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In vitro hair growth promoting activity of various leaves extract of *Hibiscus syriacus* L. on albino rats

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Abstract

The objective of this study is to ascertain the hair growth promoting activity of *Hibiscus syriacus L.* leaves extract with different solvent like petroleum ether, benzene, chloroform, methanol, and water. The phytochemical screening was identified the bioactive compounds of the dry extract. The leaves of *Hibiscus genus* are traditionally acclaimed as hair tonic in the Indian system of medicine. Studies were therefore undertaken in order to evaluate petroleum ether, benzene, chloroform, methanol, and water extract of *Hibiscus syriacus L.* leaves for its effect on hair growth in albino mice. The 10% extracts incorporated into liquid parafin base were applied topically on shaved denuded skin of albino mice. The time required for initiation of hair growth as well as completion of hair growth cycle was recorded. The results of treatment with 10% petroleum ether, benzene, chloroform, methanol, and water extracts were comparable to the control.

Key-Words: Alopecia, Hair growth, *Hibiscus syriacus L.*, Anagen phase, and Telogen phase

Introduction

In the recent times, focus on the research has all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in traditional system. Various medicinal plants have been studied using modern scientific approaches, and the results have revealed the potential of medicinal plants. [(1)]

Hair loss is a dermatological disorder that has been recognized for more than 2000 years. It is common throughout the world and has been estimated to affect nearly 2% of the world's population. [(2)] Apart from metabolic and hereditary causes, alopecia has been observed as a major side effect of anticancer, immunosuppressant and many others drug treatments. Presently, minoxidil [(3)] and finasteride [(4)] are the two USFDA approved synthetic drugs finding concomitant use for treatment of androgenic alopecia, although their side effects have abbreviated their usage. There are many products available prepared by combination of one or more herbal drugs that find acceptability as hair tonics, hair growth promoters, hair conditioners, hair cleansing agents, antidandruff agents and for the treatment of alopecia and lice infection.[(5)] The traditional system of medicine in India acclaims a number of herbal drugs for hair growth promotion. However, lack of sound scientific evidence limits their use. The fatty oil from the seeds of the plant has reportedly been used by tribes for preventing premature hair loss. [(6)]

The genus *Hibiscus* is widely distributed over Korea, china, India, and Siberia. The dried root of *Hibiscus syriacus L.* are used as a fork medicine in the orient [(7)] for the cure of hematochezia, dysentery, obstruction due to wind phlegm, and vomiting of food.[(8)] *Hibiscus syriacus L.* (Rose-of-Sharon) is valued for large flowers produced in summer when few other shrubs bloom. It is useful as a garden accent due to its strict, upright habit. The open, loose branches and light green leaves make Rose-of-Sharon ideally suited to formal or informal plantings, and with a little pruning makes an attractive, small specimen tree. The plant grows in sun or partial shade and in any soil. Rose-of-Sharon grows 8 to 10 feet tall and spreads 4 to 10 feet. The growth rate ranges from slow to moderate, and transplanting is easy. Several roots are usually located just beneath the soil surface. The insects can be dislodged with high pressure water sprays from the garden hose or controlled by pinching off the part of the twig with the insects. Over-fertilizing increases aphid infestations.[(9)]

In the present study, the effect of petroleum ether, benzene, chloroform, methanol and water extract of *Hibiscus syriacus L.* leaves was investigated on hair growth initiation and promotion.

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Material and Methods

Selection of plant

Plant was selected on the basis of literature survey.

Collection of plant materials

The leaves of *Hibiscus syriacus L.* was collected from the month of august-September from the Garden of Jawaharlal Institute of Technology and G. R. Y. Institute of Pharmacy vidya vihar Borawan district Khargone madhaya Pradesh,

Authentication of plant

The plant *Hibiscus syriacus L.* was identified and authenticated by Dr S.K. Mahajan, (Retd) Botanist from Government College, Khargone Madhaya Pradesh. The herbarium of the plant specimens were prepared and deposited in the Department of Pharmacognosy, G. R. Y. Institute of Pharmacy vidya vihar Borawan district Khargone madhaya Pradesh, India, under voucher no. G.R.Y.I.P. 43.

Preparation of extract

Extraction of organic component

The leaves were initially separated from the main plants body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. It was then passed through the 40 mesh sieve Dried and powered plant defatted firstly to remove fatty material for this purpose 1000 g of weighed powered plant of *Hibiscus syriacus L.* was packed in Soxhlet apparatus and extracted with petroleum ether at 60-80°c for 36 hrs. The marc was removed and dried then it was subjected to continuous hot extraction with another organic solvent like benzene, chloroform, and methanol extracts in soxhlet apparatus for 36 hrs according to their polarity index. After complete extraction the solvent was evaporated and concentrated to dry residue. The petroleum ether, benzene, chloroform, and methanolic extract of *Hibiscus syriacus L.* leaves yielded greenish brown and deep blue semi solid residue then it were filtered with the help of muslin cloth. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus and concentrate the extract in reduce pressure.

Extraction of aqueous component

The marc was removed and dried then it was subjected to boil with 2 liter of water for 24 h and then filtered through sterile filter paper, evaporated by using solvent distillation apparatus. The extract was got with the help of a muslin cloth. [10]

Phytochemical analysis of extract

The methods described by Harborne (1978) with slight modifications were used to test for the presence of the active ingredients in the test sample.

Test for steroids

A 10 ml of chloroform extract of the test plant leaves was evaporated to a dry mass and the mass dissolved in 0.5 mL of chloroform. Acetic anhydride (0.5 mL) and 2 mL of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for terpenoids

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids.

Test for tannins

i.) 1 mL of freshly prepared 10% KOH was added to 1 mL of the extract. A dirty white precipitate indicated the presence of tannins.

ii.) Extract of the test plant (1.0 g) was weighed into a beaker and 10 mLof distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl3 were then added. Production of greenish precipitate indicated the presence of tannins.

Test for flavonoids

A small piece of magnesium ribbon was added to ethanol extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colors varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones.

Test for alkaloids

The extract of the plant (0.5 g) was stirred with 5 mL of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 mL with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

Test for saponins

Extract of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of saponins.

Test for glycosides

Extract was added into two separate beakers. To one of the beakers was added 5 mL of dilute sulphuric acid while 5 mL of water was added to the other beaker. The two beakers were heated for 3 – 5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate and the absence

of such precipitate in the aqueous filtrate were regarded as positive for glycosides.(14)

Animals

Albino rats of either sex of age 21 days weighing between 150 and 160 g were fed on the standard diet and water *ad libitum*. The animals were housed at 20-26^{O}C room temperature.

Preparation of samples

10 gm leaves extract of petroleum ether, benzene, chloroform, methanol, and water extract of *Hibiscus syriacus L.* were dissolved in 100ml of liquid paraffin to produce the 10% active compound and it was further used for the evaluation of potential hair growth promoting activity in vitro

Treatment

The wistar albino rats were divided into seven groups of six rats each. A 4-cm2 area of the hair from dorsal portion of all the rats was removed with hair remover cram (vizon hair remover cram) and wiped with cotton and surgical spirit. 10% prepared extract in liquid paraffin and the placebo (liquid paraffin) were applied to the denuded area of the respective groups once a day and a control group received no treatment. This treatment was continued for 30 days during which time; hair growth pattern was observed visually and recorded. Skin biopsies were taken on the 10th, 20th and 30th day of sample application for follicular analysis.

Group I was received topical application of liquid paraffin of 10% petroleum ether extract.

Group II was received topical application of liquid paraffin of 10% benzene extract.

Group III was received topical application of liquid paraffin of 10% chloroform extract.

Group IV was received topical application of liquid paraffin of 10% methanol extract.

Group V was received topical application of liquid paraffin of 10% water extract.

Group VI was received topical application of liquid paraffin without extract.

Toxicity studies

Toxicity studies were carried out and all the extract of *Hibiscus syriacus L.* when applied in a concentration of up to 50% did not show any toxic side effects. Thus, the prepared extracts were considered safe for topical application.

Permission from the institutional ethical committee was taken before starting animal experiment.

Qualitative hair growth study

Qualitative hair growth was evaluated by visual observation of two parameters: (I) hair growth initiation time (minimum time to initiate perceptible hair growth) and (II) hair growth completion time(minimum time taken to cover the denuded skin region with new hair completely) Hair growth initiation and completion time was recorded for each group of animals. (12)

Quantitative hair growth study

The method reported by Uno (1991) was followed for the quantitative evaluation of all the extract of *Hibiscus syriacus L.* One rat from each group was aesthete after 30 days of treatment. Skin biopsies were taken from the shaved area and the specimens were preserved in 10% formalin. The specimens were fixed in paraffin wax and blocks prepared for microscopy. After fixation, vertical sections of the skin were cut. (13)

The sections were stained with hematoxylin and eosin. The number of hair follicles per millimeter area of skin and the ratio of hair follicles in different cyclic phases like anagen and telogen were determined using the microscope. Hair folliculogram was prepared by observing growth cycle of 100 hairs and length of hair follicle.

Statistical analysis

Data are reported as mean ± SD. Statistical analysis of data was carried out by analysis of variance (ANOVA) test.

Results and Discussion

Preliminary phytochemical screening

Our preliminary phytochemical screening of *H.syriacus L.* leaves (table 1) revealed the presence of carbohydrate, glycosides, steroids, triterpenes, flavonoids, alkaloid, tannins, saponins, and were absent.

Table 1: Phytochemical screening of *H.syriacus L.* Chemical constituents *H. syriacus* L. eaves

S.No.	Phytochemical	Pet.ether	Benzene	Chloroform	Methanol	Water
1.	Carbohydrates	-	+	+	+	+
2.	glycosides	-	-	-	+	+
3.	Steroids	+	+	+	-	-
4.	Proteins	-	-	+	+	-
5.	Flavonoids	-	-	-	+	+
6.	Tannins	+	+	+	+	+
7.	**Alkaloids**	-	-	-	-	+

Qualitative studies on hair growth

Hair growth initiation and completion time was considerably reduced upon treatment with all the extract of *Hibiscus syriacus L.* In the control group animals, hair growth was initiated in denuded area in the 2nd week; whereas it was noted in the 1st week in the extract treated groups. Hair growth was initiated on the 7th day with 10% preparation of the petroleum ether extract of *Hibiscus syriacus L.* Hair growth initiation was recorded on the 8th day of the Methanol extract of *Hibiscus syriacus L.*, whereas it was on the 9th day with 10% preparation of the water extract of *Hibiscus syriacus L.* whereas it was on the 11th day with 10% preparation of the chloroform extract of *Hibiscus syriacus L.* 10% preparation of n-hexane and benzene extract of *Hibiscus syriacus L.* hair growth initiation was observed on the 14th day, so that n-hexane and benzene extract not shown more effective compare to other extract while petroleum ether and methanol extract shown very effective were observed on the 7th and 8th days and on the 15th day in the control group.

The time taken for complete hair growth on the shaved area was also influenced by 10% preparation of all the extract of *Hibiscus syriacus L.* Complete hair growth was observed on the 15th day with 10% extract petroleum ether and on the 18th day with 10% preparation of methanol extract. It was observed on the 20th day with 10% water extract treatment, on the 22nd day with 10% chloroform, n-hexane and benzene extract of *Hibiscus syriacus L.* treatment. In control group animals, complete hair growth was noted after 26 days.

Table 2: Hair Length Determination

Name of extract	Hair length determination (mm)			
	Day 15	Day 20	Day 25	Day 30
Petroleum ether extract	3.3	7.9	13.8	**16.6**
Benzene extract	1.1	2.8	5.8	**10.7**
Chloroform extract	2.2	4.6	9.1	**11.3**
Methanol extract	3.2	7.5	12.2	**15.8**
Water extract	**2.8**	**6.3**	**10.5**	**14.6**

Length of hair follicles

Treatment with 10% preparation of all the extract of *Hibiscus syriacus L.* had a remarkable effect on the length of hair follicles. In the control group, only 24 ± 0.4% had an average length of 0.5 mm, whereas in the petroleum ether and methanol extract treated groups 46 ± 0.3% and 48 ± 0.1% hair population with more than 0.5 mm was observed with 10% extract treatment, respectively. The results of treatment were comparable with the control group where 24 ± 0.5% hair population had a length of 0.5 mm and above.

Table 3: Anagen-Telogem Ratio

Name of extract	Percentage of Hair Follicles					
	Day 10		Day 20		Day 30	
	Anagen	Telogen	Anagen	Telogen	Anagen	Telogen
Petroleum ether extract	43	47	53	41	57	**36**
Benzene extract	25	30	30	27	34	**25**
Chloroform extract	28	37	35	33	37	**30**
Methanol extract	40	56	53	43	60	**40**
Water extract	32	46	47	37	54	**33**
Control	**25**	**30**	**31**	**24**	**34**	**23**

Topical application with 10% preparation of all the extract of *Hibiscus syriacus L.* reduced the time required for hair growth initiation and was superior to extract was more effective with petroleum ether and methanol. The quality of hair in the petroleum ether extract treated group was coarse, rough and hard, whereas the methanol extract treated group resulted in soft and silky hair. Methanol extract was the best in inducing hair growth initiation. The study confirms that the methanol extract (10%) treatment revitalizing the growth of hair in rats. The remarkable improvement in length of hair follicles also supports the hair growth promoting effects of the herb.

Androgenetic alopecia (AGA) is a dihydrotestosterone (DHT) mediated process characterized by continuous miniaturization of androgen reactive hair follicles and accompanied by perifollicular fibrosis of follicular units in histological examination. [15] Retention of late

anagenic follicles as well as increase in follicular length and prevention of their miniaturization may therefore be attributed to 5-alpha-reductase inhibitory activity. The present study validates the ethnomedical use of plants for hair loss treatment. Further studies on utilization of the petroleum ether amd methanol extract and its incorporation in a formulation are warranted for commercial utilization of *Hibiscus syriacus L*.

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