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Preliminary phytochemical and pharmacognostic analysis of *Bauhinia tomentosa*

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ABSTRACT

Bauhinia tomentosa is one of the best, versatile and most commonly used house hold remedy for many manifestations. The present study is aimed to investigate the pharmacognostic analysis of the leaves and the phytochemicals present. The leaves were fixed in TBA, sectioned and then stained with toluidine blue. The leaves were dried and extracted with petroleum ether, ethyl acetate, ethanol and water. Phytochemical analysis was done with all four extracts using standard procedures. The pharmacognostical studies revealed the presence of calcium oxalate crystals and different cell components were studied and measured. The extractive yield was found to be high in aqueous and ethanol extracts. Most of the phytochemicals were found to be present in the ethanol extract. Further studies are to be carried out to assay the bioactivity of the phytochemicals and the medicinal values of the various phytochemicals present in Bauhinia tomentosa.

Key words: *Bauhinia tomentosa*, pharmacognosy phytochemicals, ethanol extract

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INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization [1].There is a widespread belief that the herbal medicines are healthier and safer than synthetic ones [2].There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurveda, Siddha, Unani and Chinese medicine. Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs [3, 4].

Bauhinia is a genus of more than 200 species of flowering plants in the sub family *Cesalpinioidae* of the large flowering plant family, *Fabacae* with a tropical distribution.

Bauhinia tomentosa commonly known as Yellow bell orchid tree belongs to *Fabaceae* family is one of the best, versatile and most commonly used household remedy for many manifestations [5]. The generic name commemorates the bauhin brothers Jean and Gaspard, the swiss botanists; the two lobes of the leaf exemplify the two brothers. *Tomentosa* derived from tomentose, meaning with dense, interwoven hairs. It is commonly known as 'Kanchini' in Tamil and 'Phalgu' in Sanskrit [6].

It is usually a scrambling, many stemmed shrub or small tree reaching 4m (max.8) in height, the branches often drooping, with many slender twigs [7].Leaves are deeply divided for almost half their length, with a small apical appendage between the lobes; each lobe is oval to almost elliptic, most often small about 2.5 x 2.5 cm, but may be up to 8cm, the colour of the leaf is pale fresh green. The apex of each lobe is broadly tapered. The base of the whole leaf is shallowly lobed and the length of the leaf stalk is usually around 10-30 mm long. [8].

The dried leaf, flower bud and a decoction of the root and bark of *Bahuinia tomentosa* is used by the medical practitioners. In India and Srilanka, the root bark is used internally for gastric problems, while the flower is used as a remedy for dysentery and diarrohea [9, 10].

The present study is focused to investigate the pharmacognostic and phytochemical analysis of leaves of *Bauhinia tomentosa*.

EXPERIMENTAL SECTION

2.1-Sample Collection and Authentication

The leaves of *Bauhinia tomentosa* Linn were collected from Villivakkam, Chennai and authenticated by Dr.S. Jayaraman, Director of Plant and Anatomy Research Centre, West Tambaram, Chennai (PARC/2014/2294)

2.2- Pharmacognostic Analysis

2.2.1- Preparation of Sample

Care was taken to select healthy plants. The required samples of leaf were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml).After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol [11]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.2.2- Sectioning

The paraffin embedded specimens were sectioned with the help of rotary Microtome to a thickness of 10-12 µm. Dewaxing of the sections was by customary procedure [12]. The sections were stained with Toluidine blue [13].The staining results were remarkably good; and some cytochemical reactions were also obtained. Wherever necessary, sections were also stained with safranin and fast-green and Iodine (for Starch)

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured.

2.2.3-Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [14].

2.3-Phytochemical Analysis

2.3.1- Preparation of plant extract

The leaves of *Bauhinia tomentosa* Linn. Were first washed, shade dried and then ground into coarse powder using a mechanical grinder. 50 g of the powdered leaf material was extracted with petroleum ether, ethyl acetate, ethanol and water successively by hot percolation method [15]

2.3.2-Qualitative Phytochemical Analysis

Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, proteins and anthraquinones were performed according to the standard procedure [16],

Alkaloids: Mayer's Test: To 1ml of extract, add 2ml of Con.HCL then few drops of Mayer s reagent was added. Green colour or white precipitate shows the presence of alkaloids.

Flavonoids: To 1ml of extract add 2ml of 1% aluminium solution. Appearance of yellow colour indicates the presence of flavonoids.

Steroids: To 1ml of the filtrate add 10ml of Chloroform and 10ml of sulphuric acid slowly by the sides of the test tube. Red colour in upper layer and sulphuric acid layer showed the yellow colour with green fluorescence shows the presence of steroids.

Tannins*:* To 1ml of extract, add 2ml of 0.1% Ferric chloride. Positive test shows brownish green or blue black colouration

Saponins: To 1ml of extract, add 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicates the presence of saponins.

Anthraquinones*:* To 1ml of the extract, add 10ml of benzene, filter it and add 5ml of 10% (v/v) ammonia to the extracts and shake well. Development of pinkish coloured solution indicates the presence of anthraquinones.

Phenols*:* To 1ml of extract add 5ml of Folin s ciocalteau reagent followed by 4ml of sodium carbonate Appearance of blue colour shows the presence of phenols**.**

Carbohydrates*:* a) To 1ml of extract add few drops of Molisch s reagent and few drops of concentrated sulphuric acid which gives purple colour.b) To 1ml of extract, add 5ml of Benedict's reagent and boil for 5 minutes. Bluish green colour indicates the presence of carbohydrates.

Amino acids*:* To 1ml of filtrate, few drops of 0.2% ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of amino acids.

Cardiac glycosides*:* To 1ml of extract, add 1ml of Ferric chloride reagent and few drops of concentrated sulphuric acid. Greenish blue colour appears within few minutes indicating presence of cardiac glycosides.

RESULTS AND DISCUSSION

3.1 Pharmacognostic Analysis

3.1.1 Anatomy of the Lamina

The leaf is dorsiventral and heteromorphic in internal structure. The lateral veinlets (Fig 1.1), lateral vein (Fig 1.2) and the main midrib (Fig 1.3) are prominently projecting on the abaxial side of the lamina. The adaxial side of the veins is flat. The vascular system of the veins includes a single wide and thick vascular strand, which is thick in the mid part and thin on either side (Fig 1.2 and 1.3). The vascular strand consists of several vertical discontinuous rows of xylem elements. The dements are elliptical in sectional view, wide and thick walled (Fig 1.3). Phloem occurs in thick continuous arc of sieve elements at the lower part of the xylem. The entire collateral system of the vascular strand is en sheathed by a thick cylinder of fibres (Fig 1.2 and 1.3)

The midrib is tweek and slightly elongated in vertical plane. The epidermal layer of the midrib consists of small with papillate outer tangential walls (Fig 1.3). The lateral veins also have papillate epidermal cells. The ground tissue of the veins is homogenous and parenchymatous.

The lamina (Fig 2.1 and 2.2) is dorsiventral with distinct adaxial and abaxial sides. The epidermal layer consists of fairly wide, thin walled cells with thin cuticle. The mesophyll tissue consists of adaxial band of oblong columnar palisade cells. The abaxial part consists of lobed 4 or 5 layers of spongy parenchyma which are loosely arranged forming wide air chambers. The lamina is 100 µm thick (Fig 2.1)

3.1.2 Crystals in the mesophyll tissue:

Fairly large calcium oxalate druses are found located diffusely in the mesophyll tissue of the lamina. The druses are 15 µm in diameter (Fig 2.2)

3.1.3 Epidermal Cells and Stomata:

The abaxial epidermis was studied by surface view of the paradermal sections. The epidermis is stomatiferous. The epidermal cells are fairly wide and possess thin wavy anticlinal walls (Fig 3.1 & 3.2).The stomata are randomly distributed and they are mostly paracytic type. There are wing like laterally placed subsidiary cells for each stroma (Fig 3.2). The stomata have elliptical guard cells and narrow slit like stomatal aperture. The guard cells are nearly 25 x 30 µm in size (Fig 3.3)

3.1.4 Adaxial epidermis:

In surface view the adaxial epidermal cells consist of small, amoeboid cells with thin highly wavy anticlinal walls. The epidermis is apostomatic (Fig 4.1)

3.1.5 Crystal Distribution:
Calcium oxalate crystals are frequently seen in the lamina. There are 2 types of crystals located in the lamina. Prismatic crystals are restricted to the veins where the crystals are found in vertical rows all along the veins (Fig 4.2). The prismatic crystals are 25 x 15 µm in size.

The other types of crystals are druses. They occur diffusely distributed in the mesophyll tissue. The druses occur in the unmodified mesophyll cells. They are 30 µm in diameter (Fig 4.2).

3.1.6 Venation pattern of the lamina:
The lateral veins are of different thickness. The ultimate vein lets are fairly wide and polygonal with well defined vein boundaries. So, the venation appears densely reticulate (Fig 5.1). Vein terminations are seen in most of the islets. The terminations are either simple and unbranched or branched once forming 2 dichotomies. The terminations are either straight or undulate (Fig 5.2).

3.1.7 Powder Microscopy:
i) Epidermal cells and stomata :
In surface view of the abaxial epidermis, the epidermal cells appear highly lobed and amoeboid in outline, due to wavy anticlinal walls (Fig 6.1). The stomata are densely distributed with different orientation. The stomata are predominantly paracytic type. There are 2 subsidiary cells one on either side of the stoma with parallel orientation to the guard cells (Fig 6.2). The guard cells are narrowly elliptical measuring 10 x 15 µm in size. The stomatal aperture is narrow and slit like (Fig 6.3)

ii)Epidermal trichomes:
Non glandular epidermal trichomes are densely distributed on the abaxial side of the lamina (Fig 7.1). These trichomes are either straight or curved (Fig 7.2). The trichome is multi cellular, uniseriate and unbranched. The cells are vertically elongate and the cell walls are very thick and wide. The septum (cross wall) is also very thick (Fig 7.3). The trichomes are 520µm long and 10µm wide.

Fig 1.1., Fig 1.2. & Fig 1.3. Lamina

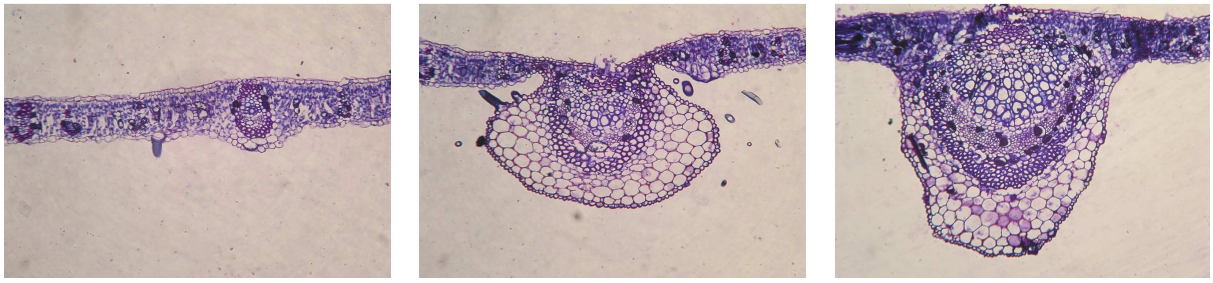


Fig 2.1. & Fig 2.2 Crystals in Lamina

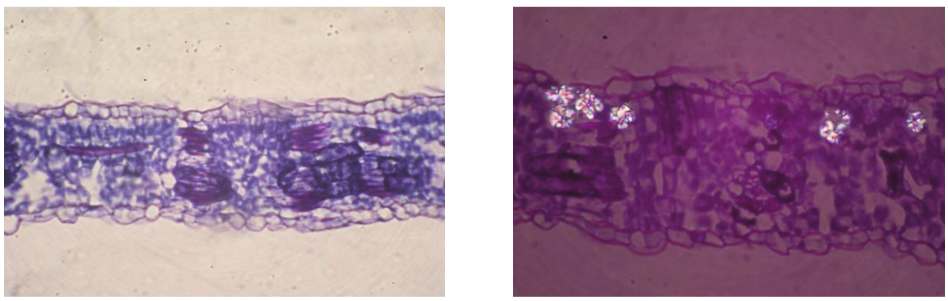


Fig 3.1., Fig 3.2. & Fig 3.3. Epidermal Cells and Stomata

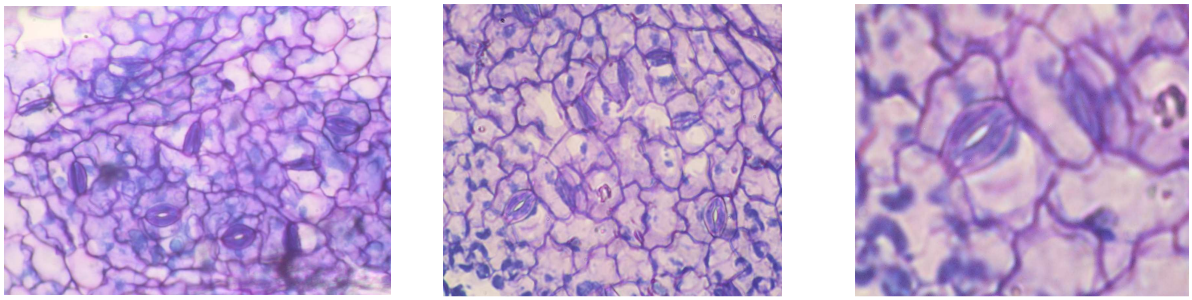


Fig 4.1. & Fig 4.2. Crystal Distribution

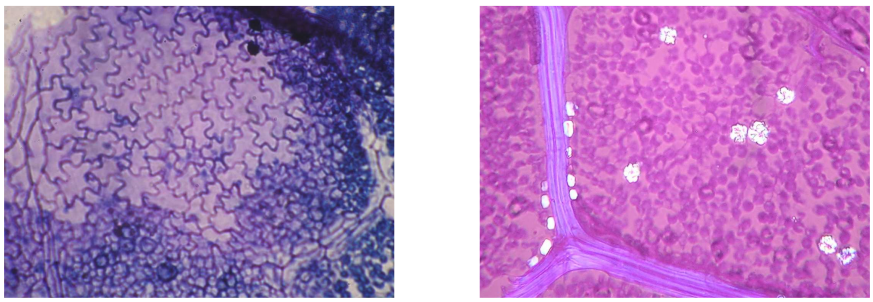


Fig 5.1. & Fig 5.2. Venation Pattern of Lamina

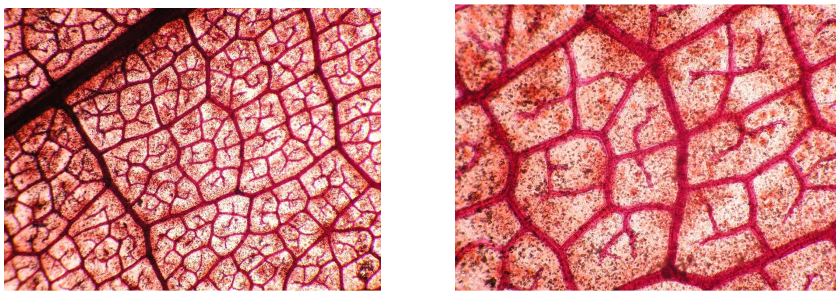


Fig 6.1., Fig 6.2. & Fig 6.3. Powder Microscopy of Epidermal Cells and Stomata

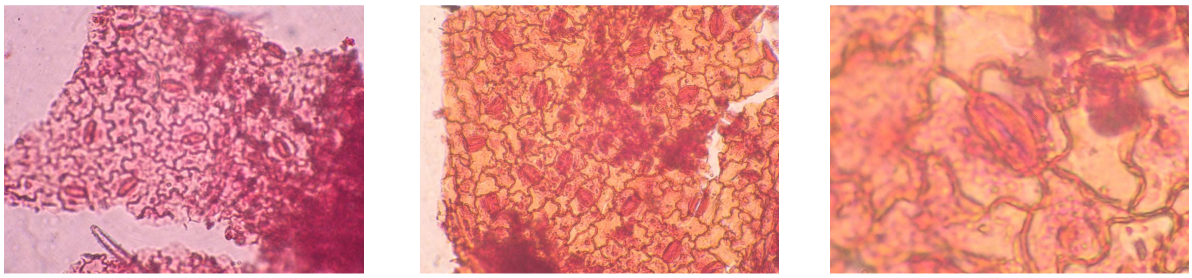
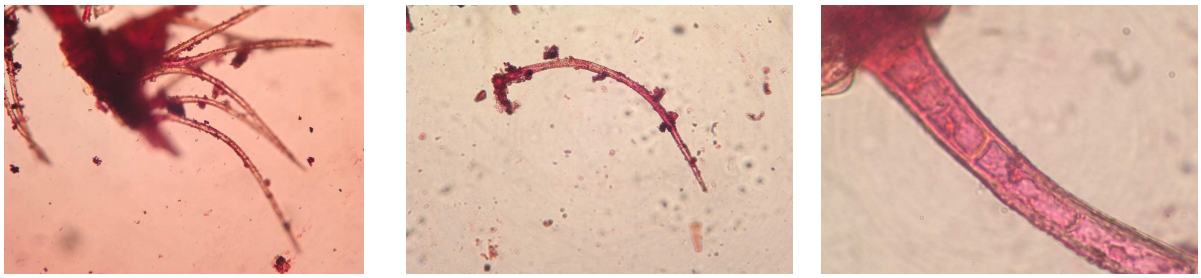


Fig 7.1., Fig 7.2. & Fig 7.3. Epidermal trichomes



3.2 Phytochemical Analysis

3.2.1 Extractive Values

The extractive values of *B. tomentosa* with various solvents were depicted in table 1. The extractive values suggest that the ethanolic extract and aqueous extract have got higher yields than the petroleum ether and ethyl acetate extracts.

Table 1.Extraction values of different extracts of *B. tomentosa*

S.no	Extract	% Yield(w/w)
1	Petroleum Ether	0.7
2	Ethyl Acetate	4.8
4	Ethanol	8.1
5	Water	9.1

3.2.2-Qualitative Analysis

It is noticed from the table 2 that the petroleum ether extracts contains steroids, saponins, phenols and cardiac glycosides. The Ethyl acetate extract contains alkaloids, steroids, phenols and carbohydrates. The ethanolic extract contains alkaloids, coumarins, tannins, flavonoids, quinones, phenols and carbohydrates. The aqueous extract contains alkaloids, coumarins, tannins, flavonoids, carbohydrates and cardiac glycosides.

Alkaloids are used medicinally. They provide information to determine lead structures of novel synthetic drugs. These compounds have antimicrobial activity by inhibiting DNA topoisomerases [17].

Tannins are a group of natural products widely distributed in plants. They are currently investigated for human medicinal use [18] to help reduce the risk of coronary heart diseases [19]. They are divided into two basic groups such as hydrolysable and condensed type. Hydrolysable tannins are normally recommended for treatment of inflammation, ulceration and tropical application for skin diseases. Tannins play an effective role in protecting the kidneys. Tannins have shown potential antiviral, antibacterial and anti-parasitic effects.

Saponins present in plants have been suggested as possible anticarcinogens. However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied [20]. It shows beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Saponins cause hemolysis of red blood cells [21].

Table 2 shows the phytochemicals present in various extracts of *Bahuinia tomentosa*

S.no	Phytochemicals	Petroleum Ether	Ethyl Acetate	Ethanol	Aqueous
1	Alkaloids	-	+	+	+
2	Steroids	+	+	-	-
3	Coumarins	-	-	+	+
4	Tannins	+	-	+	+
5	Saponins	+	-	-	-
6	Flavanoids	+	-	+	+
7	Quinones	-	-	+	-
8	Phenols	+	+	+	-
9	Proteins	-	-	-	-
10	Carbohydrates	-	+	+	+
11	Cardiac Glycosides	+	-	+	+
12	Gum	-	-	-	-
13	Starch	-	-	-	-

Flavonoids are a large family of low molecular weight polyphenolic compounds which include flavones, flavonones, isoflavones, flavonols, flavon3-ols and anthocyanins. It was widely distributed in plants fulfilling many functions. Flavonoids are generally nonnutritive agents. They possess remarkable antioxidant activities and inhibit enzyme activities like lipooxygenase, cyclooxygenase and prostaglandin synthase. Flavonoid compounds have proved of greater general interest to the plant taxonomist, both in respect of general angiosperm taxonomy and for detailed studies of gene flow at the specific and intra-specific levels. Extraction, separation and identification of these substances need to be assessed [22]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [23].

Phenols are found in the natural world, especially in the plant kingdom. The antioxidant activity of phenol is mainly due to their redox properties; Hydrogen atoms of phenols are proved to have hypotensive effects and antioxidant properties. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [24].

Naturally antioxidant in plants is in the form of phenolic compounds such as flavonoids, phenol acids, tocopherols etc. [25]. Biological activities of phenolic compounds involve free radical scavenging in cells [26], [27] [28].

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. From the results of the present study, it was clear that the alcohol and aqueous extracts have appreciable amount of phytochemicals than the petroleum ether and ethyl acetate extracts. Further studies on alcohol and aqueous extracts may provide valuable data about various biologically important phytochemicals.

CONCLUSION

The pharmacognostic studies may be helpful in the identification and authentication of this plant species. Phytochemical screening of the plant showed the presence of alkaloids, tannins, saponins, flavanoids, quinons, phenols and reducing sugars. The extracts of *Bauhinia tomentosa* could be further exploited as a source of useful phytochemical compounds and may play an important source of medicine for curing various diseases. Extensive research is required to find out the mechanisms of action as well as bioactivity of the various phytochemicals and efficacy and medicinal values of various phytochemicals.

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