

In vivo Evaluation of Hair Growth Potential of Fresh Leaf Extracts of *Naringi Crenulata*

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Abstract

Hair growth problems can affect human physical and mental health and are of particular relevance during the aging process. In an effort to resolve such problems, an attempt has been made to evaluate the hair growth promotion activity of fresh leaf extracts of *Naringi crenulata* by using rodent model. Crude extracts of 0.2 ml were applied over the shaved skin onto the backs of wistar rats and monitored for 30 days. After 30 days, rats were treated with acetone and chloroform produced a greater effect on the length and weight of hair, number of hair follicles, blood circulation and colouration of shaved skin as compared to the control groups. Therefore the results of this study suggest that, *Naringi crenulata* has hair growth promoting potential, the effect of which may be attributed to the presence of terpenoids and flavonoids in the plant extracts.

Keywords: Hair growth; Wistar rats; Hair follicles; Terpenoids; Flavonoids

Introduction

Hair is one of the vital parts of body derived from ectoderm of the skin and it is one of the protective appendages on the body [1]. It is an important of the overall appeal of the human body [2,3]. Many people suffer from hair loss or hair thinning, despite the development of several medical treatments. Therefore, it is important to develop new therapies that prevent hair loss and increase hair growth. In this respect, alternative medicine has attracted interest, although it has not yet been included into mainstream of medical care, due to the limited scientific proofs and incomplete knowledge of the mechanisms involved [4].

Many plant extracts have been traditionally used for treating hair loss in oriental medicine. However, there is no report available on hair growth promoting activity of *N. crenulata* leaves. Therefore, the present study was focused on the scientific investigation of the hair growth potential of the leaf extracts of *N. crenulata*.

Materials and Methods

Leaf collection and identification

The leaf specimens were collected in the month of August from kumbakonam, Tamil Nadu, India. The leaf specimens were authenticated by Professor N. Raaman, Herbal Science Laboratory and centre for Advanced Studies in Botany, University of Madras, Chennai. After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water.

Preparation of extracts

The fresh leaves (300 grams) were grounded into paste and were extracted with water for 12 h at room temperature. This process was repeated successively with chloroform and acetone for 72 h at room temperature until the color of the extract becomes pale. The extracts obtained were filtered separately using Whatmann No. 1 filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and dried on water bath until the constant weight with dry mass was obtained for solvent extracts. The residual extracts were stored in refrigerator at 4°C in small and sterile glass bottles.

Estimation of total phenolic content

The amount of total phenolics in leaf extracts was determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990) [5], as modified by [6]. To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteu's reagent and 2 ml of Na₂CO₃ (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a Spectronic 20D+ spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw). A standard curve was prepared from 0 to 100 mg/l gallic acid (Figure 1). Total phenolic content was expressed in mg gallic acid

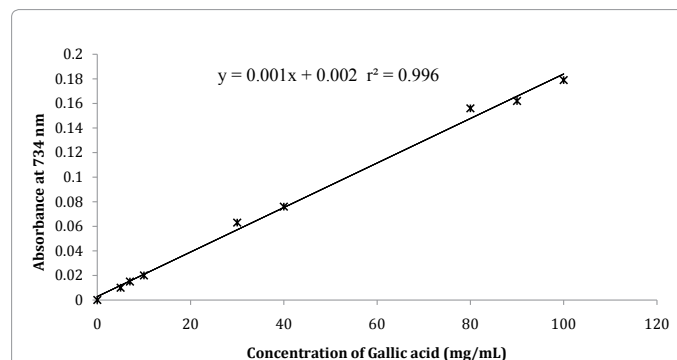


Figure 1: Calibration curve of gallic acid used to estimate the total phenolic content in solvent extracts, plotted with a 95% confidence interval.

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equivalents/g dry matter using the following equation based on the calibration curve: $y=0.001x+0.002$, $r^2=0.996$, where x was the gallic acid equivalent (mg GAE/g dw) and y was the absorbance.

Primary skin irritation test and treatment for hair growth activity *in vivo*

Wistar albino rats of either sex, weighing 180-200 g, were obtained from National Institute of Nutrition (NIN), Hyderabad and reared in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. All the animals were housed in cages at a room temperature of $25 \pm 2^\circ\text{C}$ with 12 h dark/12 h light cycles. The rats were fed with rat pellets and water *ad libitum*. The experiments were approved by the Institutional Animal Ethics Committee, Annamalai University (Proposal Number: 841). Hairs from the dorsal side of the rats were removed using a commercially available hair remover (Veet) for complete removal of hair from 4 sq cm area [7]. The shaved area was cleaned with surgical spirit and the animals did not show any toxic effects when acetone, water and chloroform extracts were applied for 48 hours post application. Hence the prepared extracts were considered safe for topical application [8]. Twenty albino rats were divided into five groups of four animals in each group. Starting the following day (day 1), 0.2 ml of leaf extracts dissolved in 3.5% DMSO was applied topically, on a daily basis, for 30 days.

Hair length determination

Hairs were plucked randomly from the shaved area of all rats on 10, 20, and 30 day after beginning the treatment. The length of 10 hairs was measured and the average length was determined. The results are expressed as the mean length \pm S.D. of 10 hairs.

Hair weight determination

After 30 days, the rats were sacrificed by cervical dislocation. 1 cm² area of dorsal skin with hair and without hair was cut from all the rats of each group and weighed by using analytical balance. After measuring, hair weight was calculated by subtracting skin weight from skin with hair weight.

Histological Studies

Three rats from each group were authenticated after 30 days of treatment. Skin biopsies were obtained from the shaved portion and preserved in 10% formalin. Pieces of skin tissues were implanted in paraffin wax and sectioned into a thickness of 7 μm . The sectioned tissues were stained with haematoxylin and eosin and the follicular phases of hairs were examined under microscope with an ocular micrometer [9].

Statistical Analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses \pm standard deviation. Correlation between the antioxidant activity and total phenolic contents was carried out using the correlation and regression in the Excel program (Microsoft Excel v. 2007). The data were analyzed using one way analysis of variance (ANOVA) and DMRT on SPSS/PC (statistical package for social sciences, personal computer) Ver. 15 and the results were considered statistically significant if the p value is less than or equal to 0.05.

Results

A calibration curve used to estimate the total phenolic content is

shown in Figure 1. The total phenolic contents (TPC) of leaf extracts of *N. crenulata* are shown in Figure 2. The total phenolic content of aqueous, acetone and chloroform solvent extracts were 161 ± 1.62 , 34 ± 1.81 and 1 ± 2.06 mg/g of GAE. Among all the extracts analyzed, a significant phenolic content was found for aqueous extract.

Hair growth initiation from the shaved area was observed in all the groups. Topical use of leaf extracts of *N. crenulata* onto the shaved area on the animals, produced a significant increase in the rate of hair growth, compared with the vehicle (DMSO) treated and control animals. Rats treated with 0.2 ml of acetone extract produced a significant effect on the hair growth when compared to other groups (Figure 3).

The length of hair for acetone, chloroform and water treated groups were 8.2, 7.5 and 5.99 mm, respectively, compared to the vehicle treated (5.75 mm) and control (5.7 mm) at the end of the experiment (Figure 4).

The weight of newly grown hairs in all the treated groups were measured and compared with that of the control group. It was found that weight of hair was highest for chloroform treated group and was measured to be 0.040, 0.039, 0.038, 0.037 and 0.035 mg/cm² area of dorsal skin for chloroform, acetone, water, DMSO treated and control rats (Figure 5). Statistical analysis (ANOVA) has shown that this increase in weight of hair in treated groups is not significantly different when compared to control and DMSO treated groups ($P > 0.05$).

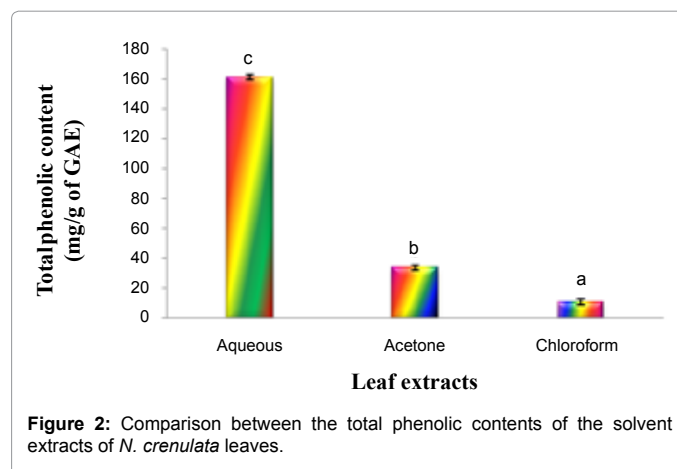
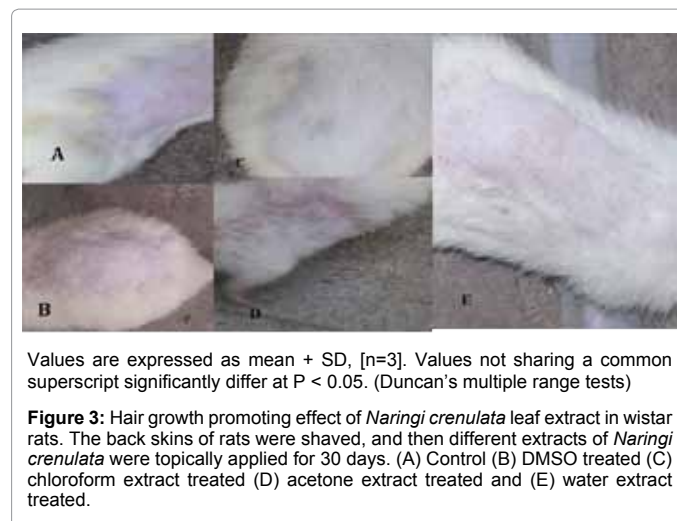


Figure 2: Comparison between the total phenolic contents of the solvent extracts of *N. crenulata* leaves.



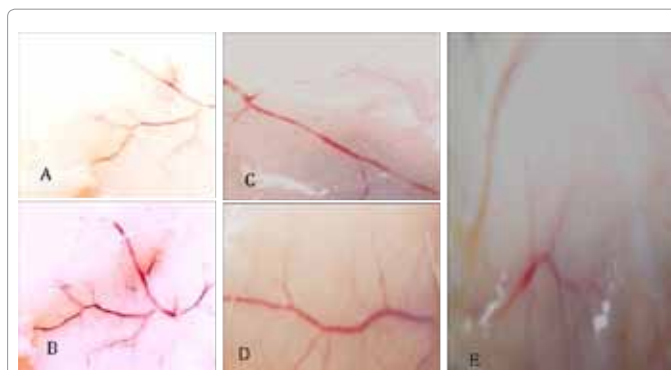
Values are expressed as mean + SD, [n=3]. Values not sharing a common superscript significantly differ at $P < 0.05$. (Duncan's multiple range tests)

Figure 3: Hair growth promoting effect of *Naringi crenulata* leaf extract in wistar rats. The back skins of rats were shaved, and then different extracts of *Naringi crenulata* were topically applied for 30 days. (A) Control (B) DMSO treated (C) chloroform extract treated (D) acetone extract treated and (E) water extract treated.

The reverse side of the dorsal skin of treated rats showed more vascularisation and colour changes as compared to control groups (Figure 6). Normally, the colour of the reverse side of telogen-phase skin is white, because hair follicles at this phase are very small. This denotes that normal hair growth cycle was stimulated. Moreover, the histological appearance of skin observed was similar in all the treated groups. There was no much difference in the number of hair follicles per millimetre of skin of the chloroform, water, and control rats. The number of hair follicles was found to be 4-7 per mm of skin of chloroform, water, and control rats and was 3-5 in acetone treated group (Figure 7).

Discussion

Hair loss is a distressing condition for an increasing number of men and women; therefore, to develop new therapies for the treatment of hair loss is of great importance. In the present study, hair growth promoting potential of the crude chloroform and acetone extracts of *N. crenulata* was demonstrated in rodent models. The exact mechanism of action or the component(s) responsible for the hair growth promoting activity in the plant extract could not be established in this study. However, a number of investigators have shown that flavonoids and terpenoids possess hair growth promoting activity by strengthening the capillary wall of the smaller blood vessels supplying hair follicles, improve blood circulation to nourish the hair follicles and thereby promoting hair growth [10-12]. In the present study increased blood



Values are expressed as mean + SD. Values not sharing a common superscript significantly differ at $P < 0.05$. [DMRT]

Figure 6: Effect of *Naringi crenulata* leaf extracts on the telogen-phase and vascularisation of back skin of wistar rats (A) Control (B) DMSO treated (C) water extract (D) acetone extract treated and (E) chloroform extract treated.



Figure 7: Histology section of albino rat skin after 10 days of treatment with leaf extract of *Naringi crenulata*. (A) control (B) acetone extract treated (C) water extract treated (D) DMSO treated (magnification x 400).

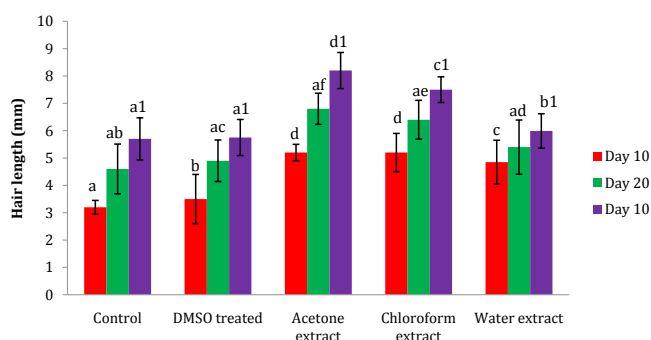
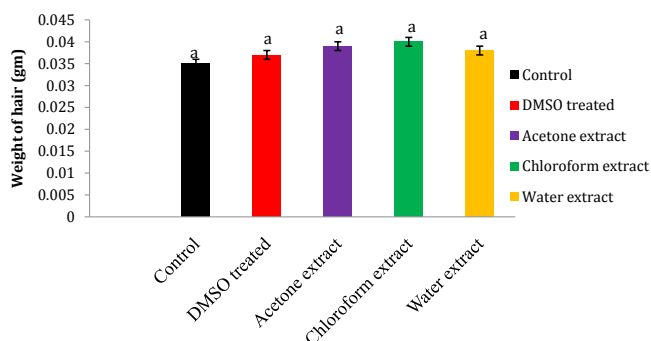


Figure 4: Effect of *Naringi crenulata* leaf extracts on the hair length of wistar rats.



Values are expressed as mean + SD. Values not sharing a common superscript significantly differ at $P < 0.05$. [DMRT]

Figure 5: Effect of *Naringi crenulata* leaf extracts on the weight of hair of wistar rats.

circulation and skin colouration was observed hence the present study is in agreement with the study of the above authors. Other workers also implicate flavonoids in stimulating telogen to anagen phase, a process involved in hair growth [13]. Sampath kumar and Ramakrishnan isolated flavonoids and terpenoids compounds from *N. crenulata* plant extracts which were found to be responsible for the various biological activities [14]. It may therefore, be possible that these flavonoids compounds and other chemical components such as triterpenes contained in these extracts may be responsible for the observed hair growth promoting effect and that, these compounds may also have stimulatory actions on these hair growth factors.

Conclusion

Finally, this study concluded that the crude chloroform and acetone leaf extracts of *N. crenulata* possesses hair growth promoting activity and further work needs to be carried out in order to establish the mechanism of action and the exact chemical component responsible for the hair growth potential of *N. crenulata*.

References

- Ebling FJ (1987) The biology of hair. Dermatol Clin 5: 467-481.
- Cash TF (2001) The psychology of hair loss and its implication for patient care. Clin Dermatol 19: 161-166.
- Stough D, Stenn K, Haber R, Parsley WM, Vogel JE (2005) Psychological

-
- effect, pathophysiology and management of androgenetic alopecia in men. Mayo Clin Proc 80: 1316-1322.
4. Bhaumik S, Jyothi MD, Khar A (2000) Differential modulation of nitric oxide production by curcumin in host macrophages and NK cells. FEBS Lett 483: 78-82.
 5. George A Spanos, Ronald E Wrolstad (1990) Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. J Agric Food Chem 38: 1565-1571.
 6. Lister E, Wilson P (2001) Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Research Institute, Lincoln, New Zealand.
 7. Roy RK, Mayank T, Dixit VK (2007) Effect of Citrullus coclocynthis Schrad. on hair growth activity of albino rats. Australian Journal of Medical Herbalism.
 8. Uno H, Kurata S (1993) Chemical agents and peptides affect hair growth. J Invest Dermatol 101: 143S-147S.
 9. Sawada M, Terada N, Taniguchi H, Tateishi R, Mori Y (1987) Cyclosporin A stimulates hair growth in nude mice. Lab Invest 56: 684-686.
 10. Kobayashi N, Suzuki R, Koide C, Suzuki T, Matsuda H, et al. (1993) Effect of leaves of Ginkgo biloba on hair growth in C 3Hstrain mice. Yakugaku Zasshi 113: 718-724.
 11. Awe EO, Makinde JM (2009) The hair growth promoting effect of Russelia equisetiformis (Schlect&Chan). J Natl Prods 2: 70-73.
 12. Kawano M, Han J, Kchouk ME, Isoda H (2009) Hair growth regulation by the extract of aromatic plant Erica multiflora. J Nat Med 63: 335-339.
 13. Roh SS, Kim CD, Lee MH, Hwang SL, Rang MJ, et al. (2002) The hair growth promoting effect of Sophora flavaescens extract and its molecular regulation. J Dermatol Sci 30: 43-49.
 14. Sampathkumar S, N Ramakrishnan (2011) GC-MS analysis of methanolic extract of Naringi crenulata (Roxb.) NICOLS. Leaves. IJPRD 3: 113-116.