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Research Article

PHARMACOGNOSTICAL STUDIES AND EVALUATION OF QUALITY PARAMETERS OF *BUTEA FRONDOSA* LEAVES

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ABSTRACT

Butea frondosa Roxb. & Koen. syn. *B. monosperma* Lam. (Leguminosae or Fabaceae) is a medium size deciduous tree, commonly known as flame of the forest (English) and palasa (Sanskrit), found throughout India. Mostly, bark, flower, seed and gum have been used in folk medicines for treatment of diabetes, snake bite, as astringent etc. The present investigation was designed to establish the pharmacognostical and quality control parameters of *B. frondosa* leaves. Various microscopic characters viz., transverse section of leaf and quantitative parameters (stomatal number, stomatal index, vein-islet and termination number and size of trichomes) were determined. Powder microscopic studies, along with fluorescence analysis, were also performed. Physio-chemical parameters such as total, water-soluble and acid-insoluble ash (3.75, 0.75 and 0.65% w/w, respectively), extractive values (aqueous and alcoholic), foreign matter (1.2%) and moisture content (0.1% w/w) were also determined. Preliminary phytochemical screening of hydro-alcoholic extract showed the presence of flavonoids, saponins, steroids and sugars. Different combination of solvents were tried to establish the fingerprint profile of the hydro-alcoholic (50%) extract. The study will provide referential information for the correct identification and purity of the crude drug.

Keywords: *Butea monosperma*, Palas, TLC fingerprinting.

INTRODUCTION

Butea frondosa Roxb. & Koen syn. *B. monosperma* Lam. (Fabaceae), commonly known as flame of forest, dhak, tesu, palash and tesh, is a deciduous tree found throughout India. Flowers, bark and gum from the stem have been widely used in traditional system of medicine, and out these flowers have been studied extensively both, chemically and pharmacologically. Traditionally, flowers have been used as astringent, aphrodisiac, diuretic, anthelmintic, in diarrhea, gynecological and various CNS disorders; bark for the treatment of dyspepsia, diarrhea, diabetes, ulcer, sore throat; leaves for cough, diabetes, menstruation and in CNS disorders; gum for leprosy; seeds in piles and skin diseases[1-4]. Various pharmacological activities of different parts of *B. frondosa* have been reported. Flowers have been reported for antiestrogenic and antifertility[5], antistress[6], anticancer[7], antibacterial[8], antiepileptic[9,10], hepatoprotective[11,12] and anti-inflammatory[13] activities. Bark has exhibited antidiarrhoeal[14], wound healing[15], osterogenic[16], antiulcerogenic[17] and aphrodisiac[18] properties. Leaves have been reported to exhibit antidiabetic[19], anti-inflammatory[20], antihelmintic[21], antimicrobial[22], antifungal[22], anticancer[23], anxiolytic and antistress activities[24]. Despite various pharmacological studies on the leaves of *B. frondosa*, studies pertaining to its pharmacognostical characters and quality parameters, have not been reported. Hence, pharmacognostic evaluation (macroscopic, microscopic, physico-chemical parameters determination and phytochemical screening) was done in the present investigations. TLC finger print profile of the hydroalcoholic extract was also established.

MATERIAL AND METHODS

Plant material

Fresh Leaves were collected from Kunihar, distt., Solan, Himachal Pradesh in the month of August 2010 and were authenticated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi; under voucher specimen no. NISCAIR/RHMD/Consult/2010-11/1633/231. A specimen was submitted in the Department of Pharmacognosy and Herbal Drug Technology, ISF College of Pharmacy, Moga.

Chemicals and reagents

All the solvents used for extraction and TLC profile of plant were of L.R. and A.R. grade, respectively. Aluminum based pre-coated TLC plate of 0.2 mm thickness (20 × 20 cm) from (E. Merck, Germany) were used for TLC studies.

Preparation of extract

The collected leaves were made thoroughly free from foreign matter, dried under shade and powdered. The powdered leaves (500 g) were extracted using 50% aqueous ethanol three times for 48 h each. The extract was filtered and concentrated under vacuum. The concentrated extract was then used for phytochemical screening and establishment of TLC profile.

Macroscopic of leaf

The leaves were studied for their macroscopic characters such as size, shape, margin, apex, surface, colour, odour, taste, nature and texture[25].

Microscopic studies

Anatomical studies, both qualitative and quantitative, were done. Free hand cut transverse sections of leaf were studied for different microscopic characters and photographs of the sections were taken with the help of Motic DMBA 300 microscope[26]. In quantitative microscopy, leaf constants like stomatal number, stomatal index, vein-islet number, vein-let termination number and size of trichomes were determined following WHO guidelines on quality control methods for medicinal plant materials[27].

Powder analysis

The shade dried leaves were powdered, and powder was passed through 100 # sieve. A small amount of powder was taken onto a microscopic slide, cleared from chlorophyll by heating with chloral hydrate solution and was mounted in 50% v/v glycerol in water. The slide was then observed under microscope to study the characteristic features[26].

Physicochemical parameters

The ash values, extractive values and loss on drying were performed according to the officinal methods the WHO guidelines for quality control methods for medicinal plant materials[27]. Fluorescence analysis was carried out according to the method of Kokoski et al.[28,29].

Preliminary phytochemical screening

The hydro-alcoholic extract were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and

phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids[30,31].

TLC profile of hydro-alcoholic extract

The TLC profile of hydro-alcoholic extract carried out by using different solvent systems. The visualization of spots was done by observing the plate under UV light (both long and short) and after derivatizing with anisaldehyde - H_2SO_4 reagent. The R_f value and color for different spots was calculated and recorded, respectively.

RESULTS AND DISCUSSION

Macroscopical characteristics

Leaves were dark green and light green in color on upper and lower surface respectively. Leaves were odour and tasteless, 8-27 cm in length and 5-23 cm wide, with entire margin and blunt apex, obtuse in shape, large stipuled, with shiny and smooth upper surface while lower surface was rough, had more prominent reticulately arranged veins and slightly sunkened midrib (Fig 1).

Fig 1: Leaves of *Butea frondosa*

Microscopical characteristics

The transverse section of leaf (Fig 2) showed dorsi-ventral condition with rectangular shaped cells of epidermis. A bi-layered upper palisade and single layer of lower palisade cells was present on the inner side of upper and lower epidermis, respectively. In the mid rib region, collenchymatous cells were present on the inner side of both upper and lower epidermis. Vascular bundles were of collateral type enclosed in a ring of fibers and prismatic crystals of calcium oxalate. Phloem was present in patches forming an incomplete ring around xylem. Long unicellular trichomes were present on both the surfaces. Surface view of both upper and lower epidermal cells showed presence of straight and wavy anticlinal walls, respectively (Fig 3). Stomata, present only on the lower surface, were paracytic in appearance (Fig 3).

Quantitative microscopy

Various quantitative microscopic parameters viz., stomatal number and index, vein islet and termination number and size of trichomes were determined as per the WHO guidelines for quality control methods for medicinal plant material. The results are given in table 1.

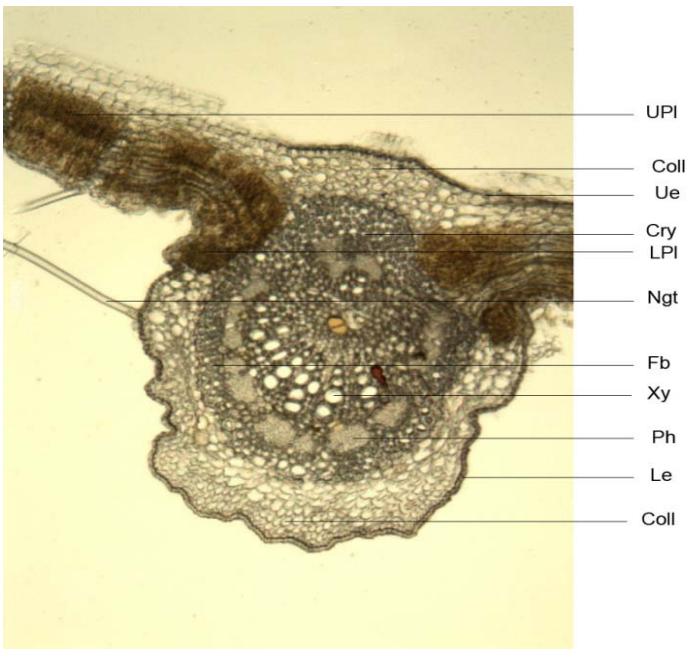


Fig. 2: Transverse section of leaf of *Butea frondosa*

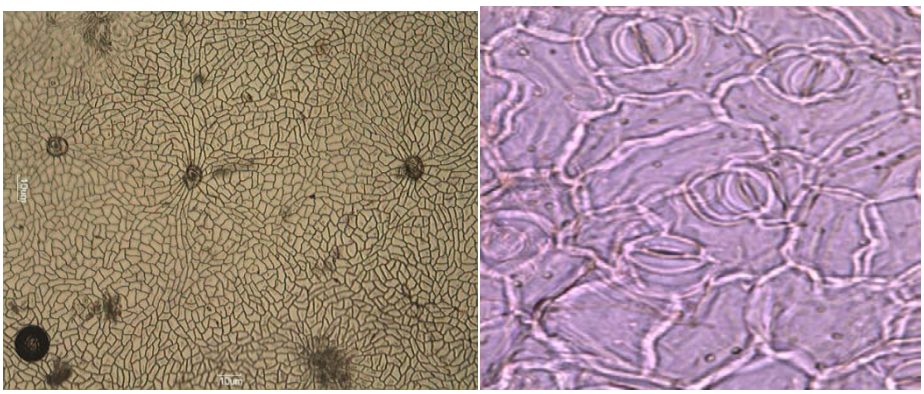
(Upl- Upper plisade; Coll- Collenchyma; Ue: Upper epidermis; Cry- Prismatic crystals; LPl: Lower palidase Ngt- Nonglandular trichome; Fb- Fibers; Xy- Xylem; Ph- Phloem; Le- Lower epidermis)

Powder analysis

The powder of leaves, passed through 100 # sieve, appeared dark green in color, odorless and had slight bitter taste. The powder microscopy (Figure 4.) showed the presence of paracytic stomata, covering trichomes, fibers and spiral vessels. Cells of upper and lower epidermis had straight and wavy anticlinal walls, respectively. The fragment of lamina, in surface view, showed cicatrices and non-glandular trichomes.

Physicochemical parameters

Various parameters such as ash and extractive values, moisture content and foreign matter were established, and the results are summarized in Table 2. The fluorescence analysis of the powder drug was also done and results are given in Table 3. The powder was treated with various reagents and the mixture was observed under UV light (366 nm) and visible light to see the type of fluorescence.



(A) **(B)**

Fig. 3: Surface view of the epidermal cells (A) Upper epidermis (B) Lower epidermis

Table 1: Quantitative microscopy parameters of *Butea frondosa* leaves

S. No.	Quantitative microscopic parameters	Values
1	Stomatal number	
	Upper surface	Nil
	Lower surface	77-98
2	Stomatal index	
	Upper Surface	Nil
	Lower Surface	14.3
3	Vein-islet number	
	Upper surface	21.4
	Lower surface	24.6
4	Vein termination number	
	Upper surface	4.2
	Lower surface	3.0
5	Size of trichomes (µm)	364

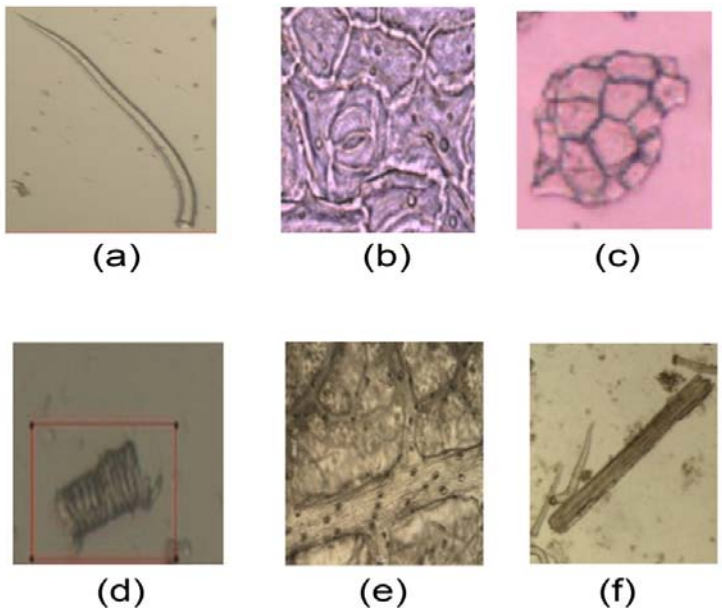


Fig. 4: Powder microscopic characters of *B. frondosa* (a) Non-glandular trichome, (b) Lower epidermal cells with paracytic stomata, (c) Upper epidermal cell in surface view, (d) Vessel, (e) Fragment of lamina showing cicatrices and trichome and (f) Group of fibers.

Table 2: Physico-chemical parameters of *Butea frondosa*

S. No	Type of Ash	Mean (%) values* (w/w) ± S.D.
1	Total ash	3.75 ± 0.7
2	Water soluble Ash	0.75 ± 0.1
3	Acid insoluble ash	0.65 ± 0.1
4	Ethanol soluble (hot)	5.04 ± 0.8
5	Ethanol soluble (cold)	1.62 ± 0.1
6	Water soluble (hot)	15.25 ± 1.2
7	Water soluble (cold)	10.81 ± 0.6
8	Foreign matter	1.2
9	Moisture constant	0.4 ± 0.03

*n=3

Table 3: Fluorescence analysis of *B. frondosa* leaves powder

Treatment	Observation under	
	Ordinary light	UV light (366nm)
Powder as such	Dark Green	Brown
Powder + 1NaOH in Methanol	Green	Brown
Powder + 1N NaOH in water	Cream	Light yellow
Powder + 1N HCl	Greenish-brown	Dark brown
Powder + HNO_3 (1:1)	Greenish-brown	Dark brown
Powder + H_2SO_4 (1:1)	Greenish-brown	Dark brown

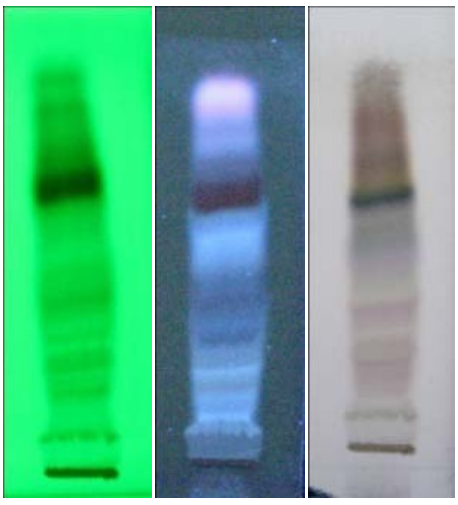
Table 4: Preliminary phytochemical screening of hydro-alcoholic extract of *B. frondosa* leaves

Phyto-constituents	Detection
Alkaloid	-
Carbohydrate	+
Flavonoids	+
Steroids	+
Saponins	+
Polyphenolic compounds	+
Anthraquinone-glycoside	-
Cardioactive glycosides	-
Amino acids	+

- : absent; + : present

Table 5: TLC analysis of hydro-alcoholic extract of *Butea frondosa* (leaves)

Inference	Number of spots	Color bands	Rf value
Under short U.V.	6	Brown, light green, light yellow, green, light brown	0.85 0.62, 0.35, 0.31, 0.27, 0.12
Under long UV	9	Purple, blue, violet, blue, pink, dark blue, light blue, pink, light blue	0.85, 0.75, 0.62, 0.35, 0.31, 0.27, 0.25, 0.22, 0.12,
Under visible light	11	Brown, Greenish yellow, Yellow, Green, light purple, light purple, light purple, light green, Brown Greenish blue, Brown	0.94, 0.85 ,0.75, 0.66, 0.64, 0.53, 0.42, 0.33, 0.32, 0.13, 0.8



(a) (b) (c)

Fig. 5: TLC profile of *Butea frondosa* leaf extract in (a & b) under short and long UV light respectively, (c) under visible light after derivatization with anisladehyde-sulphuric acid reagent.

Preliminary phytochemical screening

The hydro-alcoholic extract of leaves shows the presence of carbohydrates, flavonoids, steroids, saponins, amino acids and polyphenolic compounds (Table 4).

TLC finger printing profile

The TLC profile of hydro-alcoholic extract was optimized by testing different solvent systems**.** Amongst various solvent systems, toluene: chloroform: methanol (4.25: 5: 0.75 v/v/v) showed maximum resolution. The plate was observed under UV light (254 and 366 nm) and visible light after derivatization with anisladehyde-sulphuric acid reagent. Number, color and R_f values of spots produced are tabulated in Table 5.

CONCLUSION

The pharmacognostical and physicochemical characteristics of *Butea frondosa*, which could be used in identification and to distinguish the plant material, were determined and established. The preliminary phytochemical analysis showed the presence of various phytoconstituents such as flavonoids, steroids and polyphenolic constituents, may contribute in various pharmacological activities of this plant. The present study may be useful to supplement information with regards to its identification, standardization and in carrying out further research.

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