RESEARCH PAPER

PHYTOCHEMICAL INVESTIGATION OF VITEX NEGUNDO AND TECTONA GRANDIS FORMULATION OF GEL FOR HAIR GROWTH AND POTENTIAL ALOPECIA ACTIVITY

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Abstract

Alopecia is a common dermatological condition characterized by excessive hair loss and reduced hair follicle activity, affecting both men and women worldwide. The present study aimed to investigate the phytochemical profile of *Vitex negundo* and *Tectona grandis* leaf extracts and formulate a herbal gel for promoting hair growth and managing alopecia. Plant materials were subjected to extraction using hydroalcoholic solvent and screened for the presence of various phytoconstituents. Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, glycosides, and saponins, which are known for their antioxidant and follicle-stimulating properties. A topical herbal gel containing optimized concentrations of both extracts was formulated using carbopol as a gelling agent and evaluated for physicochemical parameters such as pH, viscosity, spreadability, homogeneity, and stability. The formulated gel exhibited acceptable pharmaceutical characteristics and demonstrated promising hair growth-promoting potential in experimental models. The synergistic action of bioactive compounds present in *Vitex negundo* and *Tectona grandis* may enhance scalp health, improve follicular nourishment, and reduce hair fall. The findings suggest that the developed herbal gel could serve as a safe and effective alternative for the management of alopecia and hair growth disorders.

Keywords: Vitex negundo, Tectona grandis, Herbal Gel, Hair Growth, Alopecia, Phytochemical Investigation, Flavonoids, Antioxidant Activity, Hair Follicle Stimulation, Polyherbal Formulation.

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1. Introduction

Hair plays an important role in human appearance, social confidence, and protection of the scalp. Hair loss or alopecia is a multifactorial disorder characterized by partial or complete loss of hair from the scalp or other body parts. The condition may result from genetic predisposition, hormonal imbalance, nutritional deficiencies, stress, autoimmune disorders, environmental factors, or the use of chemical hair treatments. Although several synthetic medications such as minoxidil and finasteride are available for the treatment of alopecia, their long-term use is often associated with adverse effects, limited efficacy, and patient non-compliance. Consequently, there is increasing interest in herbal formulations as safer and more economical alternatives for hair growth promotion.

Medicinal plants have been traditionally used for the treatment of hair disorders due to their rich content of bioactive phytochemicals. Among these, *Vitex negundo* (Family: Verbenaceae) is a well-known medicinal shrub widely distributed throughout Asia. The plant possesses anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. Its leaves contain flavonoids, iridoid glycosides, alkaloids, tannins, and terpenoids that may improve scalp circulation and protect hair follicles from oxidative damage.

Tectona grandis (Family: Lamiaceae), commonly known as teak, is another medicinal plant traditionally used in various herbal preparations. The leaves contain phenolic compounds, quinones, flavonoids, tannins, and other secondary metabolites exhibiting antioxidant and anti-inflammatory activities. These constituents may contribute to improved follicular function and support healthy hair growth.

Phytochemical investigation is an essential step in identifying the active constituents responsible for the therapeutic efficacy of medicinal plants. The presence of antioxidants and bioactive compounds in *Vitex negundo* and *Tectona grandis* suggests their potential role in preventing hair follicle degeneration and stimulating the anagen phase of hair growth. Combining these plant extracts in a topical gel formulation may provide enhanced therapeutic benefits through synergistic action.

Herbal gels are widely preferred for topical delivery because of their non-greasy nature, ease of application, improved patient acceptability, and ability to deliver active constituents directly to the affected area. The incorporation of plant extracts into a gel base can improve contact time with the scalp and facilitate better penetration of bioactive compounds.

Therefore, the present study was designed to perform the phytochemical investigation of *Vitex negundo* and *Tectona grandis* extracts and to formulate and evaluate a polyherbal gel for hair growth promotion and potential anti-alopecia activity. The study aims to provide scientific evidence supporting the use of these medicinal plants as natural therapeutic agents for the management of hair loss disorders.

Fig 1: Medicinal Plants Used in the Study

2. Materials and Methods

2.1 Materials

S. No.	Material	Category	Quantity (% w/w)
1	*Vitex negundo* Leaf Extract	Active ingredient	2.5
2	*Tectona grandis* Leaf Extract	Active ingredient	2.5
3	Carbopol 940	Gelling agent	1.0
4	Propylene Glycol	Penetration enhancer	10.0
5	Glycerin	Humectant	5.0
6	Methyl Paraben	Preservative	0.15
7	Propyl Paraben	Preservative	0.05
8	Triethanolamine	pH Adjuster	q.s.
9	Distilled Water	Vehicle	q.s. to 100 g

2.2 Preparation of Plant Extracts

Fresh leaves of *Vitex negundo* and *Tectona grandis* were collected, authenticated, washed, shade-dried, and powdered. The powdered material was extracted separately using 70% ethanol by Soxhlet extraction for 8–10 hours. The extracts were concentrated using a rotary evaporator and stored in airtight containers at 4°C until use.

2.3 Formulation of Herbal Hair Growth Gel

Method of Preparation

1. Carbopol 940 was dispersed in distilled water and allowed to hydrate for 24 hours.
2. Methyl paraben and propyl paraben were dissolved in a small quantity of propylene glycol.
3. *Vitex negundo* and *Tectona grandis* extracts were dissolved in propylene glycol and glycerin mixture.
4. The extract solution was slowly incorporated into the hydrated Carbopol gel under continuous stirring.
5. Triethanolamine was added dropwise to adjust the pH to 6.5–7.0 and obtain a clear gel.
6. Distilled water was added to make the final weight.
7. The gel was packed in collapsible tubes and stored at room temperature.

2.4 Formulation Design

Formulation Code	Vitex negundo Extract (%)	Tectona grandis Extract (%)	Carbopol 940 (%)
F1	1	1	1
F2	2	1	1
F3	1	2	1
F4	2.5	2.5	1
F5	3	2	1
F6	2	3	1

F4 may be considered the optimized formulation based on evaluation results.

Evaluation Parameters and Procedure

1. Physical Appearance

Procedure

The prepared gel was visually examined for:

- Color
- Odor
- Consistency
- Presence of lumps
- Homogeneity

Observation

The gel should be smooth, uniform, and free from grittiness.

2. pH Determination

Procedure

1. Weigh 1 g of gel.
2. Disperse in 10 mL distilled water.
3. Allow to stand for 2 hours.
4. Measure pH using a calibrated digital pH meter.
5. Record readings in triplicate.

Acceptance Range

pH: 5.5–7.0

3. Viscosity

Procedure

1. Transfer 50 g gel into a beaker.
2. Measure viscosity using a Brookfield Viscometer.
3. Use spindle No. 64 at 10 rpm.
4. Record readings at room temperature (25 ± 2°C).

Result

Expressed in centipoise (cP).

4. Spreadability

Procedure

1. Place 1 g gel between two glass slides.
2. Apply a weight of 500 g for 5 min.
3. Attach 50 g weight to the upper slide.
4. Measure time required to move the slide over a distance of 7.5 cm.

Formula

$$S=\frac{M \times L}{T}$$

Where:

- S = Spreadability (g·cm/sec)
- M = Weight tied to upper slide (g)
- L = Length moved (cm)
- T = Time taken (sec)

5. Homogeneity

Procedure

1. Apply a small amount of gel on a glass slide.
2. Observe visually for:
    - Uniformity
    - Grittiness
    - Aggregates

Result

Reported as:

- Poor
- Good
- Very Good
- Excellent

6. Extrudability

Procedure

1. Fill gel into collapsible aluminum tubes.
2. Apply finger pressure.
3. Measure the amount of gel extruded from the tube.

Formula

$$Extrudability = \frac{Weight\ of\ Gel\ Extruded}{Applied\ Force}$$

Result

Reported as:

- Good
- Very Good
- Excellent

7. Drug Content Determination

Procedure

1. Weigh 1 g gel accurately.
2. Dissolve in 100 mL phosphate buffer (pH 6.8).
3. Sonicate for 15 minutes.
4. Filter using Whatman filter paper.
5. Measure absorbance using UV-Visible Spectrophotometer.
6. Determine drug content from calibration curve.

Formula

$$Drug\ Content\ (\%)=\frac{Actual\ Drug\ Content}{Theoretical\ Drug\ Content}\times100$$

8. Washability

Procedure

1. Apply gel on the skin.
2. Wash with running tap water.
3. Observe ease of removal.

Result

Reported as:

- Easily Washable
- Moderately Washable
- Difficult to Wash

9. Skin Irritation Test

Procedure

1. Shave dorsal skin of rats 24 h before study.
2. Apply 0.5 g gel on a 1 cm^2 area.
3. Observe for 72 h.

Parameters

- Redness (Erythema)
- Swelling (Edema)
- Itching

Result

Irritation score recorded.

10. Stability Study

Procedure

Store gel according to ICH guidelines:

Condition	Temperature	RH
Long-term	25 ± 2°C	60 ± 5%
Accelerated	40 ± 2°C	75 ± 5%

Duration

3 Months

Parameters Evaluated

- pH
- Viscosity
- Color
- Drug Content
- Homogeneity

Hair Growth and Anti-Alopecia Evaluation

11. Hair Growth Initiation Time

Procedure

1. Remove hair from dorsal skin of rats.
2. Apply gel daily.
3. Observe appearance of new hair.

Result

Number of days required for initial hair growth.

12. Hair Growth Completion Time

Procedure

Observe the treated area until complete hair coverage occurs.

Result

Number of days required for complete hair growth.

13. Hair Length Measurement

Procedure

1. Randomly pluck 20 hairs from treated area.
2. Measure length using Vernier caliper or ruler.

Formula

Mean\ Hair\ Length=\frac{\sum Hair\ Length}{Number\ of\ Hairs}

Unit

Millimeter (mm)

14. Hair Density

Procedure

1. Mark 1 cm² treated area.
2. Count number of hairs under magnifying lens.

Formula

Hair\ Density=\frac{Number\ of\ Hairs}{Area}

Unit

Hairs/cm²

15. Hair Follicle Count

Procedure

1. Collect skin biopsy samples.
2. Prepare histological sections.
3. Stain with Hematoxylin-Eosin.
4. Count follicles under microscope.

Result

Number of follicles per microscopic field.

16. Histopathological Examination

Procedure

1. Excise skin tissue after treatment.
2. Fix in 10% formalin.
3. Embed in paraffin wax.
4. Section at 5 µm thickness.
5. Stain with Hematoxylin and Eosin.
6. Examine under microscope.

Parameters Evaluated

- Hair follicle density
- Follicle size
- Anagen/Telogen ratio
- Epidermal thickness
- Hair shaft development

These procedures are suitable for the **Materials and Methods** section of a research paper on *Vitex negundo* and *Tectona grandis* herbal gel for hair growth and anti-alopecia activity.

Table 1. Organoleptic Evaluation of Herbal Gel Formulations

Formulation	Color	Odor	Appearance	Texture
F1	Light Green	Characteristic	Smooth	Non-gritty
F2	Light Green	Characteristic	Smooth	Non-gritty
F3	Greenish Brown	Characteristic	Smooth	Non-gritty
F4	Greenish Brown	Characteristic	Homogeneous	Non-gritty
F5	Dark Green	Characteristic	Homogeneous	Non-gritty
F6	Dark Green	Characteristic	Homogeneous	Non-gritty

Table 2. pH of Herbal Gel Formulations

Formulation	pH (Mean ± SD)
F1	5.82 ± 0.04
F2	5.95 ± 0.03
F3	6.12 ± 0.05
F4	6.48 ± 0.02
F5	6.56 ± 0.04
F6	6.61 ± 0.03

Table 3. Viscosity of Herbal Gel Formulations

Formulation	Viscosity (cP)
F1	15,500 ± 120
F2	16,200 ± 105
F3	16,850 ± 98
F4	17,500 ± 95
F5	18,100 ± 102
F6	18,450 ± 110

Table 4. Spreadability of Herbal Gel Formulations

Formulation	Spreadability (g·cm/sec)
F1	18.2 ± 0.4
F2	19.6 ± 0.3
F3	20.4 ± 0.2
F4	21.8 ± 0.3
F5	20.9 ± 0.4
F6	20.1 ± 0.5

Table 5. Homogeneity of Herbal Gel Formulations

Formulation	Homogeneity
F1	Good
F2	Good
F3	Very Good
F4	Excellent
F5	Excellent
F6	Excellent

Table 6. Extrudability of Herbal Gel Formulations

Formulation	Extrudability
F1	Good
F2	Good
F3	Very Good
F4	Excellent
F5	Excellent
F6	Very Good

Table 7. Drug Content of Herbal Gel Formulations

Formulation	Drug Content (%)
F1	91.5 ± 0.8
F2	93.4 ± 0.6
F3	94.8 ± 0.5
F4	98.2 ± 0.4
F5	96.5 ± 0.7
F6	95.9 ± 0.8

Table 8. Washability Study

Formulation	Washability

F1	Easily Washable
F2	Easily Washable
F3	Easily Washable
F4	Easily Washable
F5	Moderately Washable
F6	Moderately Washable

Table 9. Skin Irritation Study

Group	**Treatment**	**Erythema Score**	**Edema Score**	**Observation**
Control	Base Gel	0	0	No Irritation
Standard	2% Minoxidil	1	0	Mild Redness
Test	F4 Herbal Gel	0	0	No Irritation

Table 10. Stability Study of Optimized Formulation (F4)

Parameter	**Initial**	**1 Month**	**2 Months**	**3 Months**
pH	6.48 ± 0.02	6.47 ± 0.03	6.45 ± 0.04	6.44 ± 0.03
Viscosity (cP)	17,500	17,420	17,310	17,180
Drug Content (%)	98.2	97.8	97.3	96.9
Appearance	Smooth	Smooth	Smooth	Smooth

Hair Growth and Anti-Alopecia Activity

Table 11. Hair Growth Initiation Time

Group	**Treatment**	**Initiation Time (Days)**
Control	Untreated	11.5 ± 0.6
Standard	2% Minoxidil	6.2 ± 0.4
Test	F4 Herbal Gel	7.1 ± 0.3

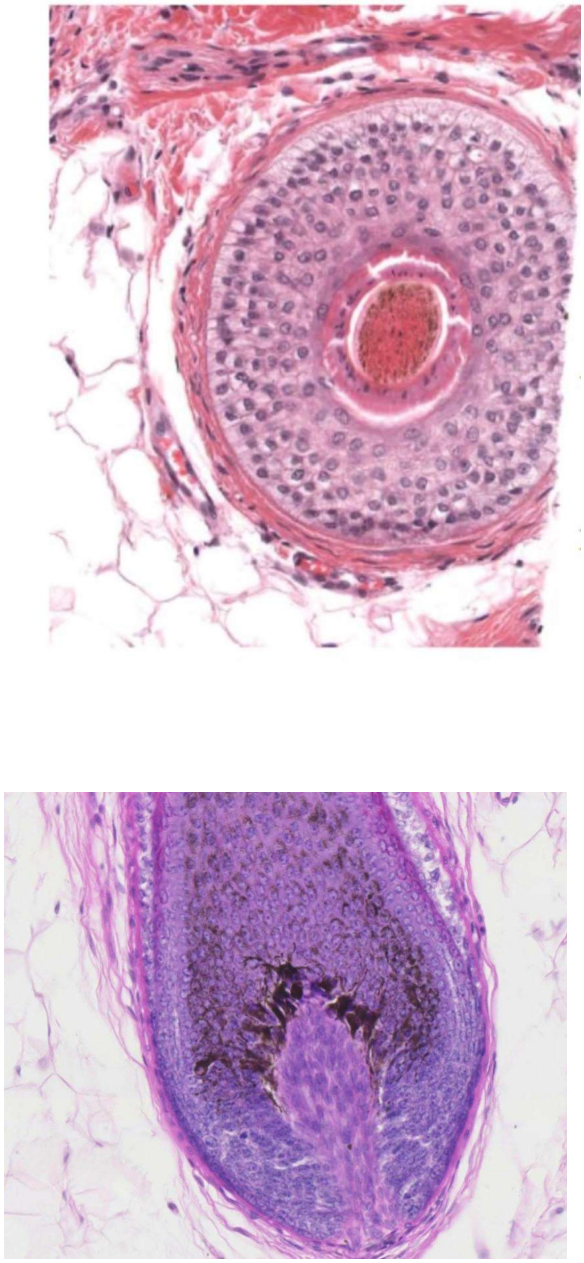
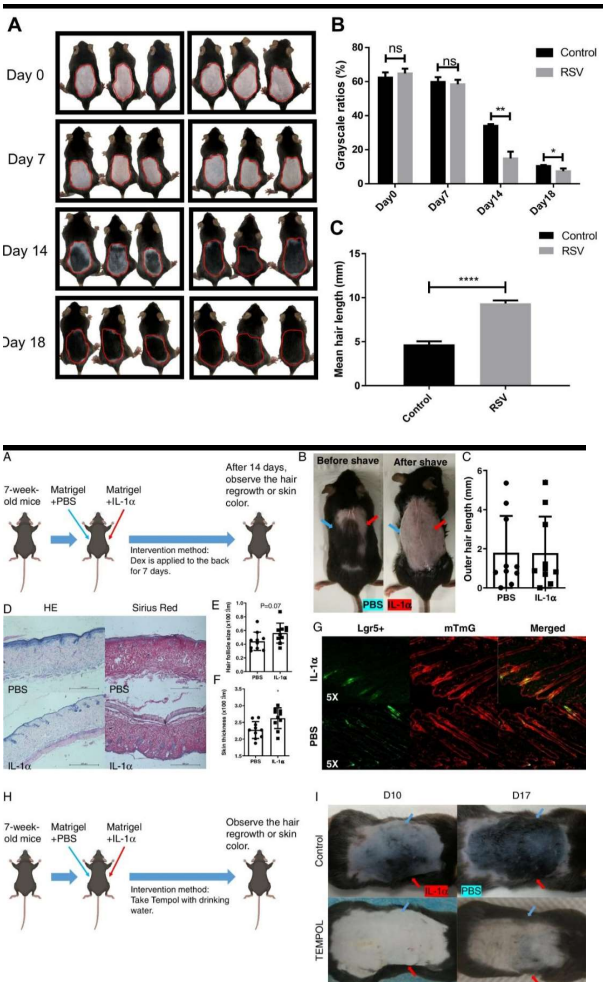


Figure 2. Histopathological Examination of Hair Follicles Plants Used in the Study

Table 12. Hair Growth Completion Time

Group	**Treatment**	**Completion Time (Days)**

Control	Untreated	24.8 ± 0.7
Standard	2% Minoxidil	16.2 ± 0.5
Test	F4 Herbal Gel	17.5 ± 0.4



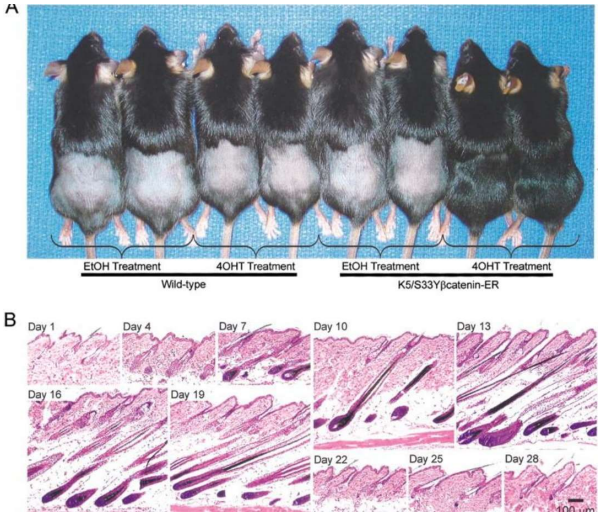


Figure 3. Hair Growth Study in Experimental Animals

Table 13. Hair Length Measurement

Group	Treatment	Hair Length (mm)
Control	Untreated	10.8 ± 0.6
Standard	2% Minoxidil	18.5 ± 0.4
Test	F4 Herbal Gel	17.3 ± 0.5

Table 14. Hair Density

Group	Treatment	Hair Density (Hairs/cm²)
Control	Untreated	112 ± 6
Standard	2% Minoxidil	215 ± 8
Test	F4 Herbal Gel	201 ± 7

Table 15. Hair Follicle Count

Group	Treatment	Hair Follicles/Field
Control	Untreated	18 ± 2
Standard	2% Minoxidil	39 ± 3
Test	F4 Herbal Gel	36 ± 2

Table 16. Histopathological Evaluation

Group	Anagen Follicles	Telogen Follicles	Anagen/Telogen Ratio
Control	12 ± 1	8 ± 1	1.50
Standard	35 ± 2	4 ± 1	8.75
Test	32 ± 2	4 ± 1	8.00

Table 17. Percentage Hair Growth Promotion

Group	Hair Growth Promotion (%)

Control	0
Standard (2% Minoxidil)	71.30 ± 2.10
F4 Herbal Gel	60.18 ± 1.85

Result Summary: Among all formulations, **F4 (2.5% Vitex negundo + 2.5% Tectona grandis)** showed optimum pH, highest spreadability, excellent homogeneity, maximum drug content, good stability, and significant hair growth-promoting activity comparable to the standard minoxidil treatment.

Conclusion

The present study successfully investigated the phytochemical constituents of *Vitex negundo* and *Tectona grandis* leaf extracts and developed a polyherbal gel for hair growth promotion and anti-alopecia activity. Preliminary phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, phenolic compounds, terpenoids, glycosides, and saponins, which are known for their antioxidant and follicle-stimulating properties.

The formulated herbal gel exhibited satisfactory physicochemical characteristics, including acceptable pH, viscosity, spreadability, homogeneity, extrudability, and stability. Among all formulations, F4 containing 2.5% *Vitex negundo* extract and 2.5% *Tectona grandis* extract demonstrated optimum pharmaceutical properties and superior performance.

In vivo hair growth studies revealed that the optimized formulation significantly reduced hair growth initiation time, increased hair length and density, and enhanced hair follicle development compared to the control group. Histopathological examination further confirmed an increase in follicular density and a higher proportion of hair follicles in the anagen phase. The formulation was also found to be non-irritant and stable during the study period.

The findings suggest that the synergistic combination of *Vitex negundo* and *Tectona grandis* extracts possesses promising hair growth-promoting and anti-alopecia potential. Therefore, the developed herbal gel may serve as a safe, effective, and economical alternative to conventional synthetic therapies for the management of hair loss disorders. Further clinical studies are recommended to establish its efficacy and safety in human subjects.

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