

Phytochemistry and Pharmacological Activities of *Bauhinia variegata* Var. *Candida*

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Abstract**Review Article**

Bauhinia is a genus of more than 500 species of flowering plants in the subfamily Cercidoideae, in the large flowering plant of Fabaceae family. *Bauhinia variegata*, also known as Kachnar (Hindi), Mountain Ebony (English), is a medium-sized deciduous tree distributed in a sub-Himalayan tract and outer Himalaya of Punjab. It is also known as Camel foot tree because of shape of its leaves which resembles foot of a camel. *Bauhinia variegata* var. *candida* belonging to the family Leguminosae, are planted in the tropics as white orchid trees. Medicinally, it has been proven to possess various pharmacological activities like antioxidant, anti-inflammatory, anti-diabetic, acetylcholinesterase inhibition, cytotoxic and antimetastatic activity. Studies revealed the presence of various phytochemical constituents mainly flavonoids (viz. quercetin, kaempferol and isorhamnetin derivatives with quercetin and kaempferol), proanthocyanidins (viz. epicatechin, epicatechin gallate, their oligomers, and epiafzelechin trimer), unsaturated fatty acids, several mono, di- and tri-hydroxy fatty acids, carbohydrates, saponins, terpenoids, alkaloids, steroids, tannins, amino acids, and proteins. The presence of lupeol in bark of this plant is responsible for anti-inflammatory action. The study on this plant is carried out to lay down the standards which could be useful for future studies.

Keywords: *Bauhinia variegata* var *candida*, Phytoconstituents, Pharmacological activity.**Copyright © 2019:** This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

INTRODUCTION

The trees of the genus *Bauhinia* (Leguminosae, Caesalpinoideae) are distributed throughout Brazil, and are used as an ornament, as medicinal plants, and as

feed for calves [11]. The varieties of the species *B. variegata* are characterized by the color of their flowers. *B. variegata* var. *candida* is the fast-growing white-orchid tree belonging to family Leguminosae.

Table-1: Taxonomic Classification [1]

Kingdom	Plantae
Genus	<i>Bauhinia</i>
Division	Tracheophyta
Species	<i>Variegata</i>
Class	Magnoliopsida
Family	Leguminosae
Species	<i>Variegata</i>
Subspecies	<i>Candida</i>
Subfamily	Caesalpinoideae
Tribe	Cercideae
Subtribe	Bauhiniinae

Table-2: Common Names [1]

Assamese	Kotora, Boga Kotora, Kanchan, Kurol
Bengali	রক্ত কাঞ্চন Raktakanchana, কাঞ্চন Kanchana
English	Ebony tree
Hindi	कंचन Kanchan, कचनार Kachnar
Kannada	ಕೆಮ್ಮು ಮಮ್ಬಾರು Kempu Mandara, ಕಮ್ಮುವಾಳ Kamchuvaala, Basavanpada
Malayalam	Chuvannamandaram, Kovidaram, Malayakatti, Mandaram
Manipuri	Chingthrao
Marathi	कंचन Or कांचन Kanchana, कोविदार Kovidara
Oriya	Borodu
Sanskrit	कांचन Kanchana, कनक Kanaka, कंचनार Kanchanara, कोविदार Kovidarah
Tamil	மந்தாரை Mantharai
Telugu	మందారీ Mandari, దేవకాంచనము Daeva-kanchanamu
Bodo	Kharmang
Others	White Orchid Tree, Kanchan (Ass.), Mountain Ebony, Sivappu mandharai, Variegated Bauhinia, White Bauhinia

Table-3: Natural History [1]

1)	Cyclicity a) Leaf fall b) Flowering c) Fruiting	March-April January-March March-May
2)	Propagation Technique	Soak seeds in cold water for 24 hrs.
3)	Dispersal	Self-Dispersers
4)	Pollinators	Insect
5)	Size o Tree height o Tree spread o Circumference of tree trunk o Seed size	20-40 ft. 20-30 ft. 192 cm 1.6 cm

Geographical distribution [1]

Bauhinia variegata var. candida is a native plant to India. It is widely distributed throughout India (except Jammu and Kashmir, Himachal Pradesh, and Sikkim), Western Himalayas, Brazil, China and Myanmar. It is less common in Zimbabwe than other *Bauhinia* species.

Cultivation and collection [2]

- **Climate-** White Orchid-Tree should be grown at a temperature of 16°C on well-drained soil
- **Soil tolerances-** Clay, Loamy sand; slightly alkaline, well-drained
- **Drought tolerance-** The drought tolerance of the tree is high
- **Aerosol salt tolerance-** Moderate
- **Pest resistance-** No pests are seen
- **Winter interest-** Tree has winter interest with persistent fruits and showy flowers

- **Pests-** Caterpillars, mites, borers.
- **Diseases-** Leaf spot, leaf scorch or mushroom root rot disease might occur.

Problems during Cultivation

- The tendency to show nutritional deficiencies, especially potassium.
- Weak wood which is susceptible to breakage in storms.
- Abundant seedlings which may germinate in the landscape.
- The litter problem created by the falling leaves, flowers, and seedpods.

The seedlings of *B. variegata var. candida* are more efficient on using their photo assimilates, when they were cultivated under full sun and it showed etiolation when cultivated under black net with 70% shading [7].

Table-4: Physical Conditions [3]

Physical conditions of <i>Bauhinia variegata var. candida</i>	Values
Temperature(°C)	16.0
Wind speed (ms ⁻¹)	0.8
Wind direction	From S80° E to N60° W
Relative Humidity (%)	57
Light intensity(lux) (below canopy)	4240
Light intensity(lux) (above canopy)	13820

Microscopy [3]

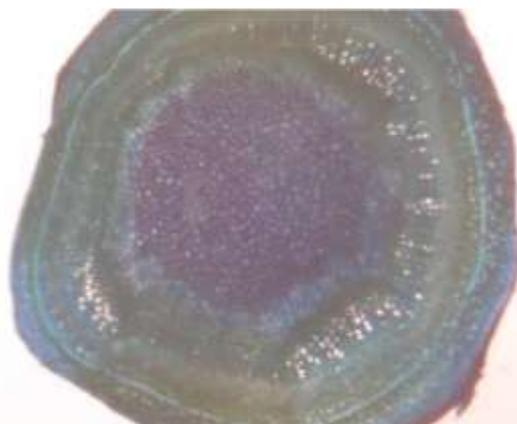
- **Dissection of flower of *B.variegata var. candida***
- Petal color- White
- No. of stamens- 5

- No. of petals- 5
- No. of sepals- 5(fused)
- No. of carpels- 1

**Dissection of fresh flower****Laminated dry specimens of dissected flowers****Cross-section (TS) of stem of *B.variegata var. candida***

- Shape- Circular
- Epidermal Hair- Absent

- Stomata Density:
Upper Epidermis- 0
Lower Epidermis- 5.4



Transpiration rate in leaves (wt. of water loss per unit area of leaf per unit time) $\text{g cm}^{-2} \text{hr}^{-1}$ = 5.18

Table-5: Morphology [2]

Sno	Part of tree	Characteristics	Morphology
1)	Leaf	Color	Green
		Arrangement	Alternate
		Type	Simple
		Margin	Bilobed, cleft
		Shape	Orbiculate
		venation	Palmate
		Type and persistence	Deciduous, semi evergreen
		Blade length	2-4 inch
		Diameter	11.12 cm
		Petiole length	2.23 cm
		Average no. of main veins on leaf	11.08
2)	Flower	Color	White
		Characteristics	Very showy, winter flowering, Bifid inflated trichomes on carpels [8]
		Width	7.17 cm
		Breadth	6.27 cm
3)	Fruit	Shape	Elongated; pod
		Length	12 inch or more
		Covering	Dry or hard
		Color	Black
		Characteristics	Showy; don't attract wildlife; fruit, twigs, or foliage cause significant litter; persistent on the tree
4)	Trunk and branches	Twig color	Brown
		Twig thickness	Medium, thin
		Characteristics	Droop as the tree grows, and will require pruning for vehicular or pedestrian clearance beneath the canopy; are routinely grown with, or trainable to be grown with, multiple trunks; don't contain thorns and are not particularly showy.
		Breakage	Susceptible to breakage; either the wood itself or tends to break at the crotch due to poor collar formation.



B. variegata var. candida Bark



B. variegata var. candida Flower



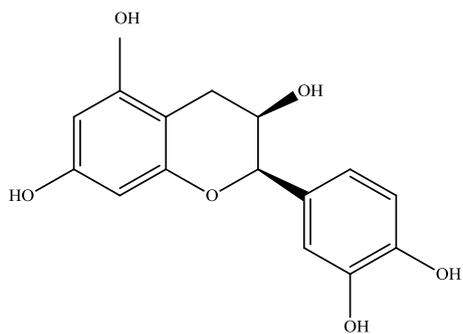
B. variegata var. candida

Phytochemistry of *Bauhinia variegata var. candida*

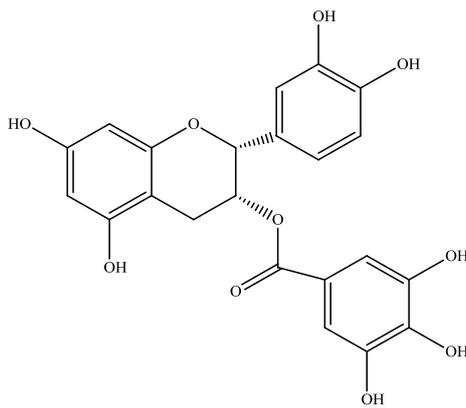
The bark of this species is phytochemically strong with phytoconstituents viz, carbohydrates, saponins, terpenoids, alkaloids, steroids, flavonoids, tannins, amino acids, and proteins. Leaf and shoot extract contains proanthocyanidins viz, epicatechin, epicatechin gallate, their oligomers, and epiafzelechin trimer. It contains unsaturated fatty acids and several monos, di- and tri-hydroxy fatty acids. It was found that shoot extracts are more enriched in flavonols i.e. rutin, quercetin, rhamnoside, epicatechin polyphenolic composition as compared to leaf extracts [7]. *Bauhinia*

species shows the presence of triterpenes viz, maslinic acid, stigmasterol, lupeol, β - sitosterol, β - amyryn and α - amyryn. The ethylacetate extract of this plant shows higher amount of stigmasterol (85%), lupeol (35%) and β - sitosterol (13%) in comparison with chloroform extract [9].

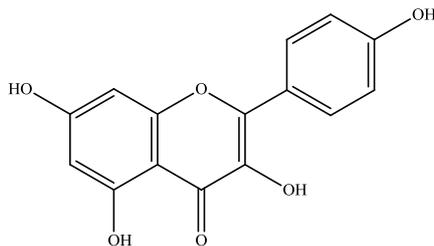
In *Bauhinia*, the flavonols are either acylated or glycosylated with one or more sugar units. Flavonols identified on MS and UV/VIS spectra were quercetin, kaempferol and isorhamnetin derivatives with quercetin and kaempferol conjugates [10].



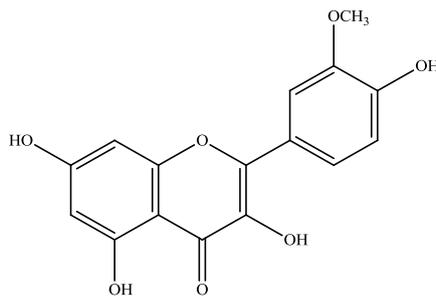
Epicatechin



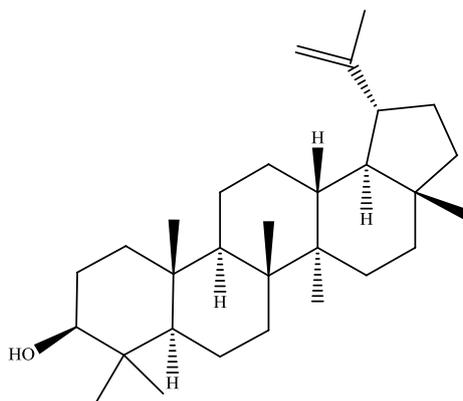
Epicatechin gallate



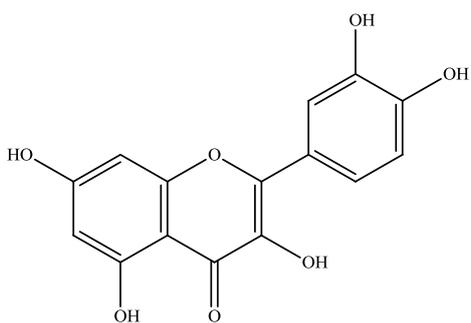
Kaempferol



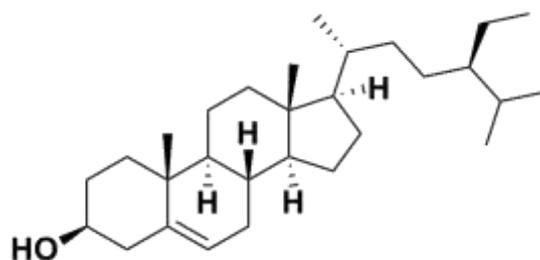
Isorhamnetin



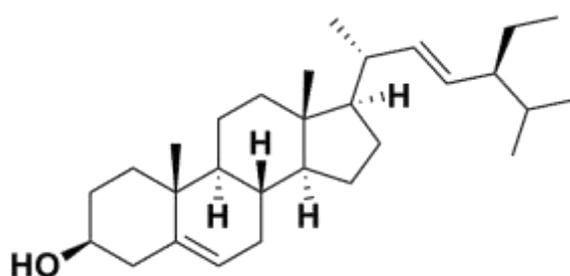
Lupeol



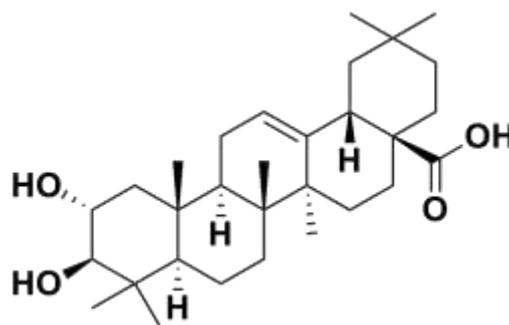
Quercetin



B- Sitosterol



Stigmasterol



Maslinic acid

Isolation and purification of Trypsin Inhibitors

The trypsin inhibitors were isolated and characterized from seeds of *B.variegata var. candida*, which showed the presence of the N-terminal amino acid sequences of six inhibitors and complete primary structure of one trypsin inhibitor. The purification used to obtain proteins which show trypsin inhibitor properties is satisfactory since the products exhibited a single band in SDS-PAGE under reductive conditions and only one N-terminal amino acid after Edman degradation. Electrophoresis control in SDS-PAGE of "pool 2" preparations from *Bauhinia variegata var. candida* trypsin inhibitor (BvcTI) displayed only one band under both reducing and non-reducing conditions and thus showed that the inhibitors were homogeneous, single-polypeptide chains of proteins with M_r 20000, without free sulfhydryl groups [11].

Isolation and Characterization of Galactoside Binding Lectin

B.variegata var. candida Lectin (BvcL) was isolated by gel filtration chromatography on Sephadex G75 and affinity chromatography on immobilized D-lactose column. The hemagglutination activity of the purified BvcL was tested with human trypsin-treated erythrocytes (A, B, AB and O) and it did not show specificity for any human blood group. The Biochemical characterization showed high content of serine, glycine, glutamic acid and low levels of methionine, histidine and aromatic amino acids and no residue of Cysteine was detected [12].

Nutrient content of *B.variegata var. candida*

Plant showed the presence of P, K, Na, Ca, Mg, Fe, Cu in higher amount and Vitamins B1, B2, and E in the lesser amount. The Total protein and Total amino acid content were found to be 15.79% and 12.74% respectively. The closest degree of essential amino acids contents in *B. variegata var. candida* to that in egg protein (the standard) was 0.85 [13]. Also, the flowers of *B. variegata var. candida* were submitted to electron beam irradiation at the doses of 0.5, 0.8 and 1 kGy, to study the effects in the nutritional and chemical profiles. It was found that the applied irradiation doses did not highly affect the nutritional profile. Thus, it proved to be promising edible wild vegetable flower with plenty of nutrients [14].

Biological activity of *B.variegata var. candida*

Antioxidant DPPH Assay

Lyophilized methanolic extract of leaf of plant was diluted with methanol covering a concentration range of 1-1000 $\mu\text{g/ml}$. Ascorbic acid was taken as positive control prepared in concentration range of 1-100 $\mu\text{g/ml}$ and measured spectrophotometrically at 492nm. The IC₅₀ was calculated for extract and standard [10].

Anti-inflammatory activity

The barks of *B.variegata var. candida* has potential to be used as an anti-inflammatory agent in future as the data obtained for decrease in paw edema volume with drug extracts in Wistar albino rats, in concentrations 200 mg/kg has the highest activity 44.4 % after 3hrs which is comparable to that of the standard diclofenac sodium solution (5mg/kg) 48% after 3 hrs. The rapid effect of anti-inflammatory of Diclofenac is due to its pharmacokinetic properties which are represented by a 1h T_{max} and 65% bioavailability with oral administration. Anti-inflammatory activity of the sample is supposed to be due to the presence of flavonoids and terpenoids (lupeol) [15].

Acetylcholinesterase inhibition

The hexane extracts of leaves, branches, and flowers of *B.variegata var. candida* were taken to investigate the potential of inhibition of the Acetylcholinesterase using the technique of thin layer chromatography. The plates were developed in the system n-hexane-acetone (80:20) followed by nebulizing with various spraying agents. The retention factor was determined using t-test to detect differences between treatments and t-student (LSD) (p-value<0.05) for comparison of means. It was observed that the Retention factor analysis shows values of 0.00aA, 0.26aA, 0.31aA for leaves, branches and flowers respectively of *B. var. candida*. It showed the flower extract of the plant to be most suitable for further studies on this inhibition [16].

Anti-diabetic activity

The insulin-secreting cell line INS-1 was used to examine the effects of crude ethanolic extracts of leaves of *B.variegata var. candida* and its major metabolite (6S, 7E, 9R)-9-hydroxymegastigma-4,7-

dien-3-one-9- β -glyco-pyranoside (Roseoside) on insulinotropic activity. INS-1 was derived by culturing rat insulinoma cells in the presence of 2-mercaptoethanol. It was immunofluorescence which showed that INS-1 cells store insulin. The ELISA kit was used for the measurement of insulin and was compared with insulin-positive controls and negative control (DMSO). As results matched the acceptance criteria of the kit and was found that the crude extract and the major metabolite were shown to increase insulin secretion in a dose-dependent manner. The statistically significance between groups was determined by application of analysis of variance followed by Bonferroni's test which showed that significant P value should be less than 0.05[17]. Also, Anti-diabetic activity was determined using methanolic extract of leaf using α -Glucosidase enzyme inhibition assay. Acarbose was used as positive control and IC₅₀ value was calculated [10].

Cytotoxic and Antimetastatic effect

The ethanolic extract of stem of *B.variegata candida* (Bvc) was taken and partitioned using hexane, ethylacetate and chloroform. These fractions were evaluated with inhibitory activity against extracellular matrix (ECM) metalloproteinase (MMPs) and the mechanism of action of these compounds was established on cell viability in human cervical carcinoma (HeLa) and human peripheral blood mononuclear cells (PBMCs). Also the migration and invasion, cell death pathways of HeLa cells and MMP-2 activity of this fraction were also evaluated. FR3 was the only fraction tested that completely inhibited MMP-2 and MMP-9 gelatinolytic activity in vitro, reduced the cervical cell viability and inhibited HeLa cell migration in wound closure assay. It showed to be most promising compound for cancer treatment as on exposure to FR3, it induces cell death via TNFR-1 and RIP1. This activity shows selective cytotoxic action on MOLT-4 leukemia cell line when correlated with the presence of ethyl-d-glucopyranoside and palmitic acid [18].

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