



# Pharmacognostic and Phytochemical Investigations of *Bahunia Variegata* Leaves (Kovidra)

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## Abstract

*Bahunia variegata* L belonging to the Caesalpinoideae family, commonly called as kovidra in sanskrit and kachnar in hindi. Kovidra is a medium sized deciduous tree mostly observed at altitude 1300 to 1800 in Himalayas. The plant is extensively utilized by the tribals all over in India and accepted in medicinal systems like Ayurveda, Unani and Homoeopathy. Secondary metabolites like carbohydrates, tannins, alkaloids, and flavonoids are present in *Bahunia variegata* plant. The aim of the current research is to develop different pharmacognostic and phytochemical analysis for the evaluation of leaf of this plant. Microscopy, Phytochemical analysis of leaf drug was performed and results were maintained. In microscopical view of *Bahunia variegata* leaf, the presence of thin walled epidermis, spongy paranchymatous cells, plenty of solitary calcium oxalate crystals, unicellular and multicellular covering trichomes were observed. Phytochemical tests indicated the occurrence of flavonoids, tannins, steroids and phenols etc. Further physicochemical analysis such as extractive value was determined which includes petroleum ether soluble extractive value, chloroform, methanol, and aqueous soluble extractive values. The results obtained from standardization of leaf drug recognized the macro and microscopical parameters, physicochemical parameters. Fluorescence analysis of leaf drug distinguishes the authenticity of plant drug; therefore these techniques are helpful for rapid recognition of crude drug. The outcome of the research study possibly will be helpful in setting investigative indices for the identification and grounding of a monograph of the plant drug.

**Keywords:** *Bahunia Variegata*; Leaf Microscopy; Physicochemical Analysis; Phytochemical Screening; Fluorescence Study

## Introduction

*Bahunia variagata* belonging to the family fabaceae and sub family caesalpinoideae is frequently identified as mountain ebony in English, kachnar in hindi and raktkanchan in marathi. It is inhabitant to moderate and tropical Indian Subcontinent, Southeastern countries of Asia and China [1]. Approximately 15 species of this genus found in India [2]. Kachnar is well described in ancient ayurveda and its stem, bark & flowers are widely used as remedy in a variety of formulations like kanchanar guggul. Bark of kanchanar is used in disorders like lymphadenopathy, goitre, tumor, and kapha-Pitta dosha disorders while flowers are used to treat pittaghna i.e. pitta dosha, dysfunctional uterine bleeding, and kshyaghna i.e. tuberculosis. Further it is also practiced by

ayurvedic practitioners in management of various carcinomas [3]. The tribal communities of diverse areas use this plant for the curing skin diseases such as leucoderma and leprosy, wounds, ulcers, cough, dysentery, snakebite, tumours, flatulence, indigestion, piles [4]. Reported phytochemical constituents of the *Bahunia variegata* are lupeol, tannins, kaempferol-3glucoside, amides, carbohydrates, crude protein, vitamin C, fibers, calcium, phosphorus, rutin, quercetin, apigenin, apigenin-7-O-glucoside, dotetracont-15-en-9-ol and heptatriacontan-12,13-diol [5-7].

Plant drugs usually adulterated with similar looking drugs therefore it is mandatory to use plant drugs after standardization for research work. This research paper deals with macroscopical characteristics of kachnar as well

as microscopy of leaves of kachnar plant. In addition it also consists of phytochemical screening of successive extracts of the kachnar plant [8].

## Materials and Methods

### Collection of Plant Material

Leaves of *Bauhinia variegata* were collected from Vadgaon area of Pune district in Maharashtra in the month of September and October 2019, and the samples were authenticated at the Agharkar Research Institutes Pune. The specimen voucher was preserved as herbarium in university.

### Macroscopical and Microscopical Evaluation

After botanical authentication of *Bauhinia variegata* plant, the macroscopic evaluation of the leaves was carried out as per standard procedure. Size & shape, colour, surfaces, venation, apex, margin, base, lamina, texture, and organoleptic properties were examined [9,10]. Fresh leaves were preferred for the microscopical examination. Transverse sections were taken out by free hand section cutting method. Various temporary and permanent slides of the microscopical sections of the specimen were prepared and examined microscopically [11].

### Powder Drug Investigation

The powder leaf drug was independently boiled with 5% phloroglucinol, 5% alcoholic potassium hydroxide and 5% Chloral hydrate solution, iodine- iodide for starch, sudan IV for lipophilic substances and in ruthenium red for presence of mucilage [12]. In addition to that quantitative microscopy of *Bauhinia variegata* leaves were carried out. For that purpose fresh leaf was independently mounted in glycerin to determine the presence of lignified cells, calcium oxalate crystals and starch grains.



Figure 1: *Bauhinia variegata* Tree.

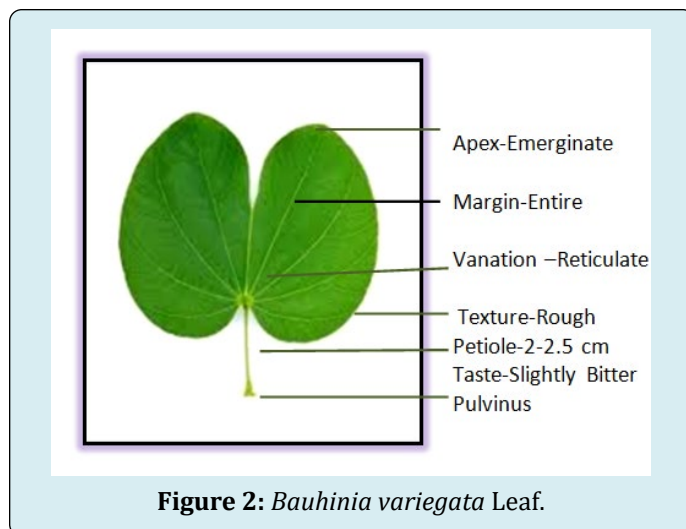


Figure 2: *Bauhinia variegata* Leaf.

### Physicochemical Parameters

1gm of dried powdered leaves was used for total value determination by keeping it in muffle furnace at 400°C temperatures. Total ash was studied for different inorganic constituents like fraction of total ash, acid- insoluble ash, water soluble ash, sulphated ash and loss on drying were calculated as per the Indian Pharmacopoeia [13]. Successive extracts of *Bauhinia variegata* leaves were prepared for the examination of extractive values of the leaves. Powdered leaves were further analyzed for fluorescence analysis according to the standard methods [14,15].

### Successive Solvent Extraction

Successive extraction of leaf drug was carried out by using powder of dried *Bauhinia* leaves along with organic solvents. 100 gm powdered leaves were subjected to continuous soxhlet extraction unit with various organic solvents as per polarity such as petroleum ether (60-80), chloroform, and methanol and water respectively [16].

### Phytochemical Investigation

The obtained successive extracts were further concentrated under vacuum using rotary vacuum evaporator, dried and weighed for calculating percentage yield. Phytochemical analysis of each extract was performed for presence of secondary metabolites, viz. proteins, carbohydrates, tannins, glycosides, flavonoids, saponins, fats and amino acids by usual prescribed methods [17,18].

### Fluorescent Studies of Powder Drugs

The fluorescence analysis of powdered drug was carried out by UV Spectroscopic method under 254 nm and 365 nm after treatment with various reagents [19].

### Total Phenolic Content

Powdered leaves were studied for concentration of phenolic compounds with the help colorimetric method of Folin-Ciocalteu. The absorbance was calculated at 765 nm against blank, using Shimadzu UV spectrophotometer. The total polyphenolic content was thus measured as gallic acid equivalents and expressed in % as gallic acid [20].

### Total Tannin Estimation

Total tannin content of *Bauhinia variegata* leaf extract was estimated with Folin-Denis method. The absorbance was calculated at 760 nm using Shimadzu UV spectrophotometer. Experiment was performed in triplicate and the estimated quantity was measured from calibration curve obtained with tannic acid. Total tannin content was expressed as g / 100g of tannic acid equivalent [21].

## Results and Discussion

### Macroscopical Evaluation

The leaves of *Bauhinia variegata* plant is of fresh green in colour on both side in fresh state and light brown in dry condition. Size and shape of leaves are 12-14 in length and 13-15 cm in breadth, cleft 1/4 to 1/3 of the way down into lobes are divided in to 2 obtuse portions, base is pulvinus with ridged petiolate, linear- lanceolate with entire margin along with presense of soft stipules. Apex of the leaf is wide and emarginated. The outer surface of leaf is moderately rough. In addition to that leaf showcased reticulate and palm-divergent venations as shown in figure 2. Taste of leaf is bitter to some extent and having characteristic of odour.

### Microscopical Characteristics

In the transverse section of *Bauhinia variegata* leaf (Figure 3) mesophyllic stratum in the laminar portion of leaf showcased the presence of palisade cells in 2 to 3 layers below the upper epidermis and spongy parenchyma above the lower epidermis. Calcium oxalate crystals are present throughout the mesophyllic membrane in prismatic manner. Midrib is slightly concave in shape. Midrib contains well developed vascular bundle at the centre surrounded by sclerenchymatic cells. The presence of the xylem at the upper side and phloem at the lower side of vascular bundle could be seen. Collenchymatous cells are scattered in between upper and lower epidermis along with calcium oxalate crystals as characteristic feature. In addition to that covering trichomes are present with peculiarity i.e. they are unicellular and multicellular uniseriate as well as unicellular sessile. They are majorly observed on the lower epidermis than upper epidermis.

### Powder Microscopy

In the powder microscopy, prism shaped calcium oxalate crystals, long multicellular uniseriate trichomes; lignified spiral xylem vessels and fraction of epidermal cells were observed.

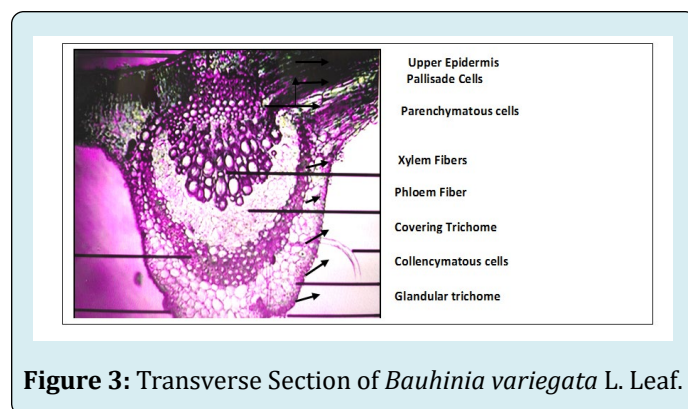


Figure 3: Transverse Section of *Bauhinia variegata* L. Leaf.

### Physicochemical Parameters

The percentage of total ash, acid insoluble ash, water soluble ash and loss on drying are summarized in Table 1. Successive solvent extraction values in various organic solvent were calculated as petroleum ether 3.15 %, chloroform 4.30 %, methanol 6.86 % and water is 8.90 % as shown in Table 2.

S.No.	Parameters	Values (%)
1	Total Ash Value	8.15
2	Water insoluble ash value	6.25
3	Water soluble ash value	2.55
4	Acid insoluble ash value	5.4
5	Loss on drying	6.55

Table 1: Physiochemical Parameters of *B. Variegata* L.

Type of Extract	Colour of extract	Extractive value % w/w (Mean a ± SEM)
Petroleum ether soluble	Sticky Greenish-Brown	3.15 ± 0.05
Chloroform soluble	Sticky Greenish	4.30 ± 0.05
Methanol soluble	Light Brown	6.86 ± 0.05
Water soluble	Water soluble Brown	8.90 ± 0.05

Table 2: Extractive value of Successive Leaf Extracts of *B. Variegata* L.

### Phytochemical Analysis

The results confirmed presence of alkaloids, flavonoids, saponin, tannins, steroids, phenols and carbohydrates

in *Bauhinia variegata* L leaf. The presences of variety of secondary metabolites in various organic and aqueous extracts are summarized in Table 3.

Sr. No	Phytochemical tests	Petroleum ether Extract	Chloroform Extract	Methanolic Extract	Aqueous Extract
1	Carbohydrates	+	+	-	-
2	Saponins	++	-	-	-
3	Glycosides	-	-	-	-
4	Tannins	-	-	++	++
5	Flavonoids	-	-	++	++
6	Phenols	-	-	+	+
7	Alkloides	-	+	-	-
8	Fixed Oil	+	-	-	-

**Table 3:** Phytochemical Analysis of Successive Leaf Extracts of *B.Variagata* L.

### Fluorescence Analysis

Result of fluorecence analysis of *Bauhinia variegata* leaf powder was observed at UV short wavelength and long

wavelength i.e at 254 and 366 nm respectively. The response at different wavelengths was recorded and summarized in Table 4.

S. No	Powdered drug with Different Reagent	Observation under visible light	Observation Under UV 254 nm	Observation Under UV 366nm
1	Drug Powder	Greenish Brown	Green	Light Brown
2	Drug+ Pet Ether	Slight Green	Green	Slightly Brown
3	Drug+5%Iodine	Light Brown	Light Green	Deep Green
4	Drug+5% FeCl <sub>3</sub>	Dark Brown	Green	Deep Green
5	Drug+ 1M H <sub>2</sub> SO <sub>4</sub>	Green	Light Green	Brownish
6	Drug + Dilute Ammonia	Light Green	Fluorescent Green	Brownish
7	Drug+ Methanol	Dark Green	Green	Brownish
8	Drug+ KOH	Yellowish Green	Light Green	Blackish Brown
9	Drug+ Picric Acid	Light Green	Light Green	Dark Green
10	Drug+ Chloroform	Light Green	Light Green	Brownish

**Table 4:** Fluorescence Analysis of *B. Variegata* Linn. Leaf Powder with different reagents observed under UV-radiation.

### Total Phenolic and Tannin Content

The total polyphenolic content was thus measured as gallic acid equivalents and obtained  $82.71 \pm 0.5$  per 100gm of gallic acid. Total tannin content was obtained  $89.25 \pm 5.25$ /100g of tannic acid equivalent.

### Conclusion

*Bauhinia variagata* Linn is the traditional therapeutic plant with a great potential and acts as remedy to cure diverse ailments. The present research work was focused on

performance of standardization parameters for recognition of original drug. These analytical techniques include macroscopical & microscopical examination idiosyncratic identification of drug. In addition to that physicochemical analysis was performed with parameters like ash values, loss on drying, extractive values. Further, qualitative phytochemical investigation was also performed on successive leaf extracts. These standardization parameters were studied and data obtained possibly will be helpful for the quality assessment of plant drug material, secondly, this data would be helpful for determination of adulterated and

substituted drug. The pharmacognostic approach of study of *Bauhinia variagata* Linn; sets qualitative and quantitative standards. It would be definitely establishes distinctiveness, eminence and purity of this plant drug in intimately correlated species.

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