



FORMULATION AND EVALUATION OF *EMBLICA OFFICINALIS* GAERTN, GEL ON HAIR GROWTH PROMOTION IN RATS

Aruna Boreddy¹, Dr. Sonali Mallick², Nirmala A³, Bhuvaneshwari J⁴

1,2 – Spurthy College of Pharmacy, Bengaluru - 562106

3 - Research Scholar, JSS AHER, Mysore - 570004

4 - Al-ameen College of pharmacy, Hosur road, Bangalore-560027.

Abstract: Hair loss or Alopecia, is a dermatological condition that not only affects an individual's physical appearance but also carries significant psychosocial implications. The loss of hair can have a profound impact on a person's self-esteem and overall well-being. To address this concern, traditional knowledge has often associated the fruit of *Embllica officinalis* Gaertn, commonly known as Amla, with hair growth-promoting properties. This study aimed to scientifically evaluate the potential of *E. officinalis* in promoting hair growth. The research involved the creation of a hydrogel containing a 5% hydro-alcoholic extract of *E. officinalis* and assessing its efficacy in promoting hair growth. The formulated gels were applied topically to the shaved skin of Wistar albino rats, serving as a model for evaluating the impact on hair growth. The results were striking: the Amla gel formulation demonstrated a significant improvement in both hair length and hair density. Moreover, it consistently and significantly increased hair length (p-value less than 0.001) and hair density after 30 days of treatment. This indicates that Amla extract, when incorporated into a hydrogel, is highly effective in stimulating hair growth. In summary, the study highlights the significant potential of *E. officinalis* (Amla) as a natural remedy for promoting hair growth. The use of a hydro-alcoholic extract in a hydrogel base proves to be a promising approach for harnessing the hair growth-promoting properties of Amla. Such findings are not only of scientific interest but also offer hope to individuals struggling with hair loss, as they seek effective solutions to this common dermatological concern with substantial psychological implications.

Keywords: Hair loss, Alopecia, *Embllica officinalis* Gaertn (Amla), Hydrogel, 5% hydro- alcoholic extract, Wistar albino rats, Hair growth, Self-esteem, Psychosocial implications, Dermatological concern.

INTRODUCTION

Hair is a significant cosmetic feature, and hair loss represents a prevalent dermatological issue that affects people worldwide and is a subject of considerable worry. Multiple factors, including metabolism, hormones, genetic predisposition, and adverse effects of medications such as anti-cancer and immunosuppressant drugs, can have detrimental effects on the normal growth of hair. FDA-approved drugs for promoting hair growth are often abbreviated due to their associated side effects. Therefore, there is a demand to explore natural plant-derived products that may contain substances with the potential to stimulate hair growth.

Numerous factors play a role in hair loss, with genetic predisposition and hormonal imbalances being prominent contributors. Additionally, certain medical conditions like typhoid, malaria, and jaundice can lead to temporary hair loss. The use of chemotherapy drugs is also known to trigger hair shedding. Among various factors, androgens are recognized as a significant cause of alopecia, alongside a range of other influencing elements.

Biological Source: *Emblica officinalis*, commonly known as Indian Gooseberry or Amla, is a fruit-bearing tree native to the Indian subcontinent and is considered the primary source of Amla.

Scientific Name: *Emblica officinalis* **Family:** Phyllanthaceae

Chemical Constituents: Amla (*Emblica officinalis*) contains a wide array of bioactive compounds, including: Vitamin C (Ascorbic Acid): Amla is famous for its exceptionally high vitamin C content, making it one of the richest natural sources of this essential antioxidant vitamin.

Tannins: Amla is rich in tannins, which have potent antioxidant and anti-inflammatory properties.

Polyphenols: Amla contains various polyphenolic compounds, including flavonoids, ellagic acid, and gallic acid, which contribute to its antioxidant effects.

Minerals: It contains essential minerals like calcium, phosphorus, and iron.

Carotenoids: Amla also contains carotenoids, which are precursors to vitamin A and have various health benefits.



Fiber: It is a good source of dietary fiber, which aids in digestion and supports gut health.

Alkaloids: Some alkaloids are also present in amla, with potential therapeutic properties.

Uses: Amla (*Emblica officinalis*) has a long history of use in traditional Ayurvedic medicine for its various health benefits. Some of its key uses include: **Immune System Support:** Amla is renowned for its high vitamin C content, which boosts the immune system and helps the body defend against infections and illnesses.

Antioxidant Properties: The antioxidants in amla help combat free radicals, reduce oxidative stress, and protect cells from damage, potentially lowering the risk of chronic diseases.

Digestive Health: Amla is used to improve digestion, alleviate constipation, and address various gastrointestinal issues.

Hair and Skin Care: Amla is used in hair oils, shampoos, and skincare products to promote hair growth, strengthen hair, and enhance skin health. It can help reduce hair fall and improve the complexion.

Cardiovascular Health: Some studies suggest that amla may have a positive effect on heart health by reducing cholesterol levels, improving blood vessel function, and lowering the risk of heart disease.

Diabetes Management: Amla may help regulate blood sugar levels and improve insulin sensitivity in individuals with diabetes.

Anti-Inflammatory Effects: Amla has anti-inflammatory properties, making it useful for reducing inflammation in various health conditions.

Detoxification: It is considered a natural detoxifier, assisting in the removal of toxins from the body.

Cognitive Health: Some research indicates that amla may have potential benefits for cognitive function and memory.

Amla is available in various forms, such as fresh fruit, dried fruit, powders, and supplements. Its versatility and potential health benefits make it a popular and widely used natural remedy in traditional and modern medicine.

MATERIALS AND METHODS:

COLLECTION AND AUTHENTICATION OF SAMPLES

The study utilized dried leaves of *Emblica officinalis*, which were sourced from local shops in Bangalore, Karnataka, during November 2011. Dr. Shiddamalaya N at NADRI (National Ayurveda Dietetics Research Institute) in Bangalore identified and authenticated the sample drug. The leaves were dried in the shade and stored in polythene bags.

PREPARATION OF EXTRACT BY SOXHLETATION: Dried fruits of *Emblica officinalis* Gaertn were ground using a mixer grinder and processed into a powder with a mesh size of #40 suitable for amla fruits. The powdered material was subsequently subjected to extraction using the Soxhlet method. The resulting extract was utilized for phytochemical analysis, formulation, and evaluation.

Soxhlation method: Procedure Soxhlet extraction was conducted employing the conventional Soxhlet extraction apparatus. A 10g sample designated for extraction was positioned within a thimble, and in a round-bottom flask attached to the thimble, 40ml of 70% alcohol was introduced. A condenser was positioned above the thimble. The extraction process was carried out over an 8-hour duration using 70% alcohol. Ultimately, the extract was evaporated to complete dryness, either through the use of a vacuum system or a rotary evaporator. The remaining residues were then weighed, and the percentage yield was calculated.

PHYTOCHEMICAL ANALYSIS

Qualitative Chemical tests

The hydroalcoholic extract of *E. officinalis* Gaertn underwent a series of chemical tests aimed at identifying the presence of various chemical constituents within the drug. These tests were conducted to detect alkaloids, glycosides, saponins, phenols, proteins, tannins, fixed oils, and resins.

Quantification of phytochemical constituents:

Qualitative chemical tests of the extract of *E. officinalis* Gaertn indicated the presence of flavonoids, polyphenols, triterpenoids, and sterols as the primary constituents. The results detailing the chemical constituents of the extracts are organized and presented in Table No. 1.

The extracts obtained through the Soxhlet extraction method were further evaluated for the content of various phytochemicals such as flavonoids, polyphenols, and sterols, as described in references 25 and 26. A standard calibration curve is depicted in Graphs No. 1, 2, and 3, with recorded data presented in Table No. 2..

FORMULATION OF *E.OFFICINALIS* GAERTN GEL:

The hydro-alcoholic extract of *E. officinalis* Gaertn, which predominantly contained polar constituents, led to the decision to formulate the extract into a hydrogel. This choice was made to mitigate any potential influences of fragrance, coloring agents, antioxidants, or preservatives on hair growth. Importantly, the hydrogel preparations were intentionally crafted without the inclusion of colorants, fragrances, antioxidants, or preservatives.

To create the hydrogel, the hydro-alcoholic extracts of *E. officinalis* Gaertn were employed, with Carbopol 934 serving as the polymer. A 5% concentration of the extract was used in the gel preparation, resulting in the formulation of the *E. officinalis* Gaertn 5% hydrogel.

Preparation of gel: A 5% hydro-alcoholic extract of *E. officinalis* Gaertn was utilized for creating an herbal gel to assess its potential for stimulating hair growth.

The gel was prepared as follows: One gram of Carbopol 934 was dispersed in 50ml of distilled water, ensuring continuous stirring, and left to stand overnight to achieve a smooth gel consistency. In a separate container, 2 ml of distilled water was combined with polyethylene glycol (PEG) 400. Then, 5% of the rosemary extract was blended into the above mixture, and the volume was adjusted to 100ml by adding distilled water. Subsequently, the mixture was thoroughly combined with the Carbopol gel, employing continuous stirring.

Triethanolamine was gradually added to the formulation to adjust the pH to match the scalp's pH (typically within the range of 5.5-6) and attain the desired consistency.

A similar process was employed to prepare a control gel, without the addition of the extract. It's worth noting that no colorants or perfumes were incorporated into the formulation.

This method was employed for the creation of both the herbal gel containing the *E. officinalis* Gaertn extract and the control gel.

EVALUATION OF FORMULATED AMLA GEL FOR DERMAL IRRITATION AND HAIR GROWTH PROMOTING ACTIVITY IN WISTAR ALBINO RATS²⁸⁻³⁴.

Numerous studies and traditional medicine sources have highlighted Amla's potential for promoting hair growth. Amla has been integrated into a hair cream and subjected to clinical investigation to assess its anti-dandruff properties.

Formulations, both traditional and modern, involving *E. officinalis* Gaertn, have been developed and tested, employing pre-clinical and clinical models for various conditions, including those aimed at promoting hair growth. However, there have been no documented reports regarding the evaluation of Amla's hair growth-promoting properties.

To address this gap, an evaluation of Amla's hair growth-promoting potential was carried out using Wistar albino rats. A 5% *E. officinalis* Gaertn gel was examined for dermal irritation and its ability to promote hair growth, with measurements based on hair length and hair follicle count.

Top of Form

Dermal irritation study:

Experimental Procedure: Wistar albino rats of both sexes, weighing between 200-250g, were distributed into four groups, each consisting of six animals. The hair in a 3 cm² area on the dorsal portion of all the rats was completely removed using a commercial hair remover. Subsequently, the rats' skin was cleansed with surgical spirit, and the various formulation/control gels were applied.

The grouping of the animals was as follows:

Skin Irritation Test (Table No. 3):

Group I: Control (Base) treated group Group II: Applied with 5% Amla gel

Each gel (0.5g) was applied once daily to the shaved area, covering approximately 6 cm² of skin. This was covered with a gauze patch and held in place with a semi-occlusive dressing for one hour. After one hour, the gauze was removed, and any remaining test substance was carefully wiped away without disturbing the skin's response or integrity. Observations were made after the patch was removed. Control animals were subjected to a similar process, with 0.5g of the gel

base (formulated with all ingredients except the herbal mixture) applied, and observations were conducted in the same manner. The results of the skin irritation test are documented in

Table No. 3.

The gel was applied to the skin once daily for 7 days, and observations were made for any signs of sensitivity. If any reaction occurred, it was graded as follows:

A: No reaction

B: Slight patchy erythema

C: Slight but confluent or moderate but patchy erythema D: Moderate erythema

E: Severe erythema with or without edema.

Evaluation of hair growth promoting activity of Amla gel

Wistar albino rats of either sex, with weights ranging from 200-250g, were organized into five groups, each consisting of six animals. The hair in a 3 cm² area on the dorsal portion of all the rats was entirely removed using a commercial hair remover. Afterward, the rats' skin was cleaned with surgical spirit, and the various formulation/control gels were applied.

Here's how the animals were grouped:

Group I: Control (Base) treated group Group II: Applied with 5% Amla gel

Group III: Applied with 2% minoxidil solution

Each gel (1g) and the standard drugs (1ml) were applied once a day to the shaved area. The treatment regimen continued for 30 days, during which the hair growth pattern was closely observed and documented for analysis and comparison.

Hair length determination: Hair samples were plucked randomly from the shaved dorsal region of the rats on the 15th, 20th, 25th, and 30th days of the treatment. The length of the plucked hair was carefully measured, and the outcomes were documented as the mean length \pm

SEM (Standard Error of the Mean) based on 25 hair measurements. The summary of the results regarding hair lengths is presented in Table No. 4. Additionally, the graphical representation of the results for hair length determination is depicted in Graph No. 4.

Hair density: A hole measuring 1 cm² was created in a cardboard. This cardboard was then placed on the intended depilated area on the rat's back, following 30 days of hair removal. The hair in the designated depilated area was carefully trimmed and subsequently counted manually. The outcomes related to hair density have been compiled and are presented in Table No. 5. Furthermore, a visual representation of the results, illustrating hair density, can be found in Graph No. 5.

Statistical analysis

The data were presented as the mean \pm standard error of the mean (SEM), and a statistical analysis was conducted employing an ANOVA test.

RESULTS:**COLLECTION AND AUTHENTICATION OF SAMPLES**

Emblica officinalis Gaertn was sourced locally in Bangalore. The sample drug underwent identification and authentication by Dr. Shiddamalaya N, with reference number: Drug Authentication/ SMPU/ NADRI/ BNG/ 2011-2012/ 681, in Bangalore, Karnataka.

PREPARATION OF THE EXTRACT BY SOXHLETION METHOD

The Soxhlet extract of *Emblica officinalis* was evaporated employing a rotary evaporator, resulting in a percent yield of 8.18%.

PHYTOCHEMICAL ANALYSIS

Qualitative chemical test: The extracts, prepared according to the procedures detailed in section 4.3, underwent qualitative chemical tests to identify the chemical constituents present. The outcomes of these tests have been organized and presented in Table 5.

Specifically, the hydro-alcoholic extract of *E. officinalis*, prepared through the Soxhlet extraction method, exhibited the presence of phenols, proteins, and phytosterols.

Determination of Phytoconstituents in the extract

The determination of total phenol content, total flavonoid content, and total sterol content in the hydroalcoholic extracts of *E. Officinalis* Gaertn was carried out using colorimetric methods as previously reported. The results have been compiled and are presented in Table 6.

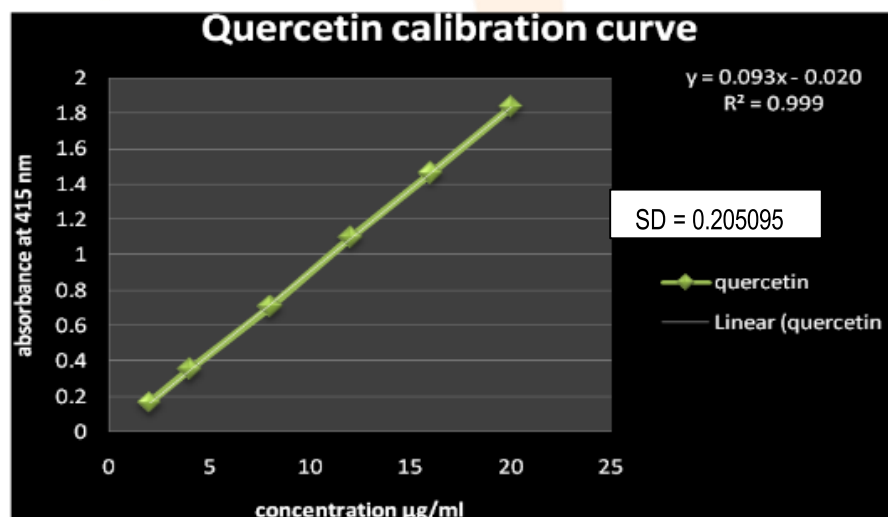
Table No:1 Qualitative Chemical Tests of the Hydro alcoholic extract of *E. Officinalis* Gaertn

Chemical constituents	Tests	AMLA
Alkaloids	Meyers test	-ve
	Dragendroff's test	-ve
	Wagner's test	-ve
	Hager's test	-ve
Carbohydrates	Molisch's test	-ve
	Benedict's test	-ve
	Fehling's test	-ve
	Barfoed's test	-ve
Glycosides	Modified Bortragers test	-ve
	Legal's test	-ve
	Lieberman buchard's test	+ve
Saponin	Foam test	-ve

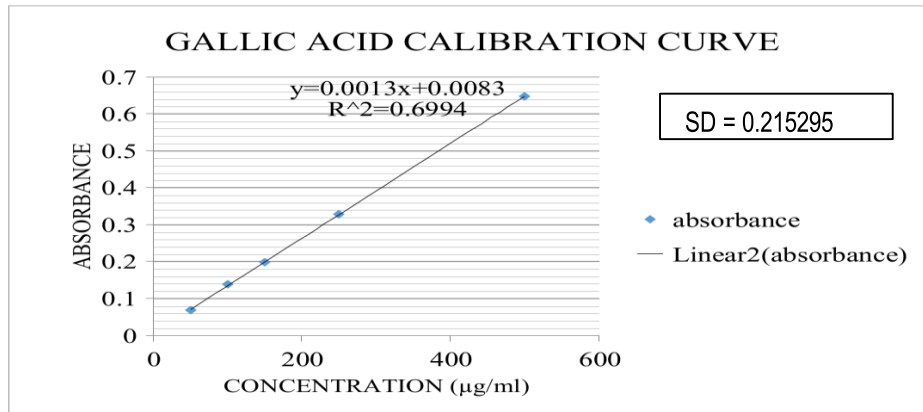
Fixed Oils And Fats	Stain test	-ve
Resins	Acetone-water test	-ve
Phenols	Ferric chloride test	+ve
	Alkaline reagents	-ve
Flavonoids	Lead acetate	-ve
	Shinado test	-ve
Protein And Amino Acid	Xanthoproteic test	+ve
	Ninhydrin test	-ve
	Biuret test	-ve
Diterpenes And Triterpenoids	Copper acetate	-ve
	Tshugajen test	-ve
Triterpenes And Phytosterols	Salkowski's test	-ve
	Lieberman buchard's test	+ve

Note: + ve Indicates presence of phytoconstituents; whereas – ve Indicates absence of phytoconstituents.

Quantitative chemical test: Standard graph for standard flavonoid, polyphenolic and sterol content



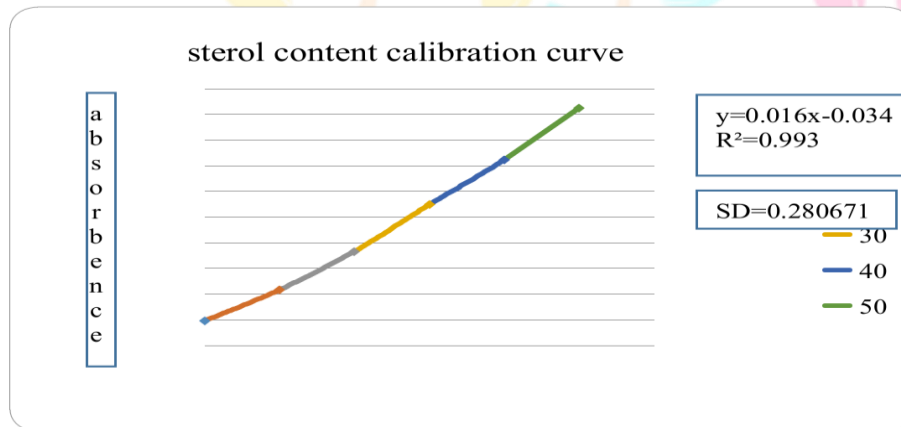
Graph no 1: The calibration curve with standard Quercetin for the determination of flavonoids by



AlCl3 colourimetry method

Graph no 2: The calibration curve of gallic acid for determination of polyphenol by Folin Ciocalteau method.

Graph no:3 The calibration curve of Cholesterol for determination of sterol content using Liberman- Burchard



reagent.

Table no 2: Content of total phenols, total flavonoid and total sterol content in hydro alcoholic extract of *E. Officinalis* GaFORMULATION OF *E. Officinalis* Gaertn GEL:

Preparation of Gels: The gel formulations are prepared according to the procedure mentioned in the methodology.

Extract	Total flavonoids % as quercetin	Total phenols % as gallic acid	Total sterols %
<i>E. Officinalis</i> Gaertn	8	39.5	0.12

EVALUATION OF FORMULATED GELS OF *E. Officinalis* Gaertn FOR DERMAL IRRITATION AND HAIR GROWTH PROMOTING ACTIVITY IN WISTAR ALBINO RATS.

E. Officinalis Gaertn was assessed for dermal irritation and its potential to stimulate hair growth in Wistar albino rats. The evaluation included the recording of data related to hair length and hair density.

Dermal irritation study: The application of gels onto the shaved skin of rats, as documented in Table No. 3, did not result in any signs of irritation. Both the control group and the experimental rats exhibited no indications of tremors, convulsions, or reflex abnormalities. Additionally, food intake per day remained normal during the 7-day evaluation for repeated dose dermal toxicity. Importantly, there were no of the two gels.

Table no: 3 Data showing the skin irritation of the two gels

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Amla gel(5%)	A	A	A	A	A	A	A

A- No reaction

Hair length determination:

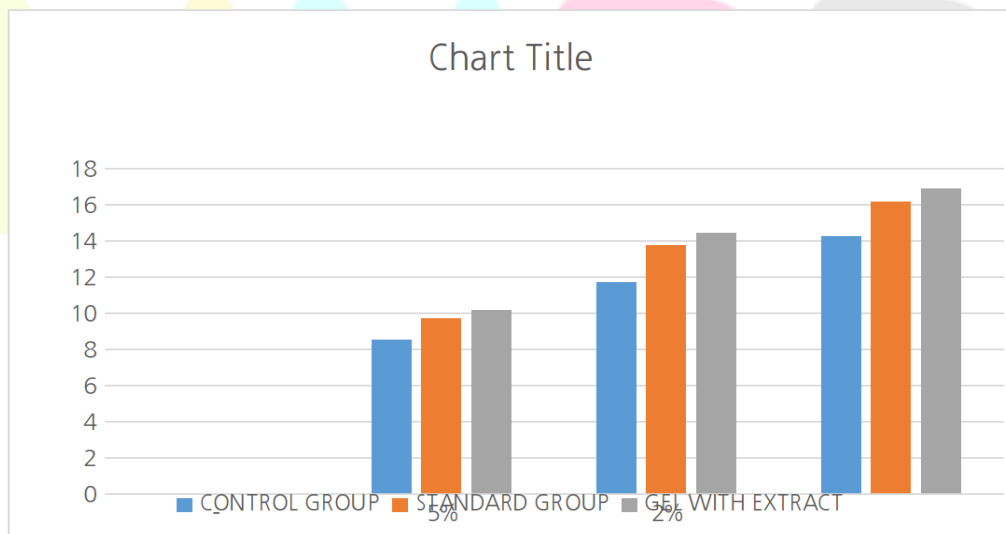
The experiment is carried out according to the procedure and the results are tabulated in the Table no 4.

Table no:4 Effects of 2%Minoxidil, Rosemary on hair growth in Wistar albino rats.

Treatment Group	Dose (%)	15th Day	20th Day	25th Day	30th Day
Group-1Control	-	5.86±0.93	8.5±0.56	11.7±0.56	14.26±0.360
Group-3 Amla (5%) gel	5%	7.92±0.48	10.18±1.04	14.41±0.56	16.88±0.239
Group-3 minoxidil (1ml)	2%	6.5±0.4	9.70±0.92	13.77±0.63	16.16±0.399

values mean± SEM, *p < 0.01, **p < 0.05, ***p < 0.0001. Compared to control group by ANOVA(n=25 hairs)

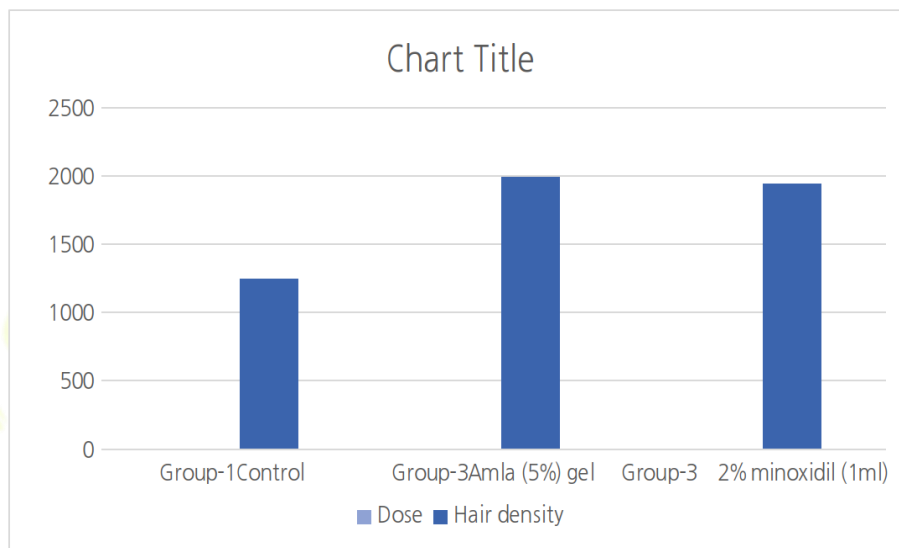
In the current study, the formulations containing 5% Amla demonstrated a substantial capacity to stimulate hair growth, particularly in terms of hair length. Notably, the use of Amla gel exhibited improved hair growth compared to the control group, starting from the 15th day of treatment. This improvement was more significant than that observed in the group treated with the standard minoxidil. Animals treated with the gel formulation exhibited superior hair growth compared to the group treated with standard minoxidil.



Graph no:4 The graph showing hair length determination of 15th,20th, 25th and 30th day treatment**Hair density:****Table No:5 Effects of Control, 2%Minoxidil and Amla on hair density in Wistar albino rats.**

Treated Group	Dose	Hair density
Group-1Control	-	1245±46
Group-3Amla (5%) gel	5%	1994***±30
Group-3 2% minoxidil (1ml)	2%	1944***±27

The experiment is carried out according to the procedure and the results tabulated in the Table no: 5

**Graph no:5 The graph showing hair density after 30th day of the treatment.**

values mean± SEM, *p < 0.01, **p < 0.05, ***p < 0.0001. Compared to control group to formulation treated groups by ANOVA(n=6)

Statistical analysis:

Values as expressed in mean ± SEM

***p < 0.0001 for ANOVA for both parameters like hair length determination and hair density determination.

DISCUSSION

Numerous endeavors have been made to encourage hair growth, with the use of herbs for promoting hair growth being a practice that dates back for centuries. Amla, an essential herb commonly found in various traditional and commercial hair care products, has not been scientifically evaluated for its potential to stimulate hair growth as a gel. This study aims to formulate and assess the effects of Amla when used in a gel form for promoting hair growth.

The Amla used in this study was sourced from local vendors, air-dried in the shade, and then finely powdered to a particle size of 40 mesh. The hydro-alcoholic extract of Amla was employed for the formulation and evaluation of its hair growth properties. Alcohol was chosen as the solvent for extraction due to its effectiveness in extracting most phytoconstituents. The hydro

-alcoholic extract of Amla was prepared using 70% alcohol through soxhlation for 8 hours. Phytochemical analysis of the Amla extract revealed the presence of phenols, proteins, and phytosterols. The quantification of phytoconstituents, namely

polyphenols, flavonoids, and sterols, was performed using established colorimetric methods. The Amla extract exhibited a polyphenol content of 39.5% w/w GAE and a flavonoid content of 8%. Notably, the polyphenol content of Amla aligns with reported analytical specifications for commercial Amla extracts, although flavonoid and sterol content had not been previously reported for Amla.

Numerous formulations, both custom-made and commercially available, are used for promoting hair growth. In this study, the selected Amla extract was rich in polar constituents and largely water-soluble. Consequently, it was decided to formulate the extract into a hydrogel using Carbopol 934 as the gelling agent. PEG 400 was used to dissolve the herbal extract, and triethanolamine was employed to adjust the pH to that of the scalp (5.5-6). The formulated Amla gel was clear and met the necessary physicochemical criteria for a hydrogel. To evaluate the effects of the hydroalcoholic Amla extract on hair growth, the formulation did not incorporate fragrances, antioxidants, preservatives, or colorants. The resulting product was designated as "Amla 5% Gel."

The formulated gel underwent initial testing for skin irritation and was then evaluated for its impact on hair growth. The gel was found to be non-irritating when applied to shaved rat skin. Both control and experimental rats displayed no signs of tremors, convulsions, or reflex abnormalities, and their daily food intake remained normal during the seven-day repeated dosedermal toxicity assessment. No skin reddening, erythema, or lesions were observed in areas treated with the Amla gel.

The study's assessment of the Amla gel's effects on hair growth was conducted over a 30-day treatment period using Wistar albino rats. Key parameters considered included the time taken for hair growth to resume, hair length on the 15th, 20th, 25th, and 30th days of treatment, and hair density (the number of hair strands or follicles per unit area) on the 30th day. Both the treated and control groups displayed hair growth on the 7th day following shaving. Notably, the Amla gel formulation significantly improved hair length on the 15th, 20th, 25th, and 30th days compared to the control group. Moreover, the rate of hair growth in the Amla-treated groups outpaced that of the group treated with 2% minoxidil during the 30-day study period.

When examining hair density on the 30th day of treatment, it was evident that all treated animals experienced a significant increase in hair density compared to the control group. Furthermore, hair density in the treated animals surpassed that in the group treated with 2% minoxidil. This observation indicates that Amla serves as a more effective hair growth stimulator, enhancing both hair density and hair length. The hair growth-promoting properties of Amla may be attributed to its polyphenols, flavonoids, and sterol contents. Additional research is warranted to confirm the individual roles of these phytoconstituents in stimulating hair growth.

CONCLUSION

The present study aimed at formulation of *E.officinalis* Gaertn and evaluation of the same for hair growth.

Extraction of *E. officinalis* was done by soxhlation and yielded 8.18%. Phytochemical studies showed presence of phenols, proteins and phytosterols in *E.officinalis* extract.

Quantification of phyto-constituents showed that amla showed presence of 39.5% of polyphenols, 8% of flavanoids and 0.12% of sterols.

Hydrogel formulation of hydro-alcoholic extracts of Amla (5%) was prepared and gave a gel of satisfactory physicochemical parameters.

Gel formulation was tested for short term skin irritation; and was free from skin irritation.

Study for effects of the formulated gel was promoted hair growth both in terms of hair length as well as hair density in a 30 day study .This is the first report on scientific evaluation of *E.officinalis* for hair growth promoting activity.

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