Evaluation of Hair Growth Promoting Activity of Maharanga bicolor and Hibiscus rosa sinensis

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ABSTRACT
Alopecia is common and most frequently complaint dermatological disorder since the time immemorial. Various herbal and allopathic formulations are reported to have hair growth promoting activity. Minoxidil and Finasteride are only US FDA approved drugs for alopecia and hair loss. Maharanga bicolor and Hibiscus rosa sinensis are two ethnomedicinal herbs commonly used for hair growth promotion. Present study was conducted to determine hair growth promoting activity of petroleum ether extract of M. bicolor and H. rosa sinensis in Aloe vera gel. Minoxidil Sulphate and Different formulations of petroleum ether extract of M. bicolor and H. rosa sinensis was formulated in Aloe vera gel and applied to denuded area of rats for 30 days. Hair growth pattern, hair growth initiation, hair growth completion and length of hair was measured. M. bicolor (2%) formulation shows shortest hair growth initiation time on 6th day compared to 11th day in blank sample. Formulation containing 2% of each extract have shortest hair growth completion time on 19th day of sample application and showed potent hair growth pattern observed by blind investigation. The result suggests that petroleum ether extract of M. bicolor and H. rosa sinensis possess good hair growth promoting activity.

Key words: Maharanga bicolor, Hibiscus rosa sinensis, Hair growth promotion, Activity

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INTRODUCTION
Alopecia is a condition of hair loss from some or all areas of the body usually from scalp. It has been recognized for more than 2000 years as common cosmetic problem. It is neither life threatening nor painful condition but is a common and most frequently encountered patient complaint due to significant psychological and physical distress. Among various types of alopecia Androgenic alopecia can occur in both men (male pattern baldness) and female (female pattern baldness) which is characterized by diffused hair loss in scalp. Androgenic alopecia results from conversion of the hormone testosterone into dihydrotestosterone (DHT) by enzyme 5-alpha reductase. Alopecia areata is highly unpredictable autoimmune disease that results in hair loss on scalp or on whole body. Depending upon the extent and nature of hair loss alopecia areata is further classified into alopecia totalis, alopecia universalis etc. Hair is terminally differentiated, dead keratinocytes which exerts a wide range of functions including thermoregulation, collecting sensory information, protection from environmental trauma, social communication and mimicry. Human body contains 5 millions of hair follicle of which around 80,000 to 150,000 hair follicles are in scalp. Hair grows in three cyclic phases of growth (anagen), regression (catagen), and rest (telogen). Approximately 85-90% of all scalp follicle hairs are within anagen follicles. Anagen lasts for 2-6 years, catagen last for a few weeks, followed by telogen phase which lasts for 2 to 4 months. Dysfunction of cycle in telogenic or anagenic phase results in hair loss. Nutrition and their proper balance play an important role in hair growth. It has been reported that various vitamins like A, C, E, B, folic acid, biotin, etc exerted supportive effect in hair growth and treatment. Two allopathic medicines namely Finasteride and Minoxidil which are approved by FDA for hair growth promotion but unwanted side effects were reported. Herbs have been the most important source of medicine since the time immemorial. It is generally believed that herbal drugs are cheaper, locally available and devoid of any side effect. Natural products and herbs are commonly used in various cosmeceuticals and pharmaceuticals. Commonly marketed herbal products are claimed to have hair tonic, hair growth promoter, hair conditioner, hair cleansing agent, antifungal agents as well as for the treatment of alopecia and lice infection. Currently, about 1000 kinds of plant extract have been screened for hair growth. Plant derived medicines have been under investigation for hair growth promoting activities. Many plants have been described as hair tonic in several texts of traditional medicine. Maharanga bicolor which is perennial herb distributed at an altitude of 2300-2700 m in Nepal, Bhutan, North-East India and west China, used for hair growth promotion in himalayan region. Hibiscus rosa sinensis Linn. (Malvaceae) is glabrous ornamental perennial tropical herb commonly cultivated in tropical and subtropical regions of the world, is distributed in tropical and subtropical region all over the world ranging from 30 degrees north latitude to 30 degrees south latitude. H. rosa sinensis is used as medicine by every civilization in direct or indirect from ancient times. The main aim of the study was to evaluate hair growth promoting activity of M. bicolor and H. rosa sinensis.
MATERIAL AND METHODS
Crude drugs collection and Identification
The leaves of Hibiscus rosa-sinensis Linn and rhizomes of M. bicolor were collected from Lekhnath, Kaski district and Marpha, Mustang respectively. Aloe vera gel was collected from Kaski district. The crude drugs were identified in National Herbarium and Plant Laboratory, Godawari, Nepal comparing with standard herbarium specimens and with related literatures. Animal models
Healthy Wister albino rats weighing between 120-150 g were used for in vivo hair growth studies. They were purchased from the Department of plant resources, Government of Nepal, Thapathali, Kathmandu, Nepal. The rats were placed in cage and housed at room temperature, fed with standard diet and water ad libitum.
Extraction of crude drugs
Dried powdered leaves, rhizomes were soxhlet extracted with petroleum ether till completely exhausted. Briefly powder were packed in thimble paper and placed in soxhlet apparatus for extraction. 130.28g powder of M. bicolor and 215.20g of H. rosa sinensis in different lots were extracted with petroleum ether. Extraction was performed for 3 hours at 65°C. After extraction, solution was filtered and evaporate by using vacuum evaporator for under reduced pressure. The yield value was calculated for each extract and that was stored in air tight container for further studies.
Sample Preparation
Firstly different formulation was designed for different extract in Aloe vera base. Formulation design consisted of two steps:
1) Preparation of Aloe vera gel base
Aloe vera gel was prepared as base for all the preparation. Collected fresh Aloe vera leaves was washed with distilled water. The Aloe vera gel was extracted from the leaves by a Filent method. In this method, the anthraquinone containing yellow sap from the pericyclic cells was drained from the freshly cut leaves. The gel from these leaves was collected by means of longitudinal incision on the dorsal side of leaves and any foreign particles removed from the gel manually. To improve stability of the gel, it was heated to 40°C and then a blend of stabilizing agent consisting of ascorbic acid (0.5% w/w) and sodium benzoate (0.5% w/w) was added and mixed uniformly. The gel was then cooled to room temperature and stored in cool and dry place until use.
2) Preparation of test samples Nine different test samples with 2% and 5% extract of each H. rosa-sinensis Linn and M. bicolor was prepared in the Aloe vera base to obtain herbal gel formulation of each extract and mixture of extract was also made to prepare a polyherbal formulation as shown in Table 1. Two percent Minoxidil was used as positive control while blank formulation was considered as negative control.

The prepared formulation was evaluated for
1. Texture: Done by naked eye
2. Odor: smelled

RESULTS
Extract yield value
The percentage extract yield value of M. bicolor and H. rosa sinensis in petroleum ether extraction was 1.78% and 2.93% respectively.

Hair growth initiation and completion time
Hair growth initiation and completion time was found to vary among different groups (Figure 1). Hair growth initiation was observed on 6th day in F1 treated group followed by 7th day in F5, F6, and F7 treated group which is considered as shorter period. In F3 and F4 treated groups, initiation time was delayed up to 8th.
day whereas in F8 and F9 treated group, hair growth initiation were on 9th and 11th day of sample application respectively which is considered as longest period among the study. Hair growth completion was observed in all the rats within 26th day of sample application. In F5 treated group it was on 19th day of sample application which is considered as shortest period for hair growth completion. For F1 treated group it was found on 20th day of sample application whereas in F7, F2 and F6 treated groups also, it took 21st day of shorter period for hair growth completion. These groups are considered shorter period for hair growth completion. F2, F3 and F4 treated groups on the other hand, had longer hair growth completion time i.e. 23rd day as compared to the F9 treated group which was the longest period for hair growth completion i.e. 26th days of sample application.

**Blind investigation of extent of hair growth in different rats under investigation**

The 10 investigators unknown about the treatment to various rats observed the hair growth pattern and assigned them from A to D with A denoting the rats with excellent hair growth pattern (Marking 4), B denoting Good hair growth pattern (Marking 3), C denoting normal (Marking 2) and D denoting lesser comparatively (Marking 1). Higher the score obtained by each group, higher will be the hair growth pattern (Table 1). The results indicate that the hair growth pattern was different among the different groups. Among the treated groups, F5 treated groups had excellent hair growth pattern.

![Image of hair growth initiation and completion time in various treated group]

**Figure 1: Hair growth initiation and completion time in various treated group**

A: Denuded rat skin of group 9 at day 3
B: Denuded rat skin of group 4 at day 3
C: Application of formulation 1 to group 1 at day 3
D: Hair growth initiation in group 6 at day 7
E: Completion of hair growth in group 7 at day 21
F: Hair growth completion in group 5 at day 18

# P< 0.05 compared with blank control group
* P< 0.05 compared with Aloe vera gel only

**Hair growth Measurement**

The hairs were plucked randomly from all the rats of each group on 15th and 30th day of sample application. The improperly plucked hairs were discarded. The hair length was measured with the help of Vernier caliper which could read up to millimeter scale. Mean hair length of each group and standard deviation (+SD) as measure of dispersion of hair length within each group was calculated and the result is presented in Table as shown below.

**Table 1: Mean hair length and their score in various sample treated groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Hair length(cm) 15th day</th>
<th>Hair length(cm) 30th day</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% M. bicolor (F1)</td>
<td>1.5±0.11*^#</td>
<td>1.9±0.16*^#</td>
<td>30.60±2.07</td>
</tr>
<tr>
<td>5% M. bicolor (F2)</td>
<td>1.2±0.22*^#</td>
<td>1.8±0.07*#</td>
<td>30.80±3.03</td>
</tr>
<tr>
<td>2% H. rosa sinensis (F3)</td>
<td>1.4±0.42^#</td>
<td>1.9±0.15^#</td>
<td>31.00±1.58</td>
</tr>
<tr>
<td>5% H. rosa sinensis (F4)</td>
<td>1.2±0.47^#</td>
<td>1.9±0.11^#</td>
<td>30.40±2.70</td>
</tr>
<tr>
<td>2% F1+F3 Formulation (F5)</td>
<td>1.5±0.38^#</td>
<td>1.8±0.14^#</td>
<td>33.20±1.30</td>
</tr>
<tr>
<td>5% F2+F5 Formulation (F6)</td>
<td>1.1±0.04^#</td>
<td>1.9±0.14^#</td>
<td>31.80±1.64</td>
</tr>
<tr>
<td>2% Minoxidil (F7)</td>
<td>1.1±0.03^#</td>
<td>1.7±0.06^#</td>
<td>31.00±1.15</td>
</tr>
<tr>
<td>Aloe vera Gel only (F8)</td>
<td>1.3±0.14</td>
<td>1.4±0.17</td>
<td>31.00±1.58</td>
</tr>
<tr>
<td>Blank control (F9)</td>
<td>0.7±0.11</td>
<td>1.0±0.04</td>
<td>25.33±2.30</td>
</tr>
</tbody>
</table>

# P< 0.05 compared with blank control group
* P< 0.05 compared with Aloe vera gel only

**DISCUSSION**

In the present study the hair growth promoting activity of *M. bicolor* and *H. rosa sinensis* was performed using animal model. The plants were selected on the basis of their ethnomedicinal uses. Different plant extract formulation ratios were made by incorporating them in Aloe vera gel used as a vehicle and applied to denude skin of rats for one month where Minoxidil FDA approved drug was used as positive control. The measurement of hair length was done in 15th and 30th day of sample application. The hair growth initiation time as well as hair growth completion time over the denuded skin of rats was observed during the course of study. Hair growth initiation time was started from 6th to 11th day of sample application which was observed earlier i.e. 6th day in 2% *M. bicolor* (F1) treated group and later in 11th day in blank treated group whereas other groups (F2, F3, F4, F5, F6, F7 and F8) shows initiation time at 7th, 8th, 6th, 7th, 9th and 9th days respectively which indicates that hair growth initiation time was shorter in 2% *M. bicolor* (F1), 5% *M. bicolor* (F2) and 5% 1:1 mixture of *M. bicolor* and *H. rosa sinensis* (F5), than that of standard i.e. Minoxidil (F7).

Similarly the hair growth completion was observed from 18th day to 26th day of sample application which was observed earlier i.e. 18th day in 2% 1:1 formulation (F5) treated group and later i.e. 27th day in blank treated group (F9) whereas other formulations F1, F2, F3, F4, F6, F7 and F8 shows completion time at 19th, 21th, 23th, 21th, 21th, 23th and 27th days respectively. Among these group 2% 1:1 mixture of *M. bicolor* and *H. rosa sinensis* (F5), 2% *M. bicolor* (F1), 5% *M. bicolor* (F2) and 5% 1:1 mixture of *M. bicolor* and *H. rosa sinensis* (F6) shows better result as compared to standard Minoxidil (F7). Though, the initiation time of 2% *M. bicolor* (F1) is earlier, it has longer completion time in comparison to 2% 1:1 mixture of *M. bicolor* and *H. rosa sinensis* (F4). This results indicates among the studied sample, 2% *M. bicolor* is superior for hair growth initiation and 2% 1:1 mixture of *M. bicolor* is superior for hair growth completion.
which is also lind investigation acts as hair growth gel is a hair growth promoting and shows that all hair growth initiation, completion and, of epithelial cells near the base of the hair follicle and may have synergistic effects of active phytoconstituents. A, senegin II, III and senega saponin and active dimethyl 3-hydroxy flavanone, ginsenoside from red ginseng, 9.

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conclusion

The present study provides a support to the traditional claim that M. bicolor and H. rosa sinensis acts as hair growth promoter. Also Aloe vera gel is a hair growth promoting substance. Identification and isolation of molecules from the extract may provide new directions for the treatment of hair loss. Further elucidation of mechanism of action for the extract will act as milestone to prove the activity.