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### Effect of ethanolic extract of *Hibiscus rosa sinensis* L., flowers on hair growth in female wistar rats

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#### ABSTRACT

*Hibiscus rosasinensis* flower is known as hair growth promoter in traditional and folklore medicines. Petroleum ether extract of *H.rosasinensis* is reported in literature for hair growth promotion. The study was aimed to investigate the efficacy of ethanolic extract of *H.rosa sinensis* flower as hair growth promoter. Female wistar rats were selected for hair growth promotion studies. They were divided in three groups and their skin was denuded with hair removing cream, electronic shavers and hair clippers for ensuring complete removal of hair. Then 2% solutions of vehicle (control), minoxidil (standard) and ethanolic extract of *H.rosa sinensis* flowers respectively were applied on shaved denuded skin of different groups of rats twice a day for thirty days. During this period they were observed visually for pattern of hair growth studies and after treatment period their skin biopiosis were taken for follicular density and cyclic phases of hair growth. On the basis of visual observation of animals and histopathology, ethanolic extract of *H.rosasinensis* flowers showed shorter hair and take more time for growth and favours telogenic stage of hair follicles as compared to control thus it showed hair growth retarding activity inspite of hair growth promoting one. Which is also of use in retarding body hair growth for beautification in females. This study proved that ethanolic extract of *H.rosa sinensis* flower may have potential as hair growth retarding agent so it may use in preparations for hair removing creams .

**Key Words-** Hair, *H. rosa sinensis*, minoxidil, wistar rats, ethanolic.

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#### INTRODUCTION

Hair loss is a dermatologic disorder, and the surge for discovering natural products with hair growth promoting potential is continuous [1,2].Hair loss, or alopecia ,is a common patient complaint and a source of significant psychologic and physical distress[3].Androgens are

considered to be one of the most important causes for alopecia apart from a variety of other factors[4]. 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[5]. A number of herbal products have been acclaimed with hair growth promoting activity.

*H. rosasinensis* is known as japa in sanskrit, jasum in hindi, jaba in bengali, Chinese hibiscus in english, etc. It is an evergreen woody glabrous showy shrub of 1.5-2.4m in height. Flowers are axillary, solitary, campanulate, red, blue, yellow or white, 10.2-15.2 cm diameter. Capsules are rotund, many seeded.

It called Keshya (hair growth promoter) in traditional texts in ayurveda [6]. Hence; the present study is focused on the scientific investigation of the hair growth potential of the flowers of *H. rosasinensis*.

## MATERIALS AND METHODS

### Experimental

#### Procurement and Identification of Plant Material

The plant material *H. rosasinensis* flower red variety were procured in the month of July from garden in girls hostel at Dr H S Gaur University, Sagar (M.P) and identified by Dr Pradeep Tiwari (Deptt of Botany, Dr H.S. Gaur University, Sagar). A Herbarium No-Bot/H/2792 was provided to it and a specimen was preserved there for further references.

#### Drying of the powdered material

The plant materials were dried under the shade. Then it was powdered and dried in oven at 30-40 °C.

#### Preparation of extract

The powdered plant material (100gm) was extracted in soxhlet apparatus with petroleum ether (60-80°C) till complete extraction. Then defatted plant material was extracted with chloroform, ethylacetate and then with ethanol in successive manner. The successive ethanolic extract obtained was concentrated under reduced pressure to get the crude extract the yield of extract was 2.8% w/w.

#### Animals

Healthy Female wistar albino rats, weighing 120–150 g, age 3-4 months were used for hair growth promotion studies based on the guidelines of the Institutional animals ethics committee of Dr H S Gaur University, Sagar, India. The rats were placed in cages and kept in standard environmental conditions of 12h light and 12 h dark cycle, at 23±2 C and 35-60% humidity, fed with standard diet ad libitum and free access to water. They were grouped and treated accordingly. (Table 1)

**Grouping and treatment of animal**

Table 1

S. No	Treatment ( topically ) Dose (0.4 mL each )	No of animals
1.	Control(Vehicle only)	6
2.	Standard (2% Ethanolic solution of minoxidil)	6
3.	1% Ethanolic extract of <i>H. rosasinensis</i> flower	6

**Chemicals**

Minoxidil was purchased from Dr Reddy Lab, Hyderabad, India. Petroleum ether, Ethanol and Paraffin oil were obtained from CDH Delhi.

**Preparation and administration of extracts and standard solution of minoxidil**

All extracts were suspended in paraffin oil to 1% solution and 2% solution of minoxidil in ethanol was prepared.

**Toxicity studies**

Toxicity studies were carried out and the ethanolic extract when applied in a concentration up to 10% for 7 days and did not show any toxic side effects or erythema on skin surface of rats. Thus the prepared extracts were considered safe for topical administration [7].

**Treatment of animals**

A 4-cm<sup>2</sup> area of the dorsal skin of all rats was shaved off using Anne French cream (a marketed hair removal cream) then wiped with surgical spirit [8]. A 0.4 milliliter of the extract solution or vehicle or minoxidil were applied to the denuded area of the respective groups twice a day. This treatment was continued for 30 days during which time; hair growth pattern was observed visually and recorded. Skin biopsies were taken on the 30th day of sample application for follicular analysis.

**Statistical analysis**

Statistical analysis of data was carried out by one way ANOVA comparing all test groups versus control followed by Dunnett's test using Instat v 2.1 software. Data are reported as mean  $\pm$  SEM.

**Parameters for measuring hair growth**

Hair growth initiation and completion time, Length of hair, Anagen (progressive phase) to telogen (regressive phase) ratio and follicle density.

**RESULTS****Phytochemical analysis**

*Hibiscus rosa sinensis* flower ethanolic extract contain Glycosides. phenolic, amino acid, carbohydrates and flavanoids. Its chromatographic profile shows best separation in n Butanol: ethylacetate: water in ratio of 10:10:4 and give rise to five bands after derivatized with anisaldehyde. .

**Qualitative study**

All the three groups of animals were observed for hair growth initiation and completion time. (Table 2)

<b>Treatment (topical)</b>	<b>Initiation Time Days</b>	<b>Completion Time Days</b>
Control (vehicle only)	10±0.0411	23±0.0635
Standard (2% minoxidil solution)	6±0.0311***	19±0.0568***
Ethanollic extract of <i>H.rosasinensis</i> flower (1%)	16±0.0456***	29±0.0789***

*N*(no of skin section studied=6) Values are mean ± SEM, \**p*<0.05, \*\**p*<0.01, \*\*\**p*< 0.001, significance Vs control

The datas showed that *H.rosasinensis* flower ethanolic extract treated animals takes more time for hair growth to initiate and complete

**Length of hair [mm]**

These datas were based on plucking 25 hairs from 10<sup>th</sup> day and then for time interval of five days, up to 25<sup>th</sup> day when complete hair growth was observed (Table 3)

<b>Extract Applied</b>	<b>Day 10</b>	<b>Day15</b>	<b>Day20</b>	<b>Day25</b>
Control (vehicle only)	5.68±0.03266	8.79±0.0233	11.13±0.0817	12.33±0.05972
Standard (2% minoxidil solution)	6.07±0.02134***	9.18±0.04899***	13.37±0.05175***	14.36±0.04761
Ethanollic extract of <i>Hibiscus rosa sinensis</i> flower (1%)	3.28±0.02000***	6.7±0.6992***	8.33±0.0366***	9.19±0.03590

Values are mean ± SEM, \**p*<0.05, \*\**p*<0.01, \*\*\**p*< 0.001, significance Vs control

The datas showed that *H.rosasinensis* flower ethanolic extract treated animals showed smaller hair as compared to control while minoxidil treated animals showed much longer hair than control



**Figure 1**



**Figure 2**

**Quantitative studies**

The photomicrographs showed that control animals(Fig 1) had much more anagenic hair which leads to hair growth as compared to ethanolic extract of *Hibiscus rosasinensis* flower treated animals in which lesser no of anagenic hair are present which leads to balding of skin(Fig 2)

Hair growth population (anagen/telogen ratio) of 100 hair after treatment with extract and standard showed that extract treated animal posses more telogenic and less anagenic hair follicles .(Table 4)

**Table 4 Hair growth population (anagen/telogen ratio) of 100 hair after treatment with extracts**

Treatment	Telogen	Anagen	A/T
Control (vehicle only)	54.6±0.7630 %	45.4±0.7630%	0.8315
Standard (2% minoxidil solution)	33.8±0.6532***%	66.2±0.6110***%	1.9586
Ethanolic extract of <i>Hibiscus rosa sinensis</i> flower(1%)	64.7±0.7895***%	35.3±0.7895***%	0.5456

Values are mean ± SEM, \**p*<0.05, \*\**p*<0.01, \*\*\**p*< 0.001, significance Vs control

Hair follicle count/mm in section of skin of animals treated with ethanolic extract of *H.rosasinensis* showed lower follicle density as compared to control (Table 5)

**Table 5**

Treatment	Follicle density/mm
Control (vehicle only)	1.4±0.2247
Standard (2% minoxidil solution)	2.59±0.3128
Ethanolic extract of <i>Hibiscus rosa sinensis</i> flower(1%)	1.3±0.1421

Values are mean ± SEM, \**p*<0.05, \*\**p*<0.01, \*\*\**p*< 0.001, significance Vs control

**DISCUSSION**

There exists an unmet need for identification of novel hair growth promoters in light of the fact that there are only two drugs, topical Minoxidil and oral Finasteride approved by the Food and Drug Administration (FDA) for the treatment of alopecia. Large randomized placebo controlled trials on humans conducted by Upjohn Company for Minoxidil, a potassium channel opener showed efficacy in 54% of the treated patients as opposed to 34% in placebo control group. There are significant adverse dermatological effects associated with minoxidil viz. pruritis, dryness, scaling, local irritation, dermatitis .48% of hair regrowth is observed in finasteride recipients in one year. Patients receiving finasteride shows that it is generally well tolerated, but few patients withdrew the treatment due to drug related sexual disorders. Finasteride is not indicated for use in women (9). Today minoxidil is categories as lifestyle drug[10]. It do posses side effects [11] Here in this study minoxidil was standard and it showed better results as compared to control for promoting hair growth which were evaluated through hair growth initiation and completion time hair length, follicle density and follicle phase but ethanolic extract of *H.rosasinensis* did not show any hair growth promoting effect even it was below to control group of animals in hair growth promotion parameters that means it is able to retard hair growth. So it may be useful in preparations for retarding hair growth such as for massage oil for retarding growth of unwanted body hair especially in females.

### CONCLUSION

So it may concluded that ethanolic extract of *H.rosasinensis* act as hair growth retarding agent and may use for preparations meant for beautification of women by retarding unwanted body hair growth.

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