Phytochemical Investigation of *Convolvulus Pluricaulis* for Hair Growth Activity

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Abstract: Herbal cosmetics are the preparations used to enhance the human appearance. Many factors such as heredity, hormones, metabolism and side effects of antineoplastic and immunosuppressant drugs, have been negatively affecting the healthy hair growth. According to one survey, androgenic alopecia eventually affects about 50% of world's adult population. Natural products in the form of herbal formulations are available on the market and are used as hair tonic, hair growth promoter, hair conditioner, hair- cleansing agent, antidandruff agents, as well as for the treatment of alopecia and lice infection. Plants not directly used as medicinal purpose, when it processed and formulated as any one of the suitable formulation gives better therapeutic effect by means of dried powder form or extract from the plant with the advance technique. Many herbal products have been praised for their hair growth-promoting activities. Traditional Indian medicine praises many herbal remedies for hair growth promotion. Gel base formulation is a one of the topical formulation and it gives better absorption on the skin and less adverse effect comparable other formulation. When the plant formulated as gel it gives better absorption through skin and gives maximum therapeutic. Thus it is very important to develop new therapeutic formulation to stop hair loss and to increase hair growth. The current investigation was carried out to evaluate the hair growth enhancing potentiality of aqueous extract of *Convolvulus pluricaulis* plant. It is potent hair growth promoter and suggested to be an effective to synthesis hair growth promotor.

Keywords: *Convolvulus pluricaulis*, Hair growth activity, Alopecia, Gel, Herbal extract

Introduction: Hair is one of the crucial parts of body which is derived from ectoderm of the skin. It is often considered as one of the protective appendages on the body of human beings and animals. Cosmetopoeia refers to the use of plants or minerals for the care and embellishment of the body and its attributes [1]. Alopecia is a dermatological disorder that has been known for more than thousand years. It is seen all over the world and affects approximately 0.2-2% of the world population [2]. Alopecia refers to a dermatological disorder which has been known since years back and is considered to be a curse to mankind. Androgenic alopecia is a genetic condition which is observed in both populations, men as well as women. The classification of the balding scalp in men into types I-VIII proposed, it is still one of the most extensively used and well-known classification. In the male pattern baldness, Men generally start experiencing hair thinning and loss of hair as early as in their teen ages or early 20s. It is generally characterized by a receding hairline which is followed by gradual thinning and loss of hair from the crown and frontal scalp [3]. Conversely, women suffering from female pattern baldness usually do not suffer from the problem of thinning and loss of hair till their 40s or later. Generally, females experience an overall hair thinning over the entire scalp, with the most widespread hair loss at the crown [4].

In the traditional Indian system of medicine, numerous plants, herbs and herbal formulations have been reported for hair growth promotion as well as for improvement of quality of hair. Different types of ointments, plant mixtures, are used traditionally to maintain good hygiene and beautify the skin and hair of both men and women [5]. Previous studies of plants' medicinal and cosmetic potential in the region have revealed a variety of uses for hair growth, skin hydration, wound healing and toiletries. A number of genetic and environmental factors play a role in causing AGA. AGA is a frequent form of alopecia in which androgens progressively convert normal-sized scalp hair follicles to miniaturized hair follicle [6]. The hair cycle comprises three main states, the anagen or growth phase during which hair shaft elongation is induced, the catagen phase, also known as the apoptotic or involution phase, during which the epithelial cell population regresses drastically and finally the telogen or resting phase that concludes the cycle by allowing shedding of the hair follicle and preparation for entry into a new anagen phase. [7] Researchers that study hair growth focus on two key transition phases, anagen to

catagen and telogen to anagen, because potent hair growth inducers should deter entry into catagen and/or promote transitioning to a new anagen phase [8]. *C. pluricaulis* is a perennial herb that seems like morning glory. Its branches are spread on the ground and can be more than 30 cm long. The flowers are white in color (5 mm) and the leaves, which are elliptic in shape (2 mm), are located at alternate positions with branches or flowers. Known as Aloe weed in English, the herb is commonly found in India, especially in the state of Bihar. Many previous studies have reported the traditional uses of *C. pluricaulis*. This plant is reported to be a prominent memory improving drug, a psychostimulant and tranquilize, and reduce mental tension. There is a pertinent reference in Ayurvedic literature about the use of the drug as brain tonic in hypotensive syndromes. The pharmacological studies of the herb have shown varying degree of its hypotensive and tranquilizing effects. Clinical studies have exhibited demonstrable beneficial effects of *C. pluricaulis* on the patients of anxiety neurosis. The herb induces a feeling of calm and peace, good sleep and a relief in anxiety, stresses, mental fatigue, producing a significant reduction in the level of anxiety, neuroticism arising due to various levels of stresses [9-11].

MATERIAL AND METHODS

Phytochemical Studies: The plants were collected from Shivpuri Madhya Pradesh, India. The collected plants were carefully examined taxonomically identified from Dr. A. N. Rai, Prof. & HOD of Department of Botany, Dr. H.S. Gour University, Sagar, M.P.

Preparation of Extract: Extraction is the preliminary step involved in the phytochemical studies. Based on solvent's polarity metabolites are extracted and according to the solubility of the constituents in the solvent. The method of extraction is hot percolation method as about 200g of coarsely powdered plant was extracted with solvents of increasing polarity like methanol at 60-70°C. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, color and consistency of all the extracts were noted and were taken up for further detailed phytochemical and pharmacological screening. The various tudies i.e. determination of ash values, determination of extractive values, determination of moisture content [12].

Qualitative Phytochemical Analysis: Qualitative analysis for various phytoconstituents in extracts were carried out i.e. alkaloids, glycosides, steroids, carbohydrates, proteins, saponins, gums and mucilage and fixed oils and fats

Formulation of Gel base: Gelling agent Carbopol 940 2 m was dispersed in sufficient quantity 10ml of water. Propylene glycol- 5ml which is used as humectants or plasticizer was added to the dispersion. Other excipient such as methylparaben (0.15gm) and propyl paraben (0.15 gm) were added with continuous stirring. The Carbopol gels pH of the vehicle was brought to neutral by using TEA (Triethanolamine). The final weight of the gel was adjusted to 50 gm with distilled water. Then the mixture was stirred by using propeller for 2 hours at 500 rpm. After stirring, this homogenous gel appeared to be free of bubbles. It was kept at room temperature for 24 hours to check the consistency and stability of gel [13-14].

Formulation of herbal hair gel: The herbal hair gel was prepared by incorporating methanolic extract of plants liquid in to optimized Carbopol gel base. On the other hand, accurate amount of methanolic extracts of plant *Convolvulus pluricaulis* extract (1%, 1.5% and 2%), propylene glycol (5 ml) , methylparaben (0.15 gm) and propylparaben (0.15 gm) were added to the Carbopol 940 (2 gm in 10 ml water) polymeric dispersion. Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. It was then stirred by using propeller for 2 hours at 500rpm. After stirring, the prepared gel appeared to be homogenous and devoid of air bubbles. The prepared gel was kept at room temperature for 24 hours [15].

Evaluation of herbal hair gel: The prepared herbal gel was subjected to physical characterization such as color, appearance, pH, viscosity, spreadability. It was also evaluated for its stability property, antimicrobial activity and in vivo skin irritation study.

Physical appearance: The formulated herbal gel was inspected visually for their color, odor, homogeneity and consistency. All developed gels were tested for homogeneity by visual inspection after gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of pH: The pH of various formulations was determined by using digital pH meter. One gram of gel was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate [16].

Determination of Viscosity: The measurements of viscosity of prepared gels were carried out with Brookfield viscometer (Brookfield viscometer RVT) with spindle No.62.

Spreadability: Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. The other slides was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide.100gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times and the mean time taken for calculation. Spreadability was calculated by using the following the formula:

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

Where, S = Spreadability, M = Weight in the pan (tied to the upper slide) L = Length of the glass slide, T= Time (in sec) taken to separate the slides.

Bloom Strength: The bloom strength of the gel was determined by means of Texture Analyzer equipped with 5 kg load cell using a cylindrical probe of 0.5 diameter as fixture. The sample in the container was placed centrally on the platform beneath the cylindrical probe. After calibrating the height of the probe, the test was commenced. A trigger force of 10 g was used for the study.

Extrudability: The gels were incubated at room temperature for 2 h before measuring their extrudability using an HDP/FE forward extrusion cell of the TA-XT2 Texture Analyzer equipped with a 5 kg load cell. Prior to measurement, the gel was manually stirred and loaded (100 g) into the cell. The compression force was measured at the following conditions: pre-test speed 1 mm/s, test speed 1 mm/s, trigger force 10 g, post-test speed 10 mm/s, compression distance 20 mm, and outlet diameter of extrusion cell 3 mm.

Drug content studies: Herbal gel (500 mg) was taken and dissolves in 50 ml of phosphate buffer pH 7.4. The volumetric flasks were kept for 2 h and shaken well in a shaker to mix it properly. The solution was passed through the Whatman filter paper and filtrates were analyzed for galic acid content spectrophotometrically at 260 nm against corresponding gel concentration as blanks [17].

in-vitro diffusion studies: Before experiment, the cellophane membrane was washed in the running water and then soaked in distilled water for 24 h to remove glycerine present on membrane. The release of herbal gel was studied by dialysis method in pH 7.4 artificial skin pH. 2 ml samples were instilled in the dialysis bag which was screwed with two clamps at each end. The dialysis bag was dipped into the receptor compartment containing 35 ml of dissolution medium and stirred continuously at 100 rpm. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5°C. The receptor compartment was closed to prevent evaporation of the dissolution medium. The solution on the receptor side was stirred by externally driven teflon coated magnetic bars. At predetermined time intervals, 5 ml of solution from the receptor compartment was pipette out and immediately replaced with fresh 5 ml phosphate buffer. Samples were withdrawn at regular time intervals, and the same volume was replaced with fresh dissolution medium. The samples were spectrophotometricially measured at 260 nm to determine gallic acid release.

% drug release = (Conc. of drug (in mg) x Volume of receptor compartment) x 100 / Label claim (amount of drug in donor compartment)

***in vivo* study:** Albino rat of either sex were taken for the experiment. Weight was noted and placed in the animal house at a room temperature in controlled humidity conditions

with a 12:12 h light and dark cycle for at least 7-14 days prior to the experiment. Animals have access to standard laboratory diet and water ad libitum [18]

Experimental Design

The animals were brought to the experimental area and kept for half an hour. Animals were divided into following groups.

Group 1: Control

Group 2: Treated

Group 3: Formulation CGH1

Group 4: Formulation CGH2

Group 5: Formulation CGH3

Specific back portion of animals were depleted by shaving before the treatment. Control group were kept without treatment and another group was treated with different formulations at two times in a day with rubbing on skin. The dorsal part of the rats, were observed and photographed either every 3 days or at specific time intervals (Day 1, 7, 14 and 21) in order to notice the start of hair regrowth period and the hair regrowth pattern according to The effect of Gel on hair regrowth potential scored as described by Matsuda et al, 0 = no hair growth, 1 = less than 20% of hair growth, 2=20-39% of hair growth, 3=40-59% of hair regrowth, 4=60-79% of hair regrowth, 5=80-100% of hair regrowth. Another parameter was scales of hair regrowth, such as: Type 4 (high hair density, full, thick fur), Type 3 (moderate hair density with no visible skin area), Type 2 (low hair density, with the visualization of the skin), Type 1 (uneven hair growth on the test area, skin easily seen).

Results And Discussion

Physiochemical constants: The percentage of total ash, acid insoluble ash, sulphated ash and water-soluble ash were investigated. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug give an idea of the earthy matter or the inorganic composition and other impurities

present along with the drug. The loss on drying and foreign matter was 9.50 and 0.10 respectively. The extractive values are primarily useful for the determination of exhausted drugs.

Extraction of plant material: The yield of methanolic extract of *Convolvulus pluricaulis* plant was in semi-solid form. 100 g of crude plant material was taken for extraction, The extract was in semisolid- solid form. The extract was stored in tightly packed container and it was 18.87 %.

Preliminary phytochemical analysis: Investigations on the preliminary phytochemical screening of *Convolvulus pluricaulis* extracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and carbohydrates in methanolic and aqueous extracts respectively.

Optimization of Gelling Agent: Different concentrations of carbopol 940 such as 1, 1.5 and 2% were optimized to obtain gel with desired physical characteristics. Carbopol gel with 2% concentration (BG3) shows good physicochemical properties for incorporating methanolic extracts of plants. The main ingredient of the gel formulation is the gelling agent from all required chemicals. The concentration of viscosity enhancer or gel former is of huge value as a less concentration will lead to simple solution or lotion with very low consistency. The high concentration of gel forming agent may lead to formation of gels with high viscosity leading to non uniform distribution of drug and showed problem with handling of gel. As the result of all evaluation parameters, 2 % of Carbopol 934 was selected as the optimized concentration of gelling agent and this gel formulation is used for further herbal hair gel preparations.

Formulation of herbal hair gel: The herbal hair gel *Convolvulus pluricaulis* extracts was incorporated into optimized 2% Carbopol gel base. Different concentrations of ethanolic extract of plants such as 1, 1.5 and 2% were incorporated in to Carbopol gel base. Convolvulus pluricaulis extract concentration was kept constant [5 ml] in the entire Carbopol gel base.

Evaluation of herbal hair gel

Physical appearance: The formulated herbal hair gel was checked visually for color, appearance and homogeneity and the results were listed in Table 3.

Measurement of pH: The pH of all prepared formulation ranged from 5.7-5.9. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin.

Determination of viscosity: Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from gel. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 62. By keeping the viscosity below about 15,000 cps, the advantages of more appealing cosmetic characteristics and ease of accurate application through improved flow and pourability are achieved.

Spreadability: Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the herbal hair gel, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. Spreadability of different gel formulation were studied. The formulation AHG2 produced good spreadability than the other formulation.

Drug content studies: Drug content was analyzed for galic acid content spectrophotometrically at 260 nm against corresponding gel concentration as blanks.

Hair growth study at rat animal model: The prepared herbal gel formulations of the selected plant were taken for the determination of hair growth activity. The evaluation of prepared gel was carried on the animal (rat) and compared with the reference who applied gel without the extract. The growth of hair were found more in CHG3 gel formulation treated group than CGH1 and CGH2.

Conclusion: Herabal hair gel formulations of the selected plant were taken for the determination of hair growth activity. The evaluation of prepared gel was carried on the animal (rat) and compared with the reference who applied gel without the extract. The growth of hair were found more in CHG3 gel formulation treated group than CGH1 and CGH2

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Table 1: Preliminary phytochemical screening of plants

Chemical constituents	*Convolvulus pluricaulis extract*
Phenols	+
Flavonoids	-
Steroids	+
Triterpenes	-
Tannins	+
Saponins	+
Alkaloids	+
Glycosides	+
Carbohydrates	+

+ Denotes the presence of the respective class of compounds.

Table 2: Evaluation of base hair gel formulation

Parameters	Formulations		
	BG1	BG2	BG3
Feel of application	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	13.2	12.5	10.7
Consistency	Poor	Good	Good
pH	6.22	6.43	7.01
Viscosity (cps)	0.88	0.91	0.95
Extrudibility	Good	Good	Excellent

Table 3: Physical appearance of formulated herbal hair gel

Parameters	CHG1	CHG2	CHG3
Physical appearance	Transparent yellow gel	Transparent yellow gel	Transparent yellow gel
Color	Pale yellow	Pale yellow	Pale yellow
Homogeneity	Absence of aggregates	Absence of aggregates	Slight aggregates

Table 4: Physical parameters of various formulations (CHG1-CHG3)

Formulation code	pH	Viscosity [cps]	Spreadability (gm.cm/sec)	Gallic acid content (%)
CHG1	6	1428±0.1	19.37	7.37
CHG2	6	1425±0.75	21.35	8.25
CHG3	6	1358±0.25	22.13	9.43

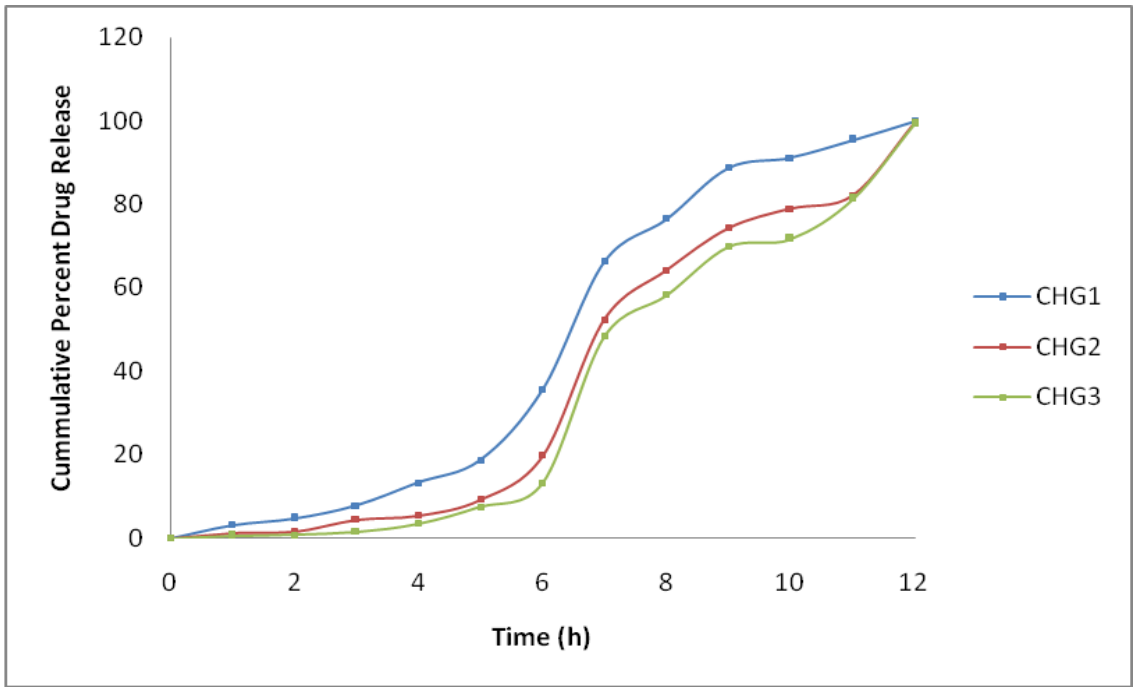


Figure 1: Zero - order kinetic plot (in-vitro drug diffusion study of herbal hair gel)

Table 5: Hair growth pattern with different formulation in Control and treated group

S. No.	Days	Control	Treated		
			CHG1	CHG2	CHG3
1	Day 1	No growth	No growth	No growth	No growth
1	Day 7	Type 1	Type 1	Type 1	Type 2
2	Day14	Type 2	Type 1	Type 2	Type 3
3	Day 21	Type 2	Type 2	Type 2	Type 4

Type 1 (uneven hair growth on the test area, skin easily seen)

Type 2 (low hair density, with the visualization of the skin)

Type 3 (moderate hair density with no visible skin area)

Type 4 (high hair density, full, thick fur)

Table 6: Percentage of hair regrowth with different formulations

S. No.	Days	Control	Treated		
			CHG1	CHG2	CHG3
1	Day 1	0	0	0	0
1	Day 7	1	1	1	3
2	Day14	2	1	2	3
3	Day 21	2	2	2	4

0 = no hair growth, 1 = less than 20% of hair growth, 2=20-39% of hair growth, 3=40-59% of hair regrowth, 4=60-79% of hair regrowth, 5=80-100% of hair regrowth.

Table 7: Hair growth pattern with different formulation in Control and treated group

Day Group / Number of Animals	Number of Animals	1	7	14	21
Control Group	6	0±0.00	1.66±0.21	1.83±0.16	2.33±0.33
CHG1	6	0±0.00	1.66±0.21	2.33±0.21	2.50±0.22
CHG2	6	0±0.00	1.00±0.00	2.33±0.21	2.50±0.22##
CHG3	6	0±0.00	2.30±0.21	3.30±0.21	3.80±0.16***

Values are mean ± SEM, n = number of rats per group, *** *P*<0.001 vs. Control; ##*P*<0.01, vs. Control;

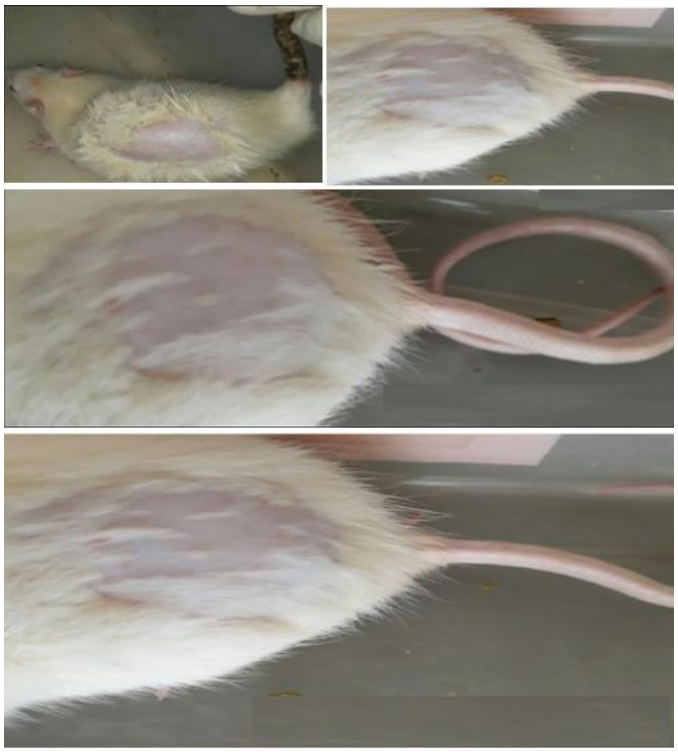


Figure 2: Hair growth study at in-vivo study data