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## Research Article

### PHARMACOGNOSTICAL STUDIES AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON THE BARK OF *BAUHINIA VARIEGATA* (CAESALPINIACEAE)

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#### ABSTRACT

*Bauhinia variegata* (Family - Caesalpiniaceae) is a medium-sized, deciduous tree, found throughout India, ascending to an altitude up to 1800m in Himalayas. The objective of the study was to develop various standardization parameters for the evaluation of Bark of this plant. Microscopy, powder characteristics of bark were observed and results were recorded. Physicochemical analysis such as extractive value includes petroleum ether, ethanol, ethyl acetate and aqueous soluble extractive values of 2.88, 6.86, 2.96, and 8.88% w/w respectively; extracts were analysed by chemical test and showed presence of flavonoids, tannins, steroids and triterpenoids etc. The preliminary phytochemical investigation indicated presence of tannins, carbohydrates, flavonoids and steroids. The results obtained from standardization of bark established the macro and microscopical parameters, physicochemical parameters, TLC profiles that characterize the genuine plant drug (*B.variegata*) so these parameters are useful for quick identification of drug and are particularly useful in the case of powdered materials.

#### INTRODUCTION

There are about one hundred species under the genus *Bauhinia* (Caesalpiniaceae) and eight are native to India, ascending to an altitude of 1300 meter in the Himalayas. Bark grey with longitudinal cracks, pale pink inside, leaves rather broader than deep, flowers variously coloured, in few-flowered, lateral, sessile or short-peduncled corymbs, pods long, hard, flat, glabrous, dehiscent, 10-15 seeded<sup>[1]</sup>. In India it is commonly known as 'Kachnar'. The bark is used as one of the ingredients of *Kanchnar-guggulu* used for various glandular swellings and in Ayurvedic preparation used for goitre<sup>[2]</sup>.

The flowers contains essential oil myrcene, linalool, borneol, limonene and eugenol<sup>[3]</sup>.

Crude extract of the bark have been studied for antidiabetic, antioxidant, antiinflammatory activity<sup>[4]</sup>. Detail pharmacognostical studies of leaf of the plant have been reported by Kamal M Modh et al.<sup>[5]</sup>

Since there is no report on systematic pharmacognostical and phytochemical studies on bark, in order to fix some standards for its identification. The present work focuses on microscopical study of the

bark surface and some reports related to physicochemical parameters of the plant.

#### MATERIALS & METHOD

##### Collection of plant material

The stems with leaves of *Bauhinia variegata* (family - Caesalpiniaceae) were collected from Nagdhar area of Pokhari district (Chamoli) in the month of September 2017, and the samples were authenticated at the Botanical Survey of Dehradun Uttarakhand India. The specimen voucher were preserved at Herbarium of UIPS.

##### Macro- and microscopy

After botanical confirmation of plant species, the macroscopic examination of the Bark was carried out according to standard procedure<sup>[6, 7]</sup>. Fresh bark was selected for the microscopical studies. sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the micro-scopical sections of the bark specimen were made and examined microscopically. Histochemical tests were carried out using hydrochloric acid-

phloroglucinol to reveal lignified tissues, iodine-iodide for starch, sudan IV for lipophilic substances, ruthenium red for mucilage (Khandelwal, 2008) [8].

#### **Powder characteristics**

Preliminary examination, behaviour of powder drug with different chemical reagent and microscopical examination was carried out [9]. A small quantity of the bark powder was heated for 10 min in chloral hydrate solution followed by staining with phloroglucinol and conc. HCl. A pinch of the powder was taken on a slide and observed under the microscope [10].

#### **Physicochemical parameters**

The total ash of the powdered bark was tested for different inorganic constituents percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash and loss on drying were calculated as per the Indian Pharmacopoeia [11]. Various extracts were prepared for the study of extractive values of the bark [12]. Fluorescence analysis of powdered bark was carried out by standard methods [13-15].

#### **Preliminary chemical analysis**

For the preliminary phytochemical analysis, 100 gm of powdered bark of *B.varigata* was extracted with petroleum ether (40-60° c), chloroform, ethyl acetate and methanol successively by successive extraction methodology by using Soxhlet apparatus. The aqueous extract was prepared by cold maceration technique. The extracts were concentrated under vacuum using rotary vacuum evaporator, dried and weighed. Each extract was tested for presence of different phytoconstituents, viz. proteins, sugars, tannins, glycosides, flavonoids and amino acids by usual prescribed methods [16-18]. The TLC pattern of petroleum ether extract and chloroform extract was studied using pre-coated silica gel plates (Merck).

#### **Quantification of total phenolics**

The concentration of phenolic compounds in powdered bark was determined by Folin-Ciocalteu colorimetric method [19]. The absorbance was measured at 765 nm against reagent blank, using Shimadzu UV-1601 spectrophotometer. The total polyphenolic content was calculated as gallic acid equivalents and expressed in % as gallic acid.

#### **Quantification of total tannins and carbohydrates**

Total tannins were estimated by using Folin-Denis method [20]. The absorbance was measured at 760 nm using Shimadzu UV-1601 spectrophotometer. Analyses were carried out in triplicate and the quantification was calculated from calibration curve

obtained with tannic acid. Total tannins were expressed as g / 100g of tannic acid equivalent.

Total carbohydrates were estimated by using phenol-sulphuric acid method [21]. The absorbance of the solution was measured at 490 nm using Shimadzu UV-1601 spectrophotometer. Glucose was used as a standard to obtain a calibration curve.

#### **Estimation of total alkaloids, mucilage, unsaponifiable matter and crude fibre content**

The total alkaloids and mucilage content was determined by the prescribed method [16] and the unsaponifiable matter of the bark was determined by the standard procedure of Indian Pharmacopoeia [11]. The total crude fibre content of the bark was determined by the procedure described by Raghuramulu et al [22].

### **RESULTS AND DISCUSSION**

#### **Macroscopy**

The bark is externally brownish and internally light reddish brown in colour. Pieces of the bark are curved or occur in the form of flat pieces with size of 7-10 cm long and about 1.5-3cm thickness (plate-1). The surface of the bark is rough. It has mucilaginous taste which is followed by bitter sensation. The odour is characteristic.

#### **Microscopical characteristics**

Transverse section of bark of this plant showed that the periderm is composed of cork cells which is 12-20 layers of radially arranged of thin walled, elongated cells. Below it there is a single layer of phellogen which is followed by wide zone of phelloderm, tangentially elongated to isodiametric cells. This zone also contains lignified fibres and stone cells. The pericyclic fibres found in the bark have narrow lumen, thickened, lignified, broad walled and tapering ends. The phloem part of the bark is characterized by sieve tubes, companion cells, crystal fibres, phloem fibres, phloem parenchyma and stone cells, transverse by unibivariate medullary rays (plate-2).

#### **Microscopical study of bark powder**

The cork cells are polygonal in shape containing occasional brownish in colour. The powder showed presence of fibres which are lignified occurs lengthwise in groups of 3-5. Some of the parenchymatous cells surrounding the group of fibres contain calcium oxalate crystals arranged in the rows. The starch grains are simple, adequate having size of 20-25 $\mu$  (plate-3).

#### **Preliminary chemical analysis**

Colours of extracts were observed (Table 1). Extracts were further analysed by performing

various chemical tests for identification of chemical constituents (Table 2). Analysis of these various extracts showed presence of tannins, flavonoid, saponins and carbohydrates. So bark of *Bauhinia variegata* show presence of specific moiety utilized for pharmacological investigation. Aqueous extract also gave positive tests for carbohydrates. Methanol, ethyl acetate and aqueous extract indicated presence of phenolic compounds.

The TLC profile of petroleum ether extract indicated presence of four compounds with the solvent system petroleum ether: ethyl acetate (95:5). Chloroform extract showed presence of three compounds with the solvent system chloroform: methanol (80:20) detection reagent Ferric Ammonium sulphate (2%) reagent (Table 3). The results of estimation of total phenolics, total tannins, total carbohydrates, total alkaloids, mucilage content, unsaponifiable matter and crude fibre content are presented in Table 4.

#### Fluorescence analysis

Fluorescence analysis helps in the analysis of chemical constituents. Bark powder was reacted with different solvents as well as chemical reagents and observed at UV short wavelength and long wavelength (274 and 366 nm). The response at different wavelengths was recorded (Table 5).

#### Physicochemical parameters

The results of determination of total ash, acid-insoluble ash, water soluble ash, sulphated ash, loss on drying are tabulated in Table-6. The qualitative analysis of ash indicated presence of calcium, aluminium, potassium, chlorides and sulphates.

#### DISCUSSION

The bark of *Bauhinia variegata* show presence of promising active constituents such as flavonoids, flavones, glycoside, cardiac glycoside etc<sup>[23]</sup>. The present study includes analysis of petroleum ether, ethyl acetate, ethanol extracts of bark of *Bauhinia variegata* and it shows presence of tannins, flavonoid, steroids and triterpenoids (Tables 1 and 2). Evaluation of physicochemical parameters is an indispensable part in the preparation of modern monograph. Thus different ash values, extractive values, loss on drying and fluorescence characteristics can be used for standardizing the crude drug samples. The TLC developed profiles can be used for identification as well as guide for isolation of various compounds from the extracts.

#### CONCLUSION

*B.variegata* Linn., commonly known as 'Kachnar', is a small sized deciduous tree with dark brown and more or less smooth bark found commonly in

various regions of Uttarakhand. The plant is reported for its various activities. The steam bark of this taxon have not been studied so far. In the present work, histological and phytochemical study of bark of *B.variegata* has been carried out. The pharmacognostic studies of this plant, however, help in the identification and authentication of taxon. The detailed phytochemical analysis of bark reveals that it contains tannins, flavonoid, steroids and triterpenoids. Microscopical study focuses on various characteristics such as cork cell, medullary rays, calcium oxalate crystal, starch grains and lignified fibers of the bark of this plant and helps in further analysis of this plant species.

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**Table 1- Extractive value**

Extract	Colour of extract	Extractive value % w/w (Mean a ± SEM)
Petroleum ether soluble	Brown	2.88 ± 0.07
Ethanol soluble	Brown	6.86 ± 0.88
Ethyl acetate soluble	Brown	2.96 ± 0.08
Water soluble	Brown	8.88 ± 0.44

\*Mean values of three readings

**Table 2- An observation of phytochemical screening of on bark of *Bauhinia variegata* Extracts**

Extract	Carbohydrates	Saponins	Glycosides	Tannins	Flavonoids	Phenols	Alkloides
Petroleum Ether	+	+	-	++	+++	++	-
Ethanol	+	++	-	+	++	+	+
Ethyl acetate	-	+	-	++	++	+++	-
Aqueous	++	++	-	++	++	+	-

Presence of constituents (+), absence of constituents (-)

**Table 3- Description of TLC pattern of extracts of *Bauhinia variegata* bark**

Extract	Visible	Visible under UV		After Spray	Rf
		254nm	366nm		
Petroleum ether extract					
1	--	-	-	Light pink	0.13
2	--	-	-	Light brown	0.16
3	--	-	White fluorescent	Dark brown	0.46
Chloroform extract					
1	--	-	-	Pink	0.53
2	--	-	-	Pink	0.4
3	--	-	Yellow fluorescent	Pink	0.73

**Table- 4- Standards of *Bauhinia variegata***

Parameter	Percentage (Mean <sup>a</sup> ±SEM)
Total phenolics	88.71 ± 1.04
Total flavonoid	132.89 ± 3.34
Total tannins	96.71 ± 6.45
Total carbohydrates	5.06±0.40
Total alkloids	3.40±0.45
Mucilage content	20.5±0.34
Unsaponifiable matter	16.67±0.56
Crude fibre content	58.3±0.56

<sup>a</sup>Mean values of three readings.

**Table 5- Behaviour of leaf powder *Bauhinia variegata* with different reagents observed under ordinary light and UV-radiation**

Interaction of powder drug with different reagent	Colour produced under visible light	Colour under UV-radiation	
		254nm	366nm
Drug (P)as such	Reddish brown	Brown	Light brown
P +Fecl3	Deep brown	Brown	Black
P+1N HCL	Reddish Brown	Reddish brown	Reddish brown
P+conc. H2SO4	Reddish brown	Reddish brown	Reddish brown
P+KOH	Yellowish green	Pale Green	Black
P+nitric acid	Yellowish brown	Pale brown	Dark green

**Table- 6- Physicochemical analysis of bark of *Bauhinia variegata***

Parameter	Obtained values (%w/w) (Mean <sup>a</sup> ±SEM)
Total ash	9.52± 0.43
Acid insoluble ash	0.57±0.03
Water soluble ash	4.7±0.04
Sulphated ash	3.45±0.05
Loss on drying	7.0±0.43

\*Results expressed as Mean<sup>a</sup> ± SEM from three observations

**Plate 1-Bark of *Bauhinia variegata***

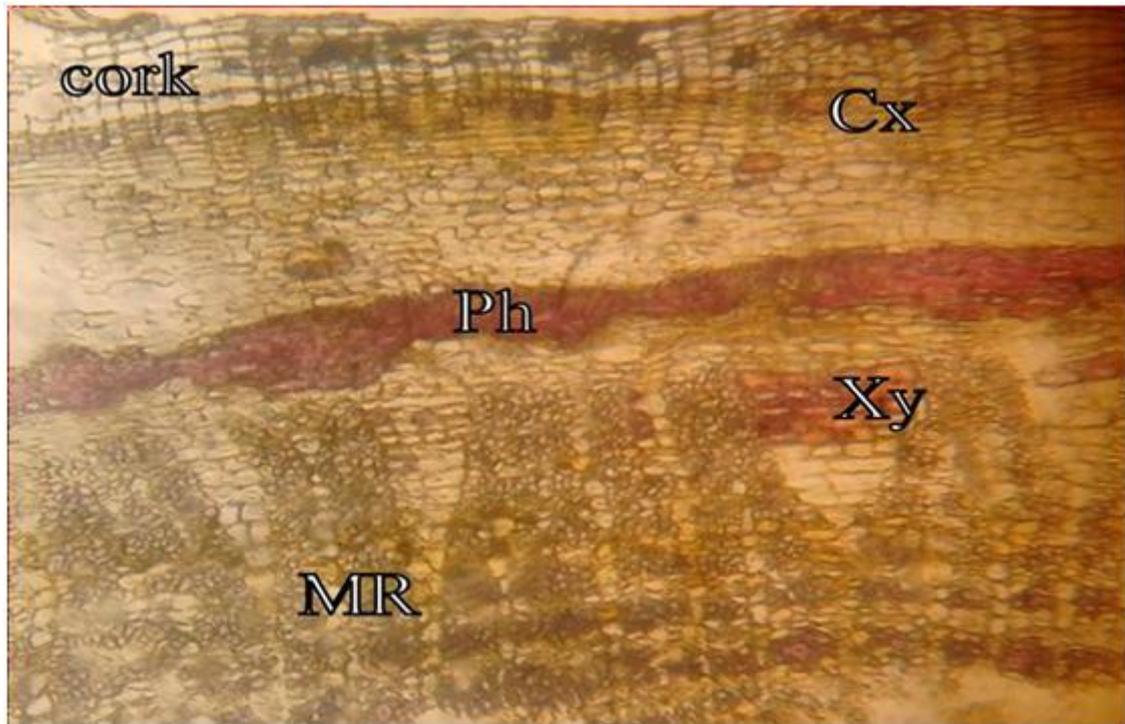


Plate 2- T.S of *Bauhinia variegata* stem bark (Abbreviations: Cx: cortex ; Ph: phloem ; Xy: xylem ; MR: medullary rays)



Plate 3- powder characteristics of *Bauhinia variegata* stem bark