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Potentiality of aqueous leaf extract of *Trichosanthes cucumerina* Linn. on hair growth promotion in Wistar albino rats

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Trichosanthes cucumerina Linn. (Snake gourd) is an annual climber which is widely distributed in Asian countries. It is a rich source of nutrition. The leaves of the plant are used by the folklore for alopecia. Hence, in the present investigation the hair growth promoting activity was evaluated on Wistar albino rats. The preliminary chemical screening of the aqueous extract revealed the presence of carbohydrates, flavonoids, saponins, flavonol glycoside and triterpenoid saponins. The animals were divided into four groups and the control group was applied with water, standard group with 2% Minoxidil and two test groups with 150 and 300 mg/kg of aqueous extract of *T. cucumerina* leaves. Qualitative and quantitative parameters were evaluated. It was observed that the hair growth completion, length of hair, percentage of hair follicles and diameter of bald patch and concentration of minerals in the blood for the test animals applied with 300 mg/kg drug was comparable with that of the animals treated with 2% Minoxidil. This study reveals that the leaves of *T. cucumerina* are a potent hair growth promoter which supports the traditional claim.

Keywords: Alopecia, Anagen, Bald patch, Hair follicles, Hair growth promoter, Minerals, Minoxidil, Snake gourd, Telogen, *Trichosanthes cucumerina*.

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Introduction

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have lead to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments[1]. Hair growth is common biological process observed in animals and human beings. Hair on scalp grows about 3-4 mm/day or 6 inches per year. The hair growth and loss is completely random and is not seasonal or cyclic. There are three stages of hair growth. The stages include anagen (growth phase), catagen (transitional phase) and telogen (resting phase). Anagen is the first phase of hair growth cycle which is also known as the growing phase. At any one time, 80-90 percent of hair follicles on scalp are in the anagen phase. During this period hair grows continuously for 3 to 7 years at the rate of half an inch a month. After the anagen phase, hair will turn into a transitional phase before going to rest. This short phase is known as the catagen phase which last for 2 to 4 weeks. During this time, hair detaches from the blood supply. The detached follicle will slowly shrink to about 1/6 its size. The hair bulb stops producing the colour pigment. The bulb will be pushed upwards towards the surface when the new hair is formed. Approximately 2-3% of hair will be in this phase in scalp. Telogen is the final phase of hair growth cycle. It is also known as the resting phase where the hair follicles will slowly fall off and replaced by a new hair. Around 10-15% of the hair in the scalp will be in telogen phase. 50-100 hairs from this phase will shed daily. This period lasts for 3 months before the hair fall out[2]. A number of things like illness or a major surgery, hormonal problems, pregnancy, anticoagulants, medicines used for gout, high blood pressure or heart problems, excess of vitamin A, birth control pills and antidepressants and fungal infections may cause hair fall.

Trichosanthes cucumerina Linn. (Family — Cucurbitaceae) commonly called as Snake gourd is a monoecious annual climbing herb with branched

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tendrils (Plate 1). It is called as *Potlakaaya* in Telugu, *Padavalanga* in Malayalam, *Chichinga* in Bengali and *Jangli Chachinda* in Hindi. It is distributed in temperate Asian regions like China, tropical regions of Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Vietnam, Indonesia, Malaysia and Philippines. *T. cucumerina* is a rich source of nutrition and contain proteins, fat, fibre, carbohydrates, vitamin A, E, carotenoids, flavonoids, lycopene, phenolics and ß-carotene. The fruit is rich in vitamin C and E. Other elements found in high amounts are Na, Mg and Zn. The triterpenes found are: 23, 24-dihydrocucurbitacin D, 23,24-dihydrocucurbitacin B, cucurbitacin B, 3β-hydroxyolean-13(18)-en-28-oic acid, 3-oxo-olean-13(18)-en-30-oic acid and the sterol 3-*O*-*β*-D-glucopyranosyl-24ξ-ethylcholest-7,22-dien-3*β*-ol. *T. cucumerina* is used in the treatment of headache, alopecia, fever, abdominal tumours, bilious, boils, acute colic, diarrohea, haematuria, skin allergy, abortifacient, vermifuge, stomachic, refrigerant, purgative, malaria, hydragogue, hemagglutinant, emetic, cathartic, bronchitis and anthelmintic[3-11]. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. Hence, in the present research work, a systematic study was carried out to evaluate the hair growth promoting activity of the plant as this activity was not proven scientifically.

Materials and Methods

Plant material

The fresh plant was collected in the month of November to February from the surrounding areas of Nalgonda District, A.P, India. The plant material was authenticated by Mr. Lakshma Reddy, Retd. Lecturer in Botany Department, Nagarjuna Government College (Affiliated to Osmania University) Nalgonda. The plant was certified as *Trichosanthes cucumerina* Linn. (Family–Cucurbitaceae), Voucher no: NCOPNLG/ph'cog/ 2009-10/002. A herbarium was prepared and deposited in the Department of Pharmacognosy, Nalanda College of Pharmacy for future reference.

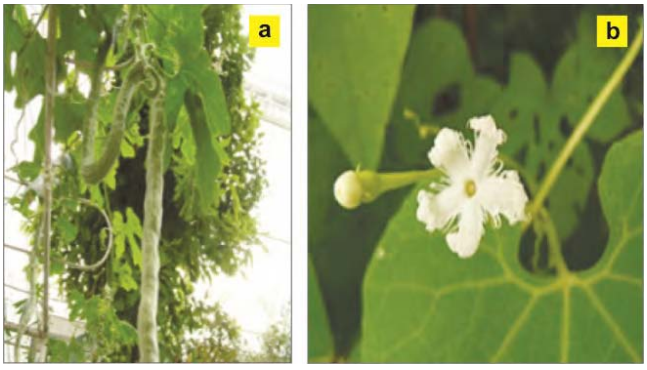

Plate 1—*Trichosanthes cucumerina*: a- Whole plant, b- Flower and leaf

Equipments and Chemicals used

Rotary vacuum evaporator (Indosatt Scientific Lab Equipments), Dhona electronic balance (Dhona Instrument Ltd./Model no. Dhona 200D, Kolkata), UV-VIS Spectrophotometer (Elico Ltd/Model no. SL 196, Hyderabad). All the drugs and chemical used in this study were of analytical grade. Commercially available 2% Minoxidil (Dr. Reddy's Laboratory) was used.

Preparation of plant extracts

The plant materials collected were shade dried and powdered. Powder (250 g) was weighed and defatted with petroleum ether and then exhaustively extracted with water to obtain the aqueous extract. It was then concentrated under reduced pressure at 40°C in a rotary vacuum evaporator to obtain a concentrated extract which was then subjected to preliminary chemical screening[12].

Experimental animals

Wistar albino rats (150-200 g) of either sex were procured from National Institute of Nutrition, Hyderabad, AP, India. The experimental protocol was approved from the Institutes animal ethics committee under the reference no. NCOP/IAEC/approval/07/2010 and then experimental studies were undergone according to their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet (Goldmohar brand, Lipton India Ltd.) and water *ad libitum.*

Hair growth promoting activity[13-20]

Wistar albino rats of either sex weighing 150-250 g were taken. They were maintained in condition below room temperature. They were caged and provided with food and water *ad libitum*. The hair on dorsal portion was clipped with scissors and removed with hair removing cream in an area of 3 cm^2. The animals

were divided into four groups with six animals in each group. Group I was applied water (negative control). Groups II and III were applied 150 and 300 mg/kg of test drug (aqueous extract of *T. cucumerina* leaf). Group IV was applied 2% Minoxidil (positive control). The drug was applied twice daily for 30 days on depilated area. The qualitative and quantitative evaluations were performed to determine the hair growth promotion.

Qualitative and Quantitative observation of hair growth

The parameter considered here is the time taken to initiate hair growth and time taken to complete hair growth was observed. The hair follicles were observed for the respective stage of hair growth. Anagen and telogen phases were considered and the percentages of follicles in both stages were accounted.

Effect of aqueous extracts on hair length and growth of albino rats

Evaluation of the average length of hair was calculated taking 25 hairs. The length of hair was noted for every ten days. The completion of hair growth was considered based on the average length of hair. The hair growth in the hair removed area was observed and the time taken to cover the bald patch was noted.

Table 1—Qualitative observation of hair growth

Treatment group	Dose	No. of days taken for hair growth initiaton	No. of days taken for hair growth completion
Group I	Water	3	23
Group II	150 (mg/kg)	3	21
Group III	300 (mg/kg)	2	18
Group IV	2% Minoxidil	2	16

Group I-Water as control, Group II-150 mg/kg of test drug, Group III-300 mg/kg test drug
Group IV-2% Minoxidil as standard. Route of administration- Topical application

Change in concentration of minerals during hair growth

The animals were anaesthetized and blood samples were collected and diagnosed for concentration of iron, zinc, total protein which aid hair growth.

Statistical analysis

The statistical calculations were performed using the soft ware Graph Pad Instat. Values are mean ± S.D., where n=6.

Results

Hair loss is one of the dermatological disorders to human race which is common throughout the world and is of great concern for decades[21]. Minoxidil, a potassium channel opener proved to be effective in 54% of the treated subjects. The treated subjects exhibited significant adverse dermatological reactions such as pruritis, dryness, scaling, local irritation and dermatitis[22]. These factors lead to the search for novel drugs that revitalize the hair growth pattern and appearance with less adverse effects. Hence, screening of medicinal plants for its potential as a hair growth promoter was brought into limelight. The aqueous extract of *T. cucumerina* was found to contain carbohydrates, flavonoids, saponins, flavonol glycoside and triterpenoid saponins by preliminary phytochemical screening. Pharmacological screening of aqueous extract infered that *T. cucumerina* produced a very good hair growth promoting activity which was almost comparable to 2% Minoxidil. The number of days taken for hair growth initiation and completion of the aqueous extract at 300 mg/kg was almost similar to that of 2% Minoxidil (Table 1). Length of hair on the 10^{th}, 20^{th} and 30^{th} days were accounted and it was observed that there was a potential increase in the hair length on the 20^{th} day of treatment (Table 2). The aqueous extract at 300 mg/kg converted telogen phase hair to anagen phase which

Table 2—Effect of aqueous extracts on hair length of Wistar albino rats

Treatment groups	Dose	Length of hair (mm)		
		Day 10	Day 20	Day 30
Group I	1ml Water	7.83±0.052*	17.83± 0.038*	20.51±0.061*
Group II	150(mg/kg)	9.17±0.033*	18.47±0.033*	21.11± 0.063*
Group III	300 (mg/kg)	9.59±0.035*	18.68± 0.055*	22.79±0.045*
Group IV	2% Minoxidal	9.48±0.057*	18.97± 0.038*	22.45±0.024*

Group I-Water as control, Group II-150 mg/kg test drug, Group III-300 mg/kg test drug, Group IV-2% Minoxidil as standard. Route of administration- Topical application. Values are mean ± S.D., where n=6. *P<0.05 compared with control.

Table 3—Quantitative observation of hair growth

Treatment Group	Dose	Density of hair folicles (%)					
		Day 10		Day 20		Day 30	
		Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Group I	Iml water	30	70	51	49	55	45
Group II	150 (mg/kg)	33	66	59	40	61	37
Group III	300 (mg/kg)	39	61	62	37	65	33
Group IV	2% Minoxidal	48	52	68	32	69	31

Group I-water as control, Group II- 150 mg/kg test drug, Group III- 300mg/kg test drug, Group IV- 2% Minoxidil as standard. Route of administration- Topical application.

Table 4—Effect of aqueous extract of *T.cucumerina* on hair growth

		Number of days									
		0	1	2	3	4	5	6	7	8	9
Treatment groups	Dose	Diameter of the bald patch (cm^2)									
Group I	1 ml water	3±0*	3±0*	3±0*	3±0*	2.7±0.1*	2.1±0.1*	1.6±0.14*	1.0±0*	0.6±0.16*	0.4± 0.1*
Group II	150 mg/kg	3±0*	3.0±0.1*	2.8±0.12*	2.3±0.16*	1.9+0.09*	1.4±0.13*	0.9±0.11*	0.6±0.17*	0.4±0.17*	0.2±0.1*
Group III	300 mg/kg	3±0*	2.9±0.2*	2.7±0.13*	2.4±0.11*	1.7±0.1*	1.6±0.16*	0.8±0.09*	0.4±0.11*	0.2±0.12*	0±0
Group IV	2% Minoxidil	3±0*	2.8±0.12*	2.7±0.16*	2.5±0.15*	1.9±0.11*	1.3±0.13*	1.0±0.11*	0.3±0.11*	0±0	0±0

Group I-Water as control, group II- 150mg/kg test drug, Group III- 300mg/kg test drug, Group IV- 2% Minoxidil as standard.Route of administration- Topical application. Values are mean ± S.D., where n=6. *P<0.05 compared with control.

Table 5—Change in concentration of minerals

Treatment groups	Dose	Minerals	Mineral conc. in blood		
			Day 0	Day 15	Day 30
Group I	1ml water	Iron µg/dL	63.0	63.5	63.9
		Zinc µmol/L	84.4	84.9	85.3
		Protein total g/dL	6.2	6.43	6.72
Group II	150 mg/kg	Iron µg/dL	64.0	65.2	65.8
		Zinc µmol/L	85.9	86.3	86.7
		Protein total g/dL	6.3	6.48	6.85
Group III	150 mg/kg	Iron µg/dL	64.2	65.3	65.9
		Zinc µmol/L	86.3	86.9	87.1
		Protein total g/dL	6.0	6.56	6.91
Group IV	2% Minoxidil	Iron µg/dL	58.1	58.9	58.1
		Zinc µmol/L	84.3	84.5	84.8
		Protein total g/dL	6.0	6.3	6.5

Group I-Water as control, Group II-150 mg/kg test drug, Group III-300 mg/kg test drug, Group IV-2% Minoxidil as standard. Route of administration-Topical application.

indicated follicle stimulatng property and can be used for non-androgenic alopecia (Table 3). The decrease in diameter of bald patch took place and the area was totally covered with hair in case of standard 2% Minoxidil and aqueous extract at 300 mg/kg on the 8th and 9th day (Table 4). A mild rise in concentration of minerals in the groups treated with the plant extract was observed during the period. The concentration of Fe, Zn and total protein in blood was increased notably during the first fifteen days of treatment (Table 5). The aqueous extract showed a result almost equal to that of standard 2% Minoxidil. The strength

and growth of hair was based on mineral concentrations in blood.

Discussion

In the present investigation the hair grwoth promotng activity on rodent model was performed and the results obtained were very promising. Herbal drugs generally exert their hair growth promoting effects by improving blood flow to scalp either by slight skin irritation or by angiogenesis (improving vasculature through endogenous substances)[23]. *T. cucumerina* might have shown the hair growth by increasing the blood flow by producing mild irritation, there by producing vasodilation. As per the results of the phytochemical screening, it was observed that the extract was rich in flavonoids and saponins, which were reported to be potent antioxidant agents. Hence, it can be assumed that the plant might have produced an antioxidant effect, which has contributory effect in hair growth. There was considerable reduction in the hair growth initiation and completion for the test drug and standard as compared to the control group of animals. A promising hair length was observed for the test drug which may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle[23]. Hair follicles are metabolically active tissues that require nutrients to support both structural and functional activities[24]. Poor nutrition may be reflected by a dull, dry, brittle or thin hair coat. Protein, phosphorus, iodine, zinc, and vitamins A, B, C and E, as well as dietary excesses of selenium, iodine, copper and cobalt are essential for hair growth[25]. Zinc is an essential element to many metalloenzymes and metabolic processes, including keratogenesis. Copper deficiency results in fibre depigmentation and loss of hair tensile strength and elasticity leading to breakage. Protein deficiency arising through starvation, low protein diet, or chronic catabolic disease results in hair production of abnormal texture and decreased length and diameter[26]. It was observed from the mineral analyses that the extract produced an increase in mineral concentrations which are essential for hair growth. As the plant has nutritional value it has reflected in the mineral content evaluated in the blood. The standard drug did not show much change in this value, which shows that minoxidil donot have any effect on mineral content in the blood.

Conclusion

From present research, it is concluded that the hair growth activity of aqueous extract of *T. cucumerina* exhibited more substantial effect which was comparable to the standard 2% Minoxidil. However, the exact mechanism is unknown and hence identification and isolation of active constituents from the extracts may guide in new directions for treatment of hair loss. Hence, further research is essential for structural interpretation and identifying the mechanism of action responsible for using *T. cucumerina* as an apparent hair growth promoter.

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