red ginseng oil promoted hair regeneration in the testosterone-induced delay of anagen entry in C57BL/6 mice, possibly contributed by major components of red ginseng oil including linoleic acid and β -sitosterol. Treatment of red ginseng oil, linoleic acid or β -sitosterol resulted in premature telogen-to-anagen transition and increased the A/T ratio in testosterone-treated mice.⁹

These research studies suggest that topical formulations which contain β -sitosterol as major component may be used as a potent therapeutic agent for treatment of androgenic alopecia. Keeping in mind the need of effective and safe option for treatment of hair loss, dandruff and premature greying, Ari Healthcare Pvt. Ltd. has developed Amarantha hair oil containing 18 standardized herbs and 2 oils. The ingredients of the formulation are reported to have activity on hair fall, dandruff and premature greying of hair.¹⁰⁻³⁰

The present study was carried out screen and confirm the presence of β -sitosterol in each and every ingredient of Amarantha hair oil and also in final formulation using HPTLC.

METHODS

Materials

Raw materials such as bibhitaka (*T. bellerica*), amalaki (*E. officinalis*), methi (*T. foenum-graecum*), tulsi (*O. sanctum*), aamrawali (*C. reflexa*), bhringraj (*E. alba*), yashtimadhu (*G. glabra*), japa (*H. rosasinesis*), nirgundi (*V. nigrundo*), gambhari (*Gmelina arborea*), madayanti (*L. inermis*), priyala (*Buchanania latifolia*), jatamansi

(*Nordostachys jatamansi*), palash (*Butea frondosa*), sahachara (*Barleria cristata*), kusumbha (*Carthamus tinctorius*), kachura (*Curcuma zedaria*), vata (*Ficus bengalensis*), til tel and coconut oil, all these herbs and oils were ordered from Amrutlal A company, Mumbai. These raw materials were screened for identity, purity and strength. Also these raw materials were screened for presence of β -sitosterol using HPTLC.

Reference standard β -sitosterol (>95%) was purchased from Natural Remedies, Bangalore. All chemicals were of analytical grade or HPLC grade purchased from Merck Chemicals, India. Stationary phase was pre-coated silica gel aluminium plate 60 F254 was obtained from Merck, Germany.

Oil formulation

The various ingredients used in the formulation of herbal oil are presented in Table 1. Accurately weighed all the dried herbs were grinded and taken in a stainless steel container. Purified water added into above materials and heated, till 1/4th volume of decoction remains. The decoction was cooled and filtered. To the decoction coconut oil and sesame oil were added and heated till all the water got evaporated. Prepared oil was cooled and filtered through muslin cloth. Finally it was packed in amber colored bottle.

Evaluation of oil

The oil was evaluated for appearance, weight per ml, refractive index, acid value, saponification value, peroxide value, TLC identification of β -sitosterol, assay (β -sitosterol content by HPTLC) and microbiological tests. The results are shown in Table 2. Accelerated stability study was also performed to check the changes during storage that are likely to influence quality, safety and efficacy. The results are presented in Table 3.

Appearance

The appearance of the oil was judged by its color and odour.

Weight per ml

Pycnometer was cleaned and rinsed with acetone. It was dried and weight of empty pycnometer was noted down. Then it was filled with the water and weight was noted. The procedure was repeated using oil in place of water. Weight per milliliter was determined by dividing the weight in air, expressed in g, of the quantity of liquid which filled the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Table 1: Composition of Amarantha hair oil.

Sr. No.	Ingredients	Plant part used	Botanical name	Quantity/100 ml
1.	Bibhitaka	Fruit	<i>T. belerica</i>	1.19 g
2.	Amalaki	Fruit	<i>E. officinalis</i>	2.38 g
3.	Methi	Seed	<i>T. foenum-graecum</i>	1.19 g
4.	Tulsi	Leaf	<i>O. sanctum</i>	1.19 g
5.	Aamrawali	Whole plant	<i>C. reflexa</i>	2.38 g
6.	Bhringraj	Whole plant	<i>E. alba</i>	2.38 g
7.	Yashtimadhu	Stem/root	<i>G. glabra</i>	1.19 g
8.	Japa	Flower	<i>H. rosasinesis</i>	1.19 g
9.	Nirgundi	Leaf	<i>V. nirgundo</i>	1.19 g
10.	Gambhari	Root	<i>G. arborea</i>	1.19 g
11.	Madayanti	Leaf	<i>L. inermis</i>	1.2 g
12.	Priyala	Seed	<i>B. latifolia</i>	1.19 g
13.	Jatamansi	Rhizome	<i>N. jatamansi</i>	1.19 g
14.	Palash	Flower	<i>B. frondosa</i>	1.19 g
15.	Sahachara	Whole plant	<i>B. cristata</i>	1.19 g
16.	Kusumbha	Flower	<i>C. tinctorius</i>	1.19 g
17.	Kachura	Rhizome	<i>C. zedoria</i>	1.19 g
18.	Vata	Hanging root	<i>F. bengalensis</i>	1.19 g
19.	Til tel	Seed oil	<i>Sesamum indicum</i>	60 ml
20.	Coconut oil	Fruit oil	<i>Cocos nucifera</i>	40 ml

Table 2: Results of various testing parameters on Amarantha hair oil.

Sr. No.	Parameters	Results
1.	Appearance	Yellowish brown coloured oil
2.	Weight per ml	0.915
3.	Refractive index	1.4638
4.	Acid value	1.28
5.	Saponification value	217.63
6.	Peroxide value	2.42
7.	Identification by TLC	Positive for β -sitosterol
8.	Assay (β -sitosterol content by HPTLC) (in %)	0.52
Microbiological test		
9.	Total microbial plate count (TPC) (in cfu/ml)	<10
	Total yeast and mould (in cfu/ml)	<10
	<i>Staphylococcus aureus</i> /g	Absent
	<i>Salmonella sp.</i> /g	Absent
	<i>Pseudomonas aeruginosa</i> /g	Absent
	<i>Escherchia coli</i> /g	Absent

Table 3: Results for accelerated stability study of Amarantha hair oil (storage condition 40 \pm 2 $^{\circ}$ /75% RH \pm 5%).

Sr. No.	Parameters	Initial	3 Months	6 Months
1.	Appearance	Yellowish brown coloured oil.	Yellowish brown coloured oil.	Yellowish brown coloured oil.
2.	Weight per ml	0.9150	0.9162	0.9155
3.	Refractive index	1.4638	1.4635	1.4630
4.	Acid value	1.28	1.32	1.35
5.	Saponification value	217.63	216.85	216.18
6.	Peroxide value	2.42	2.48	2.57

Continued.

Sr. No.	Parameters	Initial	3 Months	6 Months
7.	Identification by TLC	Positive for β -sitosterol	Positive for β -sitosterol	Positive for β -sitosterol
8.	Assay (β -sitosterol content by HPTLC) (in %)	0.52	0.48	0.43
Microbiological tests				
Total microbial plate count (TPC) (in cfu/ml)		<10	<10	<10
Total yeast and mould (in cfu/ml)		<10	<10	<10
9.	<i>Staphylococcus aureus</i> /g	Absent	Absent	Absent
	<i>Salmonella sp.</i> /g	Absent	Absent	Absent
	<i>Pseudomonas aeruginosa</i> /g	Absent	Absent	Absent
	<i>Escherchia coli</i> /g	Absent	Absent	Absent

Refractive index

It was determined by using refractometer. The refractive index was measured at $20^{\circ} \pm 0.5^{\circ}$. Prism of the refractometer was cleaned by using water and acetone. Then apparatus was calibrated against distilled water which has a refractive index of 1.3325 at 25° . Refractometer was dried and cleaned and sample was applied to determine refractive index.

Acid value

Accurately weighed 10 g of oil sample was dissolved in 50 ml of a mixture of equal volumes of ethanol (95 per cent) and ether, previously neutralised with 0.1 M potassium hydroxide to phenolphthalein solution. Flask was connected with reflux condenser and warmed slowly with frequent shaking, until the sample dissolves. 1 ml of phenolphthalein solution was added and titrated with 0.1 M potassium hydroxide until the solution remains faintly pink after shaking for 30 seconds. The acid value was calculated from the expression,

$$\text{acid value} = \frac{5.61 \times n}{W},$$

where,

n=the number of ml of 0.1 M potassium hydroxide required,

w=the weight, in g, of the substance.

Saponification value

Accurately weighed 2 g of oil sample was taken into a 250 ml flask. To it 25 ml of the alcoholic solution of potassium hydroxide was added, attached to a reflux condenser and boiled on a water bath for one hour. The cooled solution was titrated against 0.5 N hydrochloric acid using 1 ml solution of phenolphthalein as indicator (a). Similarly the blank titration was performed without taking oil sample (b).

Saponification value was calculated from the following formula,

$$\text{saponification value} = \frac{(b-a) \times 28.05}{W},$$

where,

W=the weight in g of the substance taken.

Peroxide value

Accurately weighed 5 g of oil sample was taken into a 250 ml glass-stoppered conical flask. 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform was added, swirled until dissolved and 0.5 ml saturated potassium iodide solution was added to it. Allowed to stand for exactly 1 minute, with occasional shaking, 30 ml of water was added and titrated gradually, with continuous and vigorous shaking, with 0.01 M sodium thiosulphate until the yellow colour almost disappeared. 0.5 ml of starch solution was added and continued the titration, until the blue colour just disappears (a). Similarly the blank titration was performed without taking oil sample (b).

Peroxide value was calculated from the expression,

$$\text{peroxide value} = \frac{10(a-b)}{W},$$

where,

W=weight, in g, of the substance.

TLC identification of β -sitosterol in raw material ingredients used in the oil formulation and in the *Amarantha* hair oil formulation

Identification of β -sitosterol in raw materials and also in finished product, *Amarantha* hair oil was determined using TLC. Each 1 g powdered herb of all raw materials was individually dissolved in 25 ml chloroform. The solutions were sonicated for 15 minutes and filtered by Whatman filter paper no. 1. Til tel and coconut oil solution was prepared by dissolving 0.1 g of oil sample in

20 ml chloroform. Amarantha hair oil sample was prepared by dissolving 0.1 g of oil sample in 20 ml chloroform. The solutions were sonicated for 15 minutes, filtered by Whatman filter paper no. 1 and filtrate was used for TLC identification of β -sitosterol. Then, 10 μ l of above solution was applied on TLC silica gel plate 60 F254 against 10 μ l of standard β -sitosterol using toluene:ethyl acetate (9.0:1.0 v/v) as mobile phase. Plate was sprayed with anisaldehyde sulphuric acid and dried at 105°C. Confirmation of β -sitosterol in raw materials and in the Amarantha hair oil formulation was done by comparing it with the Rf of standard β -sitosterol. The results are shown in Figures 1-3.

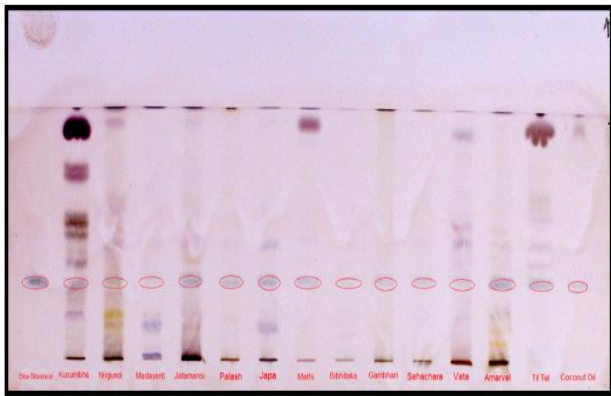


Figure 1: HPTLC fingerprint: photo-documentation after derivation with anisaldehyde sulphuric acid in white light; track number 1 standard β -sitosterol; track number 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15; various ingredients used in Amarantha hair oil.

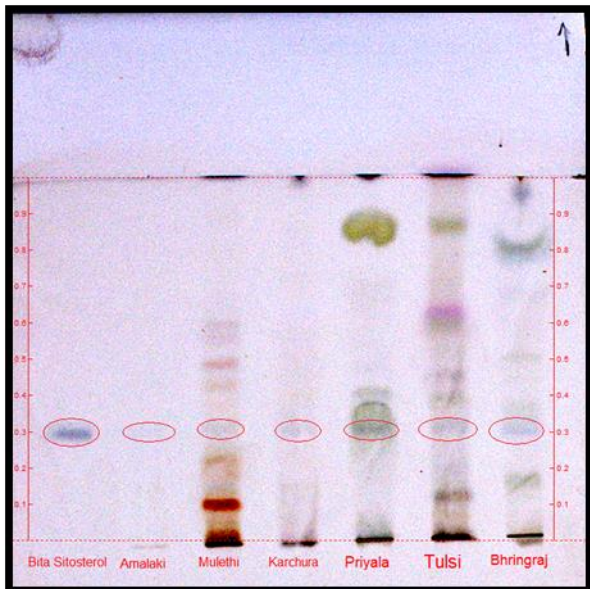


Figure 2: HPTLC fingerprint: photo-documentation after derivation with anisaldehyde sulphuric acid in white light; track number 1-standard β -sitosterol; track number 2, 3, 4, 5, 6, and 7; various ingredients used in Amarantha hair oil.

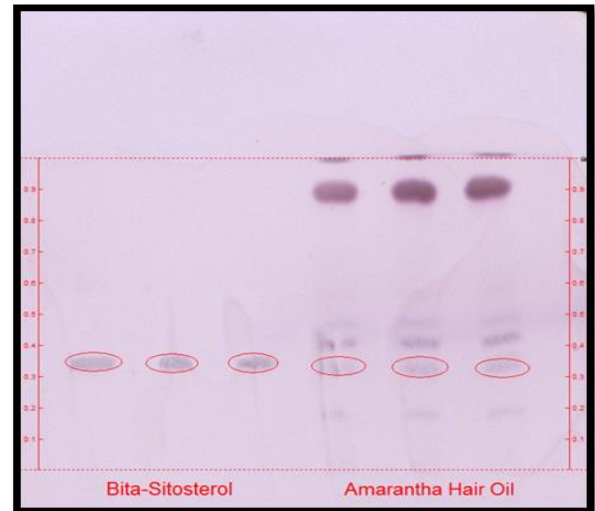


Figure 3: HPTLC fingerprint: photo-documentation after derivation with anisaldehyde sulphuric acid in white light. track number 1, 2, 3; standard β -sitosterol; track number 4, 5, 6; Amarantha hair oil formulation.

Assay (β -sitosterol content by HPTLC)

Assay of β -sitosterol in the Amarantha hair oil formulation was determined using HPTLC.

Preparation of standard solution

Accurately weighed 1.5 mg of standard β -sitosterol was dissolved in 10 ml chloroform and was sonicated and diluted with chloroform up to 20 ml.

Preparation of test solution of Amarantha hair oil

Accurately weighed 0.1 g of oil sample was dissolved in 20 ml chloroform. The solution was sonicated for 15 minutes, filtered by Whatman filter paper no. 1 and filtrate was used for estimation of β -sitosterol.

Chromatographic condition

The samples were spotted in the form of bands, width 6 mm with a Camag 100 microliter sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel pre-coated aluminum plate 60 F254 plates, using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate 0.1 μ l/s was used and the space between two bands was 10 mm. The slit dimension was kept at 6 mm \times 0.45 mm and the scanning speed was 20 mm/s. The monochromatic bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase was toluene:ethyl acetate (9.0:1.0). Linear ascending development was carried out in a 20 \times 10 cm twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase. The optimized chamber

saturation time for the mobile phase was 20 min at room temperature (25°C±2) at relative humidity 33%, saturated with MgCl₂. The length of each chromatogram run was 7 cm. Following the development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag

TLC scanner 4 in the reflectance/absorbance mode at 520 nm after derivatisation with anisaldehyde sulphuric acid reagent. Concentrations of the compounds were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The amount of β-sitosterol was computed from peak areas. The results are shown in Figure 4-6.

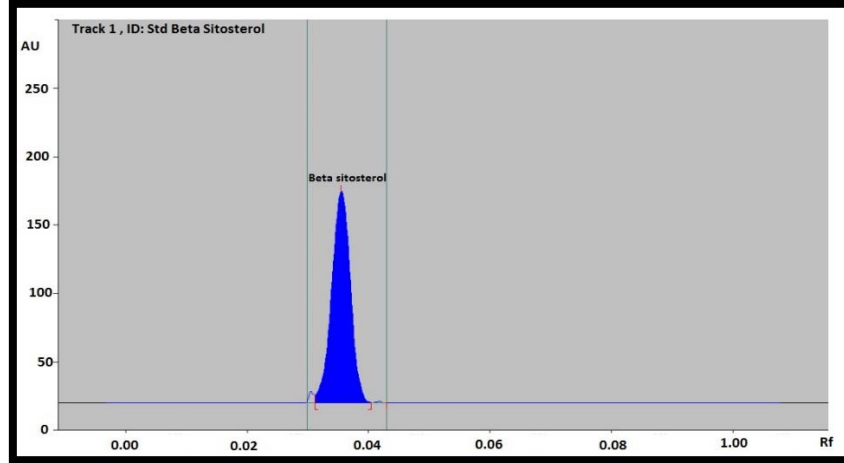


Figure 4: HPTLC chromatogram of standard β-sitosterol.

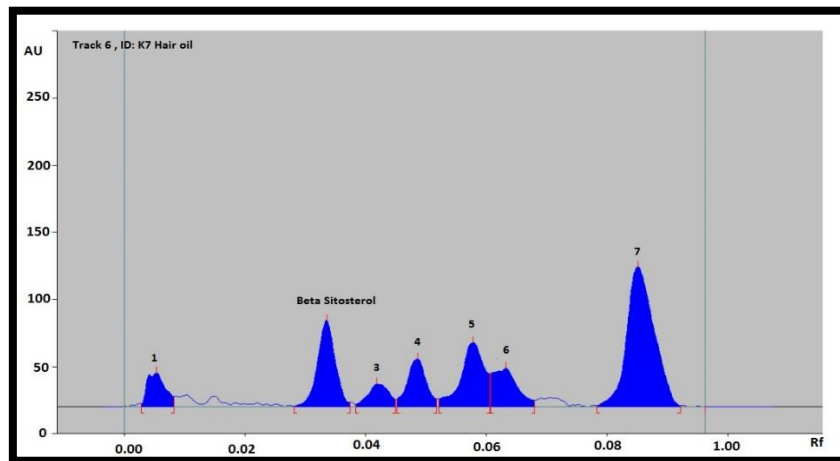


Figure 5: HPTLC chromatogram of Amarantha hair oil.

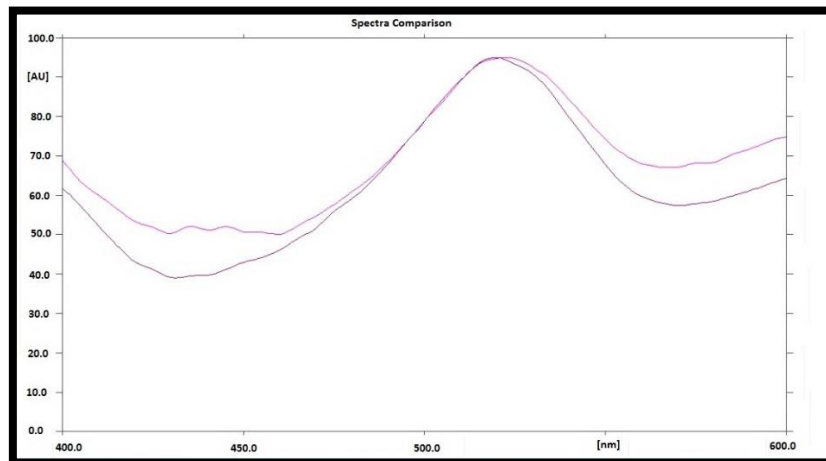


Figure 6: Overlain spectrum of standard β-sitosterol and Amarantha hair oil.

Microbiological tests

The formulated oil was subjected for microbiological limit tests, that is, total microbial plate count (TPC), total yeast and mould and pathogens like *S. aureus*, *Salmonella sp.*, *P. aeruginosa*, *E. coli*.

Accelerated stability testing

Accelerated stability study was performed on Amarantha hair oil. The stability was studied by exposing the sample to elevated conditions of temperature and humidity (40 \pm 2/75% RH \pm 5%) for 6 months. The parameters like appearance, weight per ml, refractive index, acid value, saponification value, peroxide value, HPTLC identification of β -sitosterol, assay (β -sitosterol content by HPTLC) and microbiological limit tests were performed as per ICH guidelines.

RESULTS

Appearance of Amarantha hair oil was good having smooth texture and easily spreadable property. It was yellowish brown colored oil with characteristic odour. Weight per ml of the sample was 0.915 g/ml. Refractive index of formulated oil was found to be 1.4638. The acid value was 1.28, indicating the amount of free fatty acid present in the oil. Saponification value and peroxide value of the formulated oil was found to be 217.63 and 2.42 respectively.

TLC method using toluene:ethyl acetate (9.0:1.0 v/v) as mobile phase showed the presence of β -sitosterol at refractive index 0.32 \pm 0.1 (violet colour band) under white light after derivation with anisaldehyde sulphuric acid in all the raw material ingredients and Amarantha hair oil. The results are shown in Figure 1-3.

Assay of β -sitosterol content in Amarantha hair oil was determined using HPTLC. The solutions of standard β -sitosterol and oil formulation were spotted on TLC plate in triplicate and run. The band for β -sitosterol in the samples was confirmed by comparing the Rf values and spectrum with standards. The amount of β -sitosterol in oil formulation was found to be 0.52%. The results are shown in Figure 4-6.

Amarantha hair oil was also evaluated for microbiological test; total viable count was found to be <10 cfu/ml and pathogens like *S. aureus*, *Salmonella sp.*, *P. aeruginosa*, *E. coli* was absent. All these results are tabulated in Table 2.

Accelerated stability study showed that there were no significant changes in the Amarantha hair oil during stability study and results are tabulated in Table 3. The test results obtained for Amarantha hair oil were well within the limits, which indicated good quality of product.

DISCUSSION

Amarantha hair oil is prepared using standardized herbs like bibhitaka (*T. belerica*), amalaki (*E. officinalis*), methi (*T. foenum-graecum*), tulsi (*O. sanctum*), aamrawali (*C. reflexa*), bhringraj (*E. alba*), yashtimadhu (*G. glabra*), japa (*H. rosasinesis*), nirgundi (*V. negundo*), gambhari (*G. arborea*), madayanti (*L. inermis*), priyala (*B. latifolia*), jatamansi (*N. jatamansi*), palash (*B. frondosa*), sahachara (*B. cristata*), kusumbha (*C. tinctorius*), kachura (*C. zedoria*), vata (*F. bengalensis*), til tel (*S. indicum*) and coconut oil (*C. nucifera*) as base. These ingredients were selected on the basis of Ayurvedic principles. The oil was prepared using traditional method of oil preparation mentioned in Ayurvedic texts. The present study was carried out to identify and confirm presence of β -sitosterol in ingredients as well as in Amarantha hair oil using HPTLC.

The results of the present study show that Amarantha hair oil and all its ingredients contain β -sitosterol. Literature says that β -sitosterol possessed 5- α -reductase inhibitory activity that may help in the treatment of hair loss. It was found in research studies that *T. belerica* was used as natural dyeing and coloring agent for hair.¹⁰ The phytonutrients, vitamins and minerals present in *E. officinalis* helped in increasing the scalp circulation and stimulate healthy growth. Dried amla fruits were boiled in coconut oil and then ground to form amla oil. This was a very effective conditioner and prevented balding and greying of hair. It was a potent 5- α - reductase inhibitor and hair growth promoter.¹¹

T. foenum-graecum had shown hair growth promoting activity. The results of the activity test using 10% fenugreek extract seed hair tonic showed significant difference compared to placebo. These results were comparable to that of minoxidil 2% hair tonic.¹² The experimental study on *O. sanctum* extract had reported to possess hair growth promoting activity.¹³ *O. sanctum* also reported to have anti-androgenic property.¹⁴

A study on the extracts of *C. reflexa* Roxb. in treatment of cyclophosphamide induced alopecia was shown to be capable of promoting follicular proliferation or preventing hair loss in cyclophosphamide-induced hair fall.¹⁵ β -sitosterol as a potent 5-alpha-reductase inhibitor was present in *E. alba* in appreciable quantity. Hair growth promoting activity of *E. alba* in male albino rats was established in a study. Hair growth initiation time was significantly reduced to half on treatment with the extract as compared to control animals. The result of treatment with 2% and 5% petroleum ether extracts were better than the positive control minoxidil 2% treatment.¹⁶

G. glabra was known to possess hair growth promoting activity. Animals treated with petroleum ether extract of *G. glabra* roots showed longer hair than those treated with either minoxidil or control.¹⁷ *H. rosasinesis* had shown hair growth potentiation activity in disturbed

circadian rhythm. Flower and leaves extracts have similar significant hair growth promoting characteristics with minimal chances of developing serious adverse effects.¹⁸ *V. negundo* used to treat premature greying of hair and scalp infections like pediculosis and lice.¹⁹ *G. arborea* was reported as keshya (hair growth promoting activity) in Ayurveda.²⁰

L. inermis had shown a remarkable hair growth promoting activity in terms of hair recovered area and hair length. The study concluded that the topical use of *L. inermis* powder and honey accelerated burn wound healing process in rabbit's model.²¹ In a study, *B. latifolia* leaf aqueous extract mucilage was found to have all common properties of hair conditioners. It had been found to be effective as an herbal hair conditioner with good hair texture, moisture improvement, easy to wet and dry combing with good detangling effect and improved gloss (shine) when compared with 10% (w/v) reference sample of commonly marketed herbal hair conditioner.²²

In a study named, phytochemical investigation and hair growth studies on the rhizomes of *N. jatamansi*, the extract, fraction and the isolated compounds of *N. jatamansi* showed good activities in hair growth.²³ In an investigative study for the effectiveness of a topical herbal hair loss cream application consisting of extracts of *B. monosperma* and *B. parviflora* in preventing hair loss and promoting growth of hair in men and women, it had been found to be beneficial in stimulating hair growth.²⁴

B. cristata was mentioned as keshya dravya (hair growth promoting activity) in madanpal nighantu (ancient Ayurvedic text book).²⁵ A study results shows *C. tinctorius* extract significantly increased the length of cultured hair follicles and stimulated the growth of hair with local effects in mice.²⁶ The inhibitory activity (conversion rate of testosterone to DHT) of the *C. zedoria* extract was studied in a study and *C. zedoria* extract showed 28.2% enzyme inhibition.²⁷ One invention was found related to the use of the *F. bengalensis* to increase hair growth.²⁸

A study confirmed the use of sesame seed (*S. indicum*) for hair loss and treatment of greying of hair. It had also demonstrated potential application of sesamin extracted from the sesame seeds for genetically hair loss and canities treatments.²⁹ The phytosterols, which can be found in sesame seed oil, coconut oil and the like, have the ability to lower the amount of 5- α -reductase in the liver by up to 44% and in the prostate by up to 33%.^{30,31}

From the above the discussion it was clear that the ingredients of Amarantha hair oil have been proved to be effective on hair loss and premature greying. Also majority of ingredients possess 5- α -reductase/hair growth promoting, anti-androgenic, hair conditioning, natural dyeing and colouring activities. These multiple activities of the ingredients make Amarantha hair oil an effective and safe option for treatment of hair loss.

CONCLUSION

It can be concluded from the results of the present study that all the ingredients and Amarantha hair oil contain β -sitosterol which is known to inhibit of 5-alpha-reductase enzyme thereby implying hair growth promoting capacity. Thus Amarantha hair oil is natural, safe, effective, stable option for the treatment of hair loss.

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Conflict of interest: None declared

Ethical approval: Not required

REFERENCES

1. American Hair Loss Association. Fact sheet: Introduction. Available at: https://www.americanhairloss.org/men_hair_loss/introduction.html. Accessed on 28 May 2021.
2. Patil SM, Sapkale GN, Surwase US, Bhombe BT. Herbal medicines as an effective therapy in hair loss-a review. Res J Pharmaceut Biol Chem Sci. 2010;1(2):773-81.
3. Rossi A, Cantisani C, Melis L, Iorio A, Scali E, Calvieri S. Minoxidil use in dermatology, side effects and recent patents. Recent Pat Inflamm Allergy Drug Discov. 2012;6(2):130-6.
4. Hirshburg JM, Kelsey PA, Therrien CA, Gavino AC, Reichenberg JS. Adverse effects and safety of 5-alpha reductase inhibitors (finasteride, dutasteride): a systematic review. J Clin Aesthet Dermatol. 2016;9(7):56-62.
5. Cabeza M, Bratoeff E, Heuze I, Ramirez E, Sanchez M, Flores E. Effect of beta-sitosterol as inhibitor of 5 alpha-reductase in hamster prostate. Proc West Pharmacol Soc. 2003;46:153-5.
6. Prager N, Bickett K, French N, Marcovici G. A randomized, double-blind, placebo-controlled trial to determine the effectiveness of botanically derived inhibitors of 5-alpha-reductase in the treatment of androgenetic alopecia. J Altern Complement Med. 2002;8(2):143-52.
7. Upadhyay K, Gupta NK, Dixit VK. Development and characterization of phyto-vesicles of b-sitosterol for the treatment of androgenetic alopecia. Arch Dermatol Res. 2012;304:511-9.
8. Hajhashemi V, Rajabi P, Mardani M. Beneficial effects of pumpkin seed oil as a topical hair growth promoting agent in a mice model. Avicenna J Phytomed. 2019;9(6):499-504.
9. Truong VL, Bak MJ, Lee C, Jun M, Jeong WS. Hair regenerative mechanisms of red ginseng oil and its major components in the testosterone-induced delay of anagen entry in C57BL/6 mice. Molecules. 2017;22(9):1505.
10. Gargote M. Formulation of hair colors from nature. Int J Scient Engineer Res. 2012;3(8).
11. Kumar N, Rungseevijitprapa W, Narkkhong N, Suttajit M, Chaiyasuta C. 5- α -reductase inhibition and hair growth promotion of some Thai plants

- traditionally used for hair treatment. J Ethnopharmacol. 2012;139(3):765-71.
12. Wijaya WH, Munim A, Djajadisastra J. Effectiveness test of fenugreek seed (*Trigonella foenum-graecum* L.) extract hair tonic in hair growth activity. Int J Curr Res. 2013;5(11):3453-60.
 13. Rathi V, Rathi JC, Patel A, Tamizharasi S. Hair growth activity of *Cicer arietinum* Linn. *Ocimum sanctum* Linn and *Cyperus rotundus* Linn in albino rats. J Pharmacog Phytochem. 2017;6(1):157-9.
 14. Das SK, Vasudevan DM. Tulsi: the Indian holy power plant. Nat Prod Rad. 2006;5(4):279-83.
 15. Patel S, Sharma V, Chauhan NS, Dixit VK. A study on the extracts of *Cuscuta reflexa* Roxb. in treatment of cyclophosphamide induced alopecia. Daru. 2014;22(1):7.
 16. Roy RK, Thakur M, Dixit VK. Hair growth promoting activity of *Eclipta alba* in male albino rats. Arch Dermatol Res. 2008;300(7):357-64.
 17. Upadhyay S, Ghosh AK, Vijender Singh V. Hair growth promotant activity of petroleum ether root extract of *Glycyrrhiza glabra* L (Fabaceae) in female rats. Trop J Pharmaceut Res. 2012;11(5):753-8.
 18. Pathak SS, Gala MY. Evaluation of hair growth potentiation activity of *Hibiscus rosasinensis* in disturbed circadian rhythm. Int J Curr Res. 2018;10(03):67151-5.
 19. Kumar D, Kumar R, Sharda K. Medicinal property of nirgundi. J Pharmacog Phytochem. 2018;1:2147-51.
 20. Sangeeta K, Singh DC, Safina K. A precious herb-*Gmelina arborea* linn. w.s.r to its medicinal uses and pharmacological activity. Int J Ayur Pharma Res. 2017;5(5):47-53.
 21. Djerrou Z, Mokhbi I, Hadeif KS, Boutobza N, Bouzeguine S, Brighet I, et al. Burn wound healing effect and hair growth promoting activity of *Lawsonia inermis* L. and honey in *Oryctolagus cuniculus* rabbits. Online J Biol Sci. 2016;16(2):82-9.
 22. Meghana D, Divana R. Evaluation of *Buchanania lanazan* leaf mucilage as herbal hair conditioner. IRIS. 2013.
 23. Rao GV, Annamalai T, Mukhopadhyay T. Phytochemical investigation and hair growth studies on the rhizomes of *Nardostachys jatamansi* DC. Pharmacog. 2011;7(26):146-50.
 24. Ravichandran G, Singh A, Patki PS, Mitra SK. Clinical evaluation of herbal hair loss cream in management of alopecia aerata: an open study. Med Update. 2008;15(9):31-4.
 25. Raverkar SA, Shindadkar SV. Study of keshya drugs in *Madanpal Nighantu*-a review. Int J Res Ayurveda Med Sci. 2020;3(1):41-5.
 26. Junlatat J, Sripanidkulchai B. Hair growth-promoting effect of *Carthamus tinctorius* fiolet extract. Phytother Res. 2014;28(7):1030-6.
 27. Murata K, Hayashi H, Matsumura S, Matsuda H. Suppression of benign prostate hyperplasia by *Kaempferia parviflora* rhizome. Pharmacog Res. 2013;5(4):309-14.
 28. Armani A, Armani S, Seneviratne C, Nazari R. Extracts and compounds from *Ficus Benghalensis* for increasing hair growth and decreasing hair loss. United States Patent. 2013.
 29. Manosroi J, Jantrawut P, Manosroi W, Kongtaweelert P, Manosroi A. 5 α -reductase inhibition and melanogenesis activity of sesamin from sesame seeds for hair cosmetics. Chiang Mai J Sci. 2015;42(3):669-80.
 30. Arruzazabala M, Molina V, Más R, Carbajal D, Marrero D, González V, et al. Effects of coconut oil on testosterone-induced prostatic hyperplasia in Sprague-Dawley rats. J Pharm Pharmacol. 2007;59(7):995-9.
 31. Awad AB, Hartati MS, Fink CS. Phytosterol feeding induces alteration in testosterone metabolism in rat tissues. J Nutrition Biochem. 1998;9(12):712-7.

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