

## Evaluation of Hair Growth Activity of *Buxus wallichiana* Baill Extract in Rats

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### Abstract

#### Objective(s)

The aim of this study was to evaluate antioxidant and hair growth activities of *Buxus wallichiana* Baill (Buxaceae).

#### Materials and methods

Petroleum ether, chloroform, methanol and aqueous extracts of *Buxus wallichiana* subjected to antioxidant activity by; 2, 2-diphenyl-1-picryl hydrazyl and nitric oxide methods. Methanol extract of *Buxus wallichiana* at 50, 100 mg/kg, ointment of methanol extract at 5 and 10% used for the evaluation of hair growth property.

#### Results

Methanol extract showed potential antioxidant activity. Methanol extract at 100 mg/kg showed consistent and significant increase in mean score of hair growth from day 3 to day 24. Whereas 50 mg/kg increased the mean score significantly, only from day 15 to day 24. When methanol extract at 10% applied topically, significant increase in mean hair score observed only from day 15, but at 5% showed considerable increase in mean hair score only from day 21 and 24, when compared to the control.

#### Conclusion

The result of this study suggests that Methanol extract of *Buxus wallichiana* possess good antioxidant and hair growth activity.

**Keywords:** Antioxidant activity, *Buxus wallichiana*, Hair growth activity.

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## Introduction

Hair suffers aggression: there can be some ailments to normal health of hair and cause trouble. The main problems associated with hair such as pigmentation problems (Fading), dandruff and falling of hair (1). Synthetic drug, minoxidil is a potent vasodilator appears safe for long term treatment. After 5 years use of 2 and 3 % topical minoxidil, the improvement has been shown to peak in one year with a slow decline in regrowth over subsequent years (2). Long term treatment with local side effects may be a problem with continuing use of minoxidil lotion (3, 4). Potential hair growth property is exhibited by the plants possessing antiandrogenic activity (5, 6) (testosterone, 5-alpha reductase inhibition) and antioxidant activity produced by flavonoids (7-11) (proanthocyanidines).

*Buxus wallichiana* Baill, commonly called as Himalayan boxwood, it belongs to family Buxaceae. *B. wallichiana* mainly found at high mounts, shady place and cold climates. Boxwood is an evergreen monoecious tree growing to the height of 6 meters with variable forms and leaves shape (12, 13). Traditionally *B. wallichiana* used as bitters tonic, diaphoretic, anti-rheumatic, vermifuge, antihelmentic, analgesic, purgative diuretic, antiepileptic, antileprotic and in hemorrhoids. The bark of *B. wallichiana* used as hair growth stimulant (13-16). Phytochemical reported are alkaloids buxemenol E (16), buxaltine H, Buxiramin D, buxatine, buxandrine F, buxidine F (15), (+)-16  $\alpha$ , 31-diacetylboxadine (17), semperviraminol, buxamine F (18). The steroidal alkaloid buxemenol E from *B. sempervirens* found to produce hypotensive effect in rat attributed by central and peripheral activation of muscarinic receptor and also, by the partial inhibition of acetylcholinesterase enzyme (16). The main aim of this study was to provide scientific evidence for traditional claim, hair growth activity of *B. wallichiana* bark.

## Materials and Methods

### Collection of plant materials and extraction

The wood of *B. wallichiana* collected from the Doddabetta region of Nilgiris district and identified by Dr Rajan, Botanist from

Government Arts College, Ootacamund, Tamilnadu. The specimen preserved in college herbarium, voucher no. SKVCP 15. The collected wood shade dried and grinded to a coarse powder. Successive extraction was done with petroleum ether, chloroform, methanol, and water, respectively (soxlet extraction). Preliminary phytochemical screening of all the extracts carried out (19).

### Animals

Wistar albino rats weighing about 150-250 g of either sex acclimatized to the experimental room at temperature  $23\pm 2$  °C, controlled humidity conditions (50-55%). They caged with a maximum of two animals in polypropylene cage and fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. The study conducted after obtaining ethical committee clearance from the institutional animal ethical committee of SKVCP.

### Acute toxicity (OECD guidelines 423 adoption)

Wistar albino rats of either sex divided into two groups of six animals each. Group one received 0.5% Carboxy methyl cellulose (CMC) (20 ml/kg, orally) and served as control, while other group received Methanol extract of *Buxus wallichiana* (MEBW) at 2000 mg/kg body weight respectively. Immediately the animals behavior observed continuously for 6 hr and thereafter, daily for mortality for 14 days (20). Based on acute toxicity studies, dose selected.

### Preparation of ointment

The MEBW ointment (5 and 10 %) prepared by using the ointment base (BP) containing white bees wax (20 g), hard paraffin (30 g), cetostreyl alcohol (50 g), white paraffin (900 g) and picric acid 1%. The latter used to avoid licking of ointment by the animals.

### Antioxidant studies

#### DPPH radical scavenging method

Ten  $\mu$ l aliquot of the different concentration of petroleum ether, chloroform, methanol and aqueous extracts of *B. wallichiana* added to 200  $\mu$ l of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) (100  $\mu$ M) in a 96-well microtitre

plate (Tarsons Product (P) Ltd., Kolkata, India). After incubation at 37 °C for 20 min, the absorbance of each solution determined at 490 nm, using ELISA reader (Bio-Rad Laboratories Inc., California, USA). Rutin (10.5 mg/ml in 10 µM of Dimethyl Sulfoxide (DMSO) solution) used as a positive control. The percentage of the inhibition of sample exposure determined by comparing with the control. This percentage plotted against the concentration and IC<sub>50</sub> obtained. The results expressed as the mean±SEM of 5 replicates (21).

#### *Nitric oxide radical inhibition activity*

The reaction mixture (6 ml) containing sodium nitroperoxide (10 µM, 10 ml), phosphate buffer saline (1 ml) and 1 ml petroleum ether, chloroform, methanol and aqueous extracts incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture removed, by adding 1 ml of sulphanilic acid reagent (0.33% in warm 20% glacial acetic acid), the combination mixed and allowed to stand for 5 min for the completion of diazotization. Then 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for another 30 min in diffused light. The absorbance of these solutions measured at 540 nm against corresponding blank solution in a 96-well microtitre plate (Tarsons Product (P) Ltd., Kolkata, India), using ELISA reader. Ascorbic acid (1 mg/ml in 10 µM of DMSO) used as a positive control. The percent of the inhibition plotted against the concentration and IC<sub>50</sub> obtained. The results expressed as the mean±SEM of 5 replicates (22, 23).

#### *Hair growth activity in rats*

The method described by Saraf, *et al* (24) used. Screening of hair growth potential evaluated in Wistar albino rats weighing 190 – 210 g. The hairs of the dorsal portion of the rats (of area 9 cm<sup>2</sup>) clipped with scissor and the hair removed after the application of hair removal cream (Anne French). For the oral treatment group of animals, only the hair of one area on the dorsal surface removed. For the ointment group, the hair of two areas on the dorsal removed, one side area treated with ointment and another side area did not receive any treatment (this was done to

differentiate oral and topical activities). After removal of hair, animals divided into six groups, each group containing 6 animals. Group I received 0.2 ml/100 g of 1% CMC solution orally, this group served as solvent control. Group II - III received 0.2 ml/ 100 g of 50 and 100 mg/kg orally of methanol extract of *B. wallichiana*. Group IV received topical application of ointment base. Group V and VI received topical application of 5 and 10% ointment of MEBW in only one hairless area. The other hairless area did not receive any treatment. The hair depleted region observed every three days to note the start of hair regrowth period and hair growth pattern. The effect of the extract on hair regrowth potential scored as described by Matsuda *et al* (6).

The effect of the extract on hair regrowth potential, scored as follows:

- 0 No hair growth
- 1 Less than 20% of hair growth
- 2 20-39% of hair growth
- 3 40-59% of hair growth
- 4 60-79% of hair growth and
- 5 80-100% of hair growth.

#### *Statistical Analysis*

Data expressed as mean±SEM and analysed using One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Values of *P* < 0.05 considered statistically significant.

#### **Results**

From preliminary phytochemical analysis, the results revealed the presence of alkaloids, carbohydrates and flavonoids for methanol and aqueous extracts of *B. wallichiana*. Steroids were present only in petroleum ether and chloroform extracts. By acute toxicity study it was found that, the dose of 2000 mg/kg of extract shows neither behavioral changes nor mortality.

#### *Antioxidant studies*

When assayed for antioxidant potential by DPPH and NO inhibition models, the methanol extract of *B. wallichiana* showed an IC<sub>50</sub> value of 29.2±2.10 in DPPH model, whereas rutin in the same model showed IC<sub>50</sub> of 3.41±0.11. The aqueous, chloroform and petroleum ether extracts produced IC<sub>50</sub> of

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190±5.33; 647±163.43 and 747.5±102.2 respectively. In nitric oxide inhibition model; methanol, chloroform, aqueous and pet ether extracts produced IC<sub>50</sub> values of 110±11.6, 184±22, 189±13.3 and 380±9.5 respectively, whereas, ascorbic acid IC<sub>50</sub> showed value of 39.2±4.5 (Table 1).

Table 1. Antioxidant potential of various extracts of *B. wallichiana* wood in DPPH and NO inhibition model (*in vitro* study).

Treatment	IC <sub>50</sub> (µg/ml)	
	DPPH method	NO inhibition method
PEBW	747.5 ± 102.2	380.0 ± 9.5
CEBW	647.5 ± 163.5	184.0 ± 22.0
MEBW	29.2 ± 2.1	110.0 ± 11.6
AEBW	190.0 ± 5.3	189.0 ± 13.3
Ascorbic acid	-	39.2 ± 0.49
Rutin	3.41 ± 0.11	-

Values are mean±SEM, n= 5 number tests per group

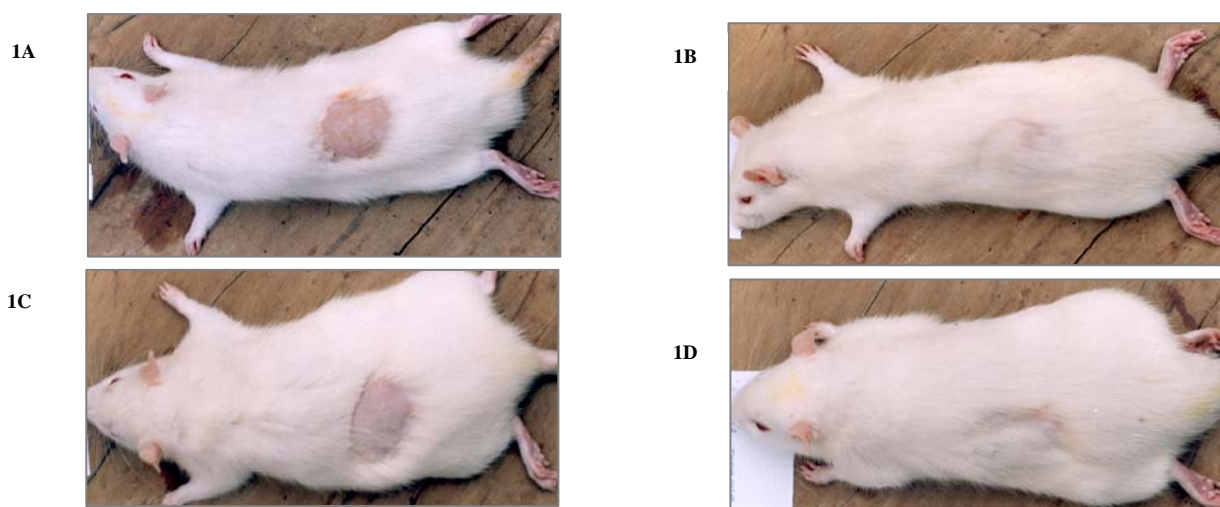
### *Hair growth activity*

Methanol extract of *B. wallichiana* when administered orally at dose level of 50 and 100 mg/kg exhibited significant increase in the mean score of hair regrowth. The result obtained for MEBW at 100 mg/kg was consistent and the from day 3 to 24 (end of the experiment), whereas, for 50 mg/kg was only from day 15 to day 24. When MEBW applied topically at 10% concentration, positive changes observed only from day 15. MEBW ointment at 5% showed considerable results only on day 21 and 24 when compared to the control. The untreated area did not show any increase in the mean increase score on hair regrowth in both the MEBW ointment groups at 5 and 10% when compared to the control (Table 2, Figure 1).

Table 2. Effect of methanol extract and ointment formulation of *Buxus wallichiana* on hair regrowth in rats.

Treatment	Dose	n	Mean score on day								
			3	6	9	12	15	18	21	24	
Control I (1% CMC 0.2 ml/ 100 g)	-	11	0.0±0.0	0.2±0.12	0.5±0.25	0.8±0.30	1.5±0.42	2±0.27	2.8±0.30	2.3±0.16	
MEBW	50 mg/kg; p.o.	6	0.0±0.0	1.0±0.0**	1.8±0.31**	2.0±0.26	2.8±0.31*	4±0.26***	4.5±0.22***	4.5±0.22***	
	100 mg/kg; p.o.	6	0.3±0.21*	0.8±.40*	1.8±0.40**	2.5±0.56*	3.0±0.37**	4.2±0.48***	4.7±0.33***	4.7±0.33***	
Control II (ointment base)	-	11	0.0±0.0	1.0±0.0	0.3±0.16	0.7±0.16	1.0±0.19	1.8±0.23	2.8±0.23	2.8±0.23	
	5%	Treated	6	0.0±0.0	0.7±0.21	1.3±0.49	1.5±0.5	2.0±0.45	1±0.45	4.2±0.4 <sup>##</sup>	3.8±0.4 <sup>#</sup>
MEBW ointment (topical)	5%	Untreated	6	0.0±0.0	0.3±0.21	0.7±0.21	0.8±0.31	1.3±0.42	2.0±0.45	3.0±0.26	3.0±0.26
		Treated	6	0.0±0.0	0.0±0.0	1±0.26	2.2±0.48	2.7±0.33 <sup>#</sup>	4.3±0.21 <sup>###</sup>	4.7±0.21 <sup>###</sup>	4.7±0.21 <sup>###</sup>
	10%	Treated	6	0.0±0.0	0.0±0.0	1±0.26	2.2±0.48	2.7±0.33 <sup>#</sup>	4.3±0.21 <sup>###</sup>	4.7±0.21 <sup>###</sup>	4.7±0.21 <sup>###</sup>
		Untreated	6	0.0±0.0	0.0±0.0	0.7±0.21	1.2±0.17	1.3±0.21	2.0±0.0	2.7±0.21	2.7±0.21

Values are mean±SEM, n= number of rats per group, \*P<0.05, \*\*P<0.01, \*\*\* P<0.001 vs. Control I; <sup>#</sup>P<0.05, <sup>##</sup>P<0.01, <sup>###</sup>P<0.001 vs. Control II; One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test. MEBW: Methanolic extract of *Buxus wallichiana*, CMC: Carboxy methyl cellulose



**Figure 1A.** Effect of control (1% CMC, p.o.) on 15th day of wood treatment, **1B.** Effect of methanol extract of *B. wallichiana*, (100 mg/kg, p.o.) on 15th day of treatment, **1C.** Effect of ointment base on 15th day of treatment. **1D.** Effect of methanol extract of *B. wallichiana*, wood 10 % ointment base on 15th day of treatment.

## Discussion

Some of the phytoconstituents which have been investigated for hair growth promoting activity are proanthocyanidins (7), 3, 4 dimethyl 3-hydroxy flavanone (10), ginsenoside from red ginseng (23), and active principles of *Polygala senega* such as senigose A, senegin II, III and senega saponin B (25). Among these, proanthocyanidins have been investigated to larger extent for their hair growth property. Proanthocyanidins are class of flavonoids formally called as condensed tannins. Proanthocyanidins like other flavonoids have potent antioxidant properties and also, potentiate other antioxidants. After several investigations on the mechanism through which proanthocyanidins promote hair growth, it has been found that proanthocyanidins; convert telogen (non-growing phase of hair growth) into anagen (growing phase of hair growth) (8). Procyanadines (procyanadines B-2 and C-1) by selectively inhibiting protein kinase C intensively promoting hair epithelial cell proliferation *in vitro* and increase anagen production *in vivo* (9). Among the proanthocyanidins, dimer and trimer exhibit the higher growth promoting activity than monomer (7). Since androgens (metabolite product of testosterone by 5-alpha reductase enzyme) have been implicated for the development of common baldness and alopecia, research also, has been conducted in search of natural compound having anti-androgenic activity or testosterone 5-alpha reductase inhibition.

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From the literature survey, it can be seen that potential hair growth property is exhibited by the plants possessing antioxidant activity produced by flavonoids (proanthocyanidins) (7). The antioxidant study carried out to evaluate the potential inhibition of reactive oxygen species and nitrogen species by the extracts of *B. wallichiana*. It was found that only MEBW possesses potential activity as a free oxygen radical scavenger, therefore, MEBW used to investigate the hair growth stimulant property based on the antioxidant property and also, at the same time phytochemical studies showed the presence of flavonoids in this extract. From the results of hair growth experiment in rat model, it can be strongly suggested that the MEBW has potential components to stimulate the hair growth. The interesting feature of MEBW was its efficiency as both systemically and topically, however, the oral treatment proved more effective than the topical application.

## Conclusion

By the study it was found that *B. wallichiana* possess good antioxidant and hair growth properties. This supports the traditional claim of plant for hair growth activity.

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