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Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *BAUHINIA PURPUREA*

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ABSTRACT
This paper presents a detailed pharmacognostical and physicochemical study of the stem bark of the plant *Bauhinia purpurea* L., Fabaceae, an important plant in Indian system of medicine. There were no pharmacognostical reports of the stem bark of this plant, particularly to determine the anatomical and other physicochemical standards required for its quality control. The stem bark were studied using light, confocal microscopy. The physicochemical, morphological and histological parameters carried out as per WHO guidelines of quality control methods for medicinal plant materials. The present study aims at developing a standardize profile of the stem bark of *Bauhinia purpurea* which would be of immense use to identify and establish the authenticity of the plant *Bauhinia purpurea.*

KEY WORDS: *Bauhinia purpurea* L., Fabaceae, Triterpenoids, Sterols.

INTRODUCTION

The well-known and well established genus Bauhinia comprises of trees and shrubs that grow in warm climate. It is rare in southern most districts, 5-7m tall tree in deciduous forests which is often planted in gardens along roadside for its large purple beat flowers. The leaves are 10–20 cm long and broad, rounded, alternate and bilobed at the base and apex. The flowers are conspicuous, pink, and fragrant, with five petals. The fruit is a pod 30 cm long, containing 12 to 16 seeds and have long seeds as pea. Flowers and fruits appear in the month of December. Synonyms/Common names of plant *Bauhinia purpurea* is Purple Orchid tree, Mandaram, etc[1].

Geographical distribution- *B. purpurea* is native to South China (which includes Hong Kong) and South-eastern Asia and it is found throughout India, ascending to an altitude of 1300m in Himalaya[2].

Plant profile**:** The different species of *Bauhinia viz., B. reticulata, B. rufescens and B. variegata* have been traditionally used to treat roundworm infections, conjunctivitis, anthrax, ulcerations, dysentery, blood-poisoning, leprosy, lung and skin diseases in Africa; while in India, extracts of the bark of *B. variegata* is used for treatment of cancer.

Leaves are used as a plate for food and fodder during lean period [3], bark used as fibre, in dyeing and tannin extraction and its decoction is used in diarrhea. The decoction of root is used for expelling gases, flatulence and gripping pain from the stomach and bowels. The decoction of flower works as a maturant for boils and abscesses [4]. Root bark of *Bauhinia purpurea* L. contains flavones glycoside [5].

MATERIALS AND METHODS

The stem bark of *Bauhinia purpurea* L. were collected from local area of Manipal, Karnataka, India during August 2011 and were authenticated by Dr. Chandrashekar KS, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal.

Preparation of extract [6, 7]

The stem bark of *Bauhinia purpurea* L. were dried in hot air oven at 50-60º C for 5-6 days and then grinded to a fine powder in a grinder. The powdered plant material (2.5 kg) was subjected to maceration using methanol. Yields of extracts were methanol (4.92%)

Organoleptic evaluation

Fresh bark was moist, flat strips, outer surface was dark brown and inner surface was white in colour. Odour was sweet and characteristic and fracture was fibrous. Dried bark was hard and contracted, outer surface was blackish brown and inner surface was brownish buff coloured. Odour was slight and fracture was splintery.

Fresh Bark

Dried Bark

Pharmacognostical study

Materials

Compound microscope, stage micrometer, glass slide, cover slip, watch glass, Leica DMLS microscope attached with Leitz MPS 32 camera, digital electronic balance (Dhaus Corp), grinding mixer, hot air oven (Osworld), sonicator (Lequitron), silica crucible, ash less filter paper (Whatman no.44), petridish, UV apparatus, stoppered conical flask, magnetic stirrer, alcohol (95%), chloroform water, chloral hydrate solution, phloroglucinol, hydrochloric acid, sudan red-III, ruthenium red, glycerin, sodium hydroxide, petroleum ether, acetone, benzene, chloroform and water.

Anatomical study[6]

Free hand sections of the stem were taken and warmed with chloral hydrate to remove the coloring matter. Then uniform and clear sections were selected and stained with phloroglucinol and HCl, mounted on a clean glass slide with glycerin and covered with cover slip. The sections were then viewed under low power 10 X and subsequently under high power 40 X.

Photomicrography

The microphotographs were taken using Olympus BX 41 microscope, attached with Olympus DP20 camera.

Powder analysis [6,8]

The powder of stem of *B. purpurea* was examined for its microscopic characters. The powder was passed through sieve no. 60 and was boiled with chloral hydrate to remove coloring matter and viewed under microscope for calcium oxalate crystals and other characters. The clarified powder was later stained with phloroglucinol in the presence of hydrochloric acid for the lignified structures like stone cells and viewed under microscope as described earlier.

Physicochemical Constants[9,10]

Ash Value

Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration. Total ash is the residue remaining after incineration. Acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. Water-soluble ash is the part of total ash, which is soluble in hot water. Total ash was determined by **a**bout 2 g of the powdered drug in a tared silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 512°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated in triplicate with reference to the air dried drug. Acid insoluble ash was determined by the following procedure**.** The ash obtained as described in the determination of total ash was boiled with 25 ml of dil. hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into a tared silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug .Water soluble ash was determined as follows. The ash obtained as described in the determination of total ash was boiled for 5 min with 25 ml of water. The insoluble matter was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a tared silica crucible and ignited at a temperature not exceeding 512°C. The procedure was repeated until a constant weight was observed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

Extractive Value determination

Coarsely powdered air-dried material 4 g was placed in a glass stoppered conical flask and macerated with 100 ml of solvents (water, methanol, and chloroform) shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through whatman No. 1 filter paper, transfer 25 ml filtrate to flat- bottom dish and evaporate solvent on a water bath. Dry at 105° C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material.

Moisture content

2gms drug was taken in a previously weighed china dish and heated in oven for 1 hr. at 105° C. China dish was cooled and weighed. Again heated in oven for 1 hr. and reading is taken. This step is repeated until the weight becomes normal.

Preliminary Screening [6.7,11]

Tests for alkaloids performed were -Mayer's test, Dragendroff's test, Wagner's test, Hager's test, Test for saponins-Foam test, Sodium bicarbonate test, Test for carbohydrates-Molisch's test, Fehling's test, Benedict's test Test for glycosides- Borntrager's test, Modified Borntrager's test, Baljet`s test, Legal`s test, Liebermann`s test, Test for steroids-Libermann-Burchard test, Salkowaski test for tannins and phenolic compounds-Dilute ferric chloride, Lead acetate, Gelatin, Aqueous bromine solution, Test for flavonoids-Shinoda's test.

TLC studies

Thin layer chromatography analysis was carried out was carried out on silica gel G (E. Mark, Germany) with petroleum ether: chloroform (90:10) as the solvent system. The spot was developed by spraying the plate with solution I (5% ethanolic sulfuric acid), followed immediately by solution II, then heated for 5-10 min at 100^{O} C under observation.

RESULTS AND DISCUSSION

Transverse Section of bark shows few layers of cork. Outer layers contains reddish brown content and inner layers are colourless. Phellogen and Phelloderm are indistinguishable from each other. Cortex is composed of 10-15 layers of parenchyma cells. Stone cells are scattered in the middle of parenchyma. Starch grains and calcium oxalate crystals are found in some parenchyma cells. Stone cell layer is found in between cortex and secondary phloem. Secondary phloem composed of phloem parenchyma, phloem fibre and medullary rays Phloem parenchyma is composed of parenchyma cells containing starch grains and calcium oxalate crystals. Medullary rays traverse radially the phloem parenchyma, 1-3 cell wide and extend upto stone cell layer (Fig 2). Powder microscopy shows fibers, parenchymatous cells, starch grains and calcium oxalate crystals (Fig 3).

Table 1- Ash value of stem bark of *Bauhinia purpurea*

Plant part	Total Ash % age	Water Soluble Ash %age	Acid Soluble Ash %age	Sulphated Ash %age
Bark	11%	7.5%	1%	13.5%

Table 2- Extractive value of stem bark of *Bauhinia purpurea*

Treatment	Percentage of Extraction	Filtrate colour	Residual nature and colour
Water	0.75%	Brown	Powdery and Blackish brown
Methanol	1.5%	Dark Brown	Semisolid and Yellowish brown
Chloroform	0.25%	Transparent	Waxy and Light Green

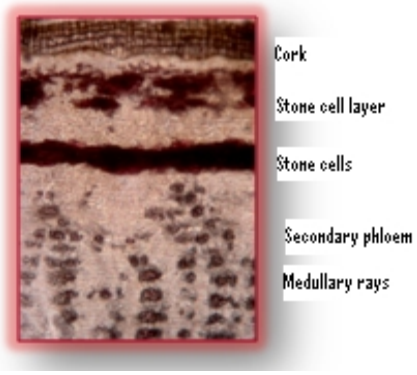
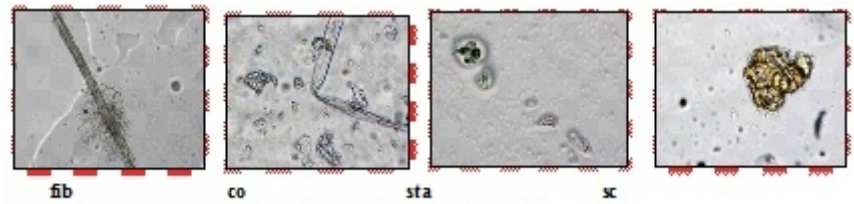


Fig 2- Transverse Section of Bark of *B. purpurea* L



Fib- phloem fibres, co- calcium oxalate, sta- starch, sc- stone cell

Fig 3- Powder microscopy of Bark of *B. purpurea* L.

19.5 % of moisture content was found in drug, as the weight of drug decreased to 1.61g from 2 g. Preliminary Screening reveled absence of alkaloids and resins in methanolic extract. Carbohydrates, Glycosides and Saponins were present in methanolic extract. Sterols and Triterpenoids were present in methanolic extract. The TLC analysis of methanol extract showed six compounds of which one was identified as Lupiol by comparing with reference standard.

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