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Pharmacognostical and Physicochemical Analysis of the Bark of *Bauhinia tomentosa* L.

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Abstract: The bark of *Bauhinia tomentosa* L. is used for inflammation, wound, dysentery, skin diseases and for microbial infections. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. Macroscopic, microscopic and physico-chemical characteters determination have been carried out, which would facilitate quick identification and selection of the drug from various adulterants.

Keywords: Pharmacognosy, *Bauhinia tomentosa* L, Physicochemical, microscopic, Florescence analysis.

Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on individual basis, and prepared drugs according to the requirement of the patients. But the scene has been changed now, Herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters[1,2] and *etc*. Correct knowledge of such crude drugs is very important aspect in the preparation, safety and efficacy of the herbal products. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained[3-5]. There is a need for documentation of research work carried out on traditional medicines[6]. Hence, it becomes extremely important to make an effort towards standardization of the plant material to be used as drugs. The process of standardization can be achieved by stepwise pharmacognostic studies.

Bauhinia is well known for the therapeutic efficacy of its different species. One of the most important species of this genus is *Bauhinia tomentosa* L. *Bauhinia tomentosa* L. is commonly known as "Kanjana" in Tamil and "Phalgu" in Sanskrit. The dried leaves, buds and flowers are prescribed in dysentery[7]. The bruised bark is applied externally to tumors and wounds. A decoction of the root-bark is administered for inflammation of the liver and it is also used as a vermifuge. An infusion of the bark is also used as an astringent gargle. The plant has been scientifically proved to have antimicrobial activity. In previous

phytochemical studies, *Bauhinia tomentosa* flowers have been reported to contain rutin, quercetin, isoquercetin, and glycosides of quercetin. The literature survey reveals that there is no systematic pharmacognostic study for this plant, The present investigation has been planned to study the pharmacognostic characters of the bark of *Bauhinia tomentosa* L.

Experimental

All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (St Louis, MO, USA), Merck (Darmstadt, Germany) and Qualigens (Mumbai, India).

The medicinal plant *Bauhinia tomentosa* L. was collected in the month of September from Courtallam Hills of Tirunelveli District, Tamil Nadu, India. The plant was identified by Prof. P. Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai, Tamil Nadu, India and the voucher specimens (MSU/PHAR/HER–138) was deposited at the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

Macroscopic studies

The macroscopic evaluation was carried out for shape, size, color, and fracture of the drug . These macroscopic characters are presented in Figure 1.

Figure 1. Bauhimia fomentosa. L.showimg its macroscopic characters.

Microscopic studies

Sectioning

The Paraffin embedded bark of *Bauhinia tomentosa* L. were sectioned with the help of rotary microtome. The thickness of the section was 10 to 12 µm. Dewaxing of the section was done by customary procedure[8]. The sections were stained with toluidine blue as per the method of O' Brien *et al*[9]. Since toluidine blue is a polychromatic stain, the staining results were remarkably good; some cytochemical reactions were also obtained. The necessary sections were also stained with safranin and fast–green and IKI (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 was carried out by partial maceration employing Jeffrey's maceration fluid[10]. Glycerine mounted temporary preparations were made for macerated materials.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used, For the study of crystals,

starch grains and lignified cells, polarized light was employed. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books[11].

Physicochemical parameters

Physico-chemical characters, such as ash value[12-15] extractive values and loss of weight on drying were determined and presented in Table 1.

Fluorescence analysis

The fluorescence analysis of the bark powders of *Bauhinia tomentosa* L. and their extracts in various solvents were examined under ordinary and Ultra Violet light (365 nm). The powder was also treated with different chemical reagents and changes in colour were studied in ordinary light and UV light. These fluorescence characters were determined according to the methods of Chase and Pratt[15]. The results are presented in Table 2.

Table 1. Physicochemical characters of the bark of *Bauhinia tomentosa* L.

S.No	Particulars	Percentage (%)
	Ash Value	
1	Loss of weight on drying	87.5
2	Total ash	4.86
3	Acid insoluble ash	0.62
4	Water soluble ash	1.96
5	Sulphated ash	6.23
6	Loss on ignition	2.28
	Extractive Value	
7	Petroleum ether extractive (40-60 °C)	12.23
8	Benzene extractive	1.5
9	Chloroform extractive	3
10	Ethanol extractive	10.9
11	Water extractive	14.42

Table 2. Fluorescent analysis of *Bauhinia tomentosa* L.

S.No.	Treatment	Under Ordinary light	Under UV light, 365 nm
1.	Powder (P) as such	Yellow	Brown
2.	P +1N NaOH in water	Yellowish brown	Dark brown
3.	P + 1N NaOH in ethanol	Brown	black
4.	P+ 1N HCl	Yellow	Dark brown
5.	P +1:1 H_2SO_4	Light brown	Dark brown
6.	P+ 1:1 HNO_3	Brown	Yellow at the edge and brown at centre
7.	Extracts:		
	Petroleum ether (40º-60ºC) extract	Yellowish brown	Brown
	Benzene extract	Black	Black
	Chloroform extract	Yellowish	Brown
	Ethanol extract	Reddish brown	Yellowish brown
	Water extract	brown	Dark brown

Results and Discussion

Macroscopic studies

Bauhinia tomentosa is an erect, branched shrub attaining a height of 1.5-3 m. The branchlets, lower surfaces of the leaves, and pods are somewhat hairy. The leaves are 4-7 cm long, about as wide, and split about one-third to the base, into two, with oval, rounded lobes. The flowers are pale lemon yellow, usually in pairs on axillary peduncles. The pods are 9-11 cm long, about 1.5 cm wide, flattened, and contain 6 to 10 small seeds. These macroscopic characters of *Bauhinia tomentosa* are presented in Figure 1.

Microscopic studies

Stem bark

The bark is fairly smooth and even surface. It is grey - white. The texture is fibrous. It has no specific odour or taste. Total thickness of the bark is 1.2 mm. The bark consists of peridern and secondary phloem.

Periderm (Figure 2a,b)

The periderm is superficial and wavy; it is continuous. The surface exhibits shallow wide fisseres. The periderm comprises twelve layers of phellem and seven or eight layers of phelloderm (Figure 2b). The phellem is homogeneous; cells are tabular in shape and suberized. The phelloderm cells are living cells with storage of ergastic substances.

Secondary phloem

It is the major part of the bark and measures about 1 mm wide. The phloem can be differentiated into outer collapsed phloem and inner non-collapsed phloem.

Collapsed phloem

It is the wider zone measuring about 850 µm thick. The collapsed phloem is characterized by wide, highly dilated, funnel-shaped phloem-rays which consist of horizontal rows of tangentially stretched cells. Alternating with the dilated rays are conical bands of sieve – elements and phloem fibres (Figure 2c). The phloem elements are crushed into dark thin tangential lines and are located in between thick fibre segments (Figure 2d).

Non-collapsed phloem

Non collapsed phloem consists of intact sieve elements and parenchyma cells. The phloem rays are narrow (not dilated) and fiber segments reduced in frequency or they are absent. The sieve elements are polyhedral in outline and are arranged in compact radial files (Figure 2e). The sieve elements are 20 µm wide. The companion cells are small and situated along the corners of the element (Figure 2e).

TLS view

In tangential longitudinal sections of the phloem, the phloem rays are nonstoried. They are mostly biseriate, less frequently uniseriate or 3-seriate (Figure 3a,b,c,d). The rays are homocellular; the cells are wide, polyhedral and compact. The biseriate rays are 220-250 µm high and 20-40 µm thick. Three-seriate are 50-60 µm thick and 250 µm high. Phloem parenchyma cells are fusiform in shape with wedge shaped ends. They are arranged in stories (Figure 3a,b,c,d).

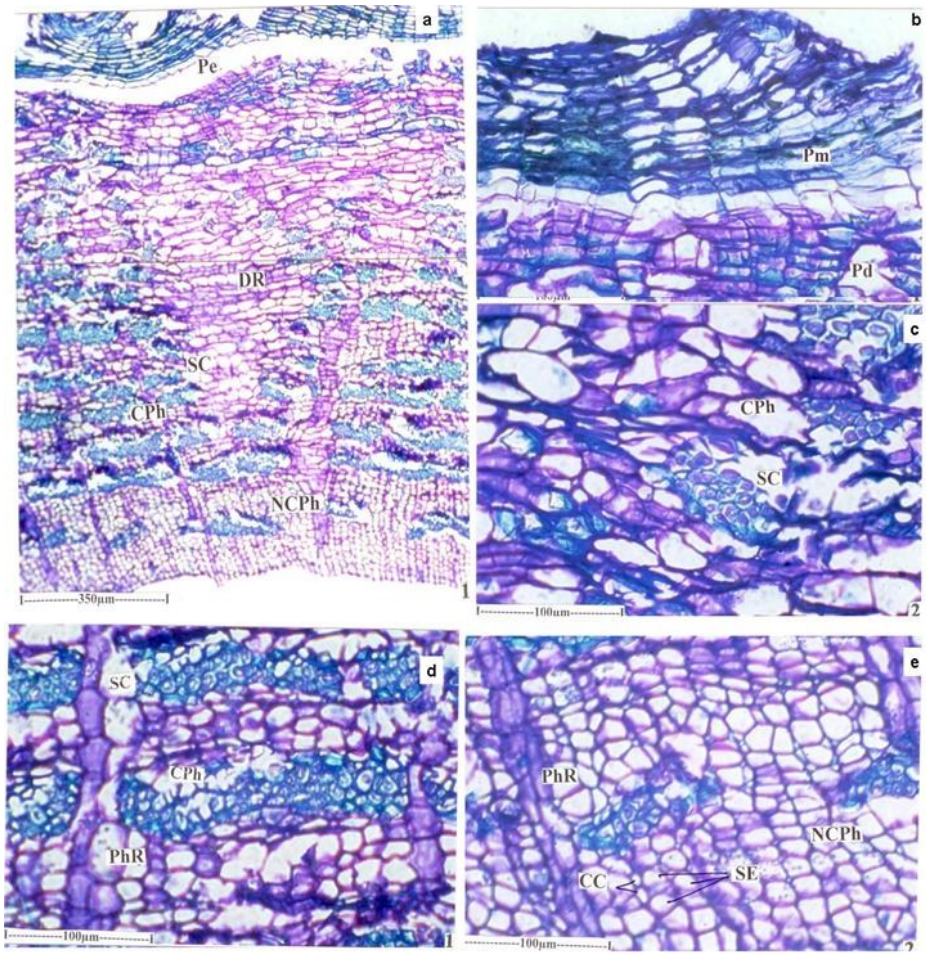

Figure 2. a) T.S. of stem bark -entire view, b) T.S. of stem bark-periderm region c) T.S. of stem bark-collapsed phloem, d) T.S. of collapsed phloem, e) T.S. of non collapsed phloem (DR: Dilated ray; Pe; Periderm; CPh: collapsed phloem; Pm: Phellem; Pd: Phelloderm; Sc: sclerenchyma; Cc: comparnion cells; NCPh: non-collapsed phloem: PhR: Phloem ray: SE: Sieve element).

Crystals (Figure 3e,f,g)

Calcium oxalate crystals are abundant in the phloem. They are exclusively prismatic type. They are located along the marginal cells of rays (Figure 3f) or occur within the lumen of the fibres (Figure 3g). The crystals are located in close vertical rows in the axial parenchyma such rows of crystals are called crystal strands (Figure 3g).

Physicochemical studies

The results of physicochemical characters such as loss of weight on drying, total ash, acid insoluble ash, water soluble ash, residue on ignition and the percentage of extractive values in various solvents such as petroleum ether (40º–60ºC), benzene, chloroform, ethanol and water of the bark powders of *Bauhinia tomentosa* L. are presented in Table 1.

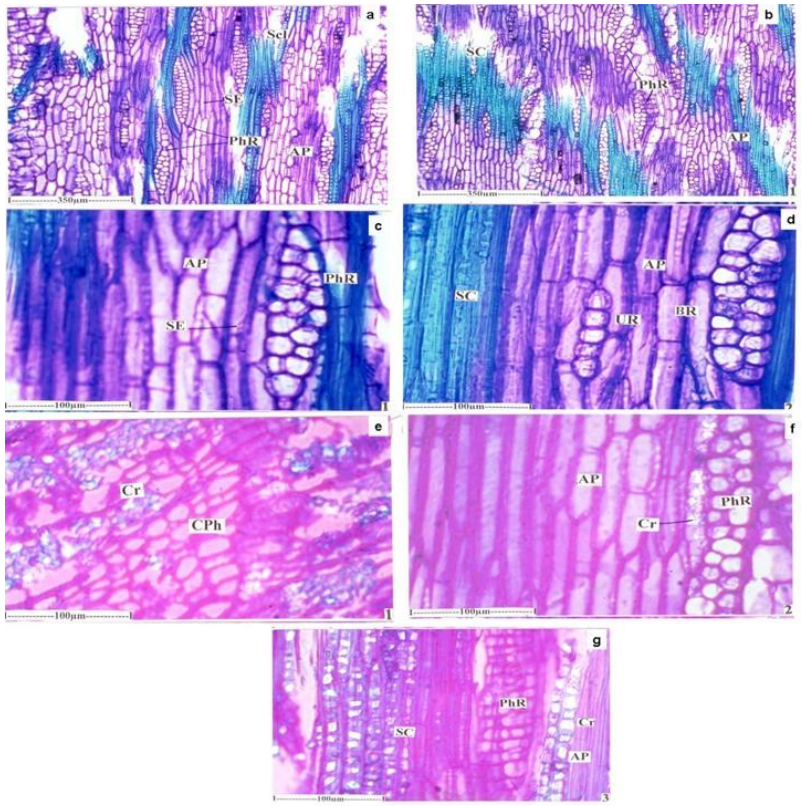

Figure 3. a and b) TLS of phloem showing the phloem rays and parenchyma, c) and d) TLS of phloem rays, e) T.S. of –showing crystals in the sclerenchyma cells, f) TLS of phloem showing crystals in the ray cells, g) crystals in the fibres and parenchyma cells. (AP; axial parenchyma; PhR: phloem; Sc: sclerenchyma; SE: sieve element; BR: Biseriate ray; PhR: phloem ray; Sc: sclerenchyma; UR: uniseriate ray; CPh: collapsed phloem; Cr: crystals;).

Fluorescence analysis

Fluorescence analysis of powdered drug material and different extracts of the bark of *Bauhinia tomentosa* L with different reagents were carried out to observe the color reactions (Table.2).

In the midrib of the bark of *Bauhinia tomentosa* L., the abaxial side is broadly conical and adaxial side is flat. Stomata occurs only in the lower epidermis and paracytic. Vein-islet varies from quarish to polygonal. The terminal part of the vein islet possesses angular or elongated sclereids. The dintal part of the petiole consists of epidermal layer, parenchymatous ground tissue and sclerenchymatous tissue enclosing the vascular strands. The T.S of stem exhibits angular outline. In stem collenchyma batches are absent and a thick continuous cylinder of sclerenchyma are present. These anatomical characters are useful for the correct identification and authentication of selected medicinal plant.

Ash values are indicating the purity of drug. The percentage of loss of weight on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash and loss on drying were found to be 87.5 %, 4.86 %, 0.62 %, 1.96%, 6.23%, 2.28% respectively (Table 1).

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The water extractive value was found to higher (14.42%) followed by petroleum ether (40º-60ºC) extractive value (12.23%), ethanol(10.9%), chloroform (3%), benzene (1.5%). (Table.1)

A characteristic yellowish-green fluorescence is noticed in the ethanol extract of *Bauhinia tomentosa*. This characteristic fluorescence can be used as a diagnostic tool for the correct identification of the crude drug and to test adulteration if any. In conclusion, it can be stated that the standardization parameters used in the present investigation will provide a way for the standardization of raw materials and prepared formulation of herbal origin as well as answer to the latest GMP norms and FDA guidelines on standardization of herbal drugs. This could also serve in the correct identification and preparation of a monograph on the plant.

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