

Research Article

Physico-chemical evaluation and phytochemical potential of a medicinal herb: *Butea frondosa* Koen. Ex Roxb (leaves)

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Abstract

Objective: The present work attempts to evaluate the physicochemical and preliminary phytochemical studies on the leaves of *Butea frondosa* Koen. Ex Roxb, family Fabaceae.

Methods: The herbal standardization was carried out on the basis of organoleptic properties, physical characteristics, and physico-chemical properties. Physicochemical parameters including ash values, extractive values, loss on drying, foreign matter were evaluated.

Results: Macroscopically leaves were observed to be compound. Lateral leaflets were obliquely ovate, obtuse, round at apex. Its texture was fairly tough. Microscopical leaves have single layered upper and lower epidermis, covered with thick cuticle. Xylem and phloem are arranged in ring. Calcium oxylate crystal were scattered throughout the cells. The total ash, acid insoluble ash and water soluble ash were found to be 10.16%, 2.83%, and 5.16% w/w respectively. Petroleum ether, Chloroform, ethanol and water soluble extractive values (hot) were 2.94%, 3.08%, 5.06%, 10.61% w/w respectively. The pH of 1% and 10% aqueous solution was found to be 6.06 and 5.76 respectively. Preliminary phytochemical screening showed the presence of sterols, tannins, flavonoids, amino acids, glycosides, phenolic compounds, carbohydrates, saponins and alkaloids. Thin layer chromatographic studies also had been done on ethanolic and aqueous extracts.

Conclusion: These studies provided referential information for correct identification and standardization of this plant material.

Keywords: *Butea frondosa*, Fabaceae, standardization, physico-chemical evaluations, qualitative parameters, TLC.

1. Introduction

Herbal medicine is the mainstay of health care in several developing countries. The efficacy and safety of herbal products therefore rely on the quality and proper identification of the raw material or the original plant source. Medicinal plants are the great source of economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country.¹ WHO estimate that about 80% of the population living in the developing countries rely upon the traditional medicine for their primary health care needs. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of the traditional medicine. In traditional medicine, there are many natural crude drugs that have the potential to treat many disease and disorders, one of them is *Butea frondosa* Koen. Ex Roxb Family Fabaceae syn *Butea monosperma* (Lam) Kuntz, popularly known as 'palash', commonly known as 'Flame of forest'. Palash is known as Dhak and Tesu in Hindi, Palash in Bengali, Palas in Marathi and Kesuda in Gujarathi.²

The tree has been planted extensively throughout the tropics as a medicinal plant. In India, the plant is traditionally used for various medicinal purposes. It is a slow growing tree. (Fig: 1) Bark is reported to possess astringent bitter, pungent, alliterative, aphrodisiac³, and anti-obese activity⁴. Roots are useful in elephantiasis and in curing night blindness and other eyesight defects. Leaves have astringent, tonic, diuretic, and antidiabetic⁵, antidiarrhoeal⁶, and antimicrobial⁷ properties. Flowers are reported to possess hepatoprotective⁸, anti-inflammatory⁹, anticonvulsant¹⁰ properties.

Fig.1: *Butea frondosa*: (a) whole tree (b) Leaves



Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample. The present work therefore, attempts to report necessary pharmacognostical and standardization parameters of leaves of *Butea frondosa* Koen. Ex Roxb., which will help to identify the drug .

2. Material and Methods

2.1 Collection and Authentication

The fresh leaves of *Butea frondosa* Koen. Ex Roxb was collected from the forest of Musabagh, Hardoi road, Lucknow during the month of March 2013. For identification and Taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI) Lucknow, India. The text report from National botanical research institute, Lucknow, confirmed the authenticity of plant material sample was *Butea frondosa* with voucher specimen no. NBRI -SOP-202 Receipt no. and date CIF-RB-3-269, 17.04.2013. The fresh leaves were used for the study of macroscopic and microscopical characters. Whereas collected plant material were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values, and preliminary phytochemical investigation was studied as per standard methods.

2.2 Extraction of plant material

100 gm coarse powdered of air dried leaves of *Butea frondosa* Koen. Ex Roxb. were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with distilled water, ethanol, chloroform , and petroleum ether for 8 hrs separately. Then each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of petroleum ether, chloroform, ethanol and the aqueous extracts were calculated.

2.3 Macroscopic and microscopic studies

The macromorphology of the leaves were studied according to standard methods.^{11,12,13} transverse section of the leaf was taken, stained and mounted following usual microtechniques¹⁴ and representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).

2.4 Physicochemical analysis

Physicochemical analysis i.e extractive values of petroleum ether, chloroform, ethanol and aqueous, fluorescent analysis^{15,16} total ash, acid-insoluble ash, water-soluble ash, foreign matter and moisture content were carried out.¹⁷ Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts .

2.5 Preliminary phytochemical screening

Preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous was carried out for the detection of various compounds by using standard procedures described by Harborne¹⁸ and Khandelwal.¹⁹

2.6 Thin layer chromatography

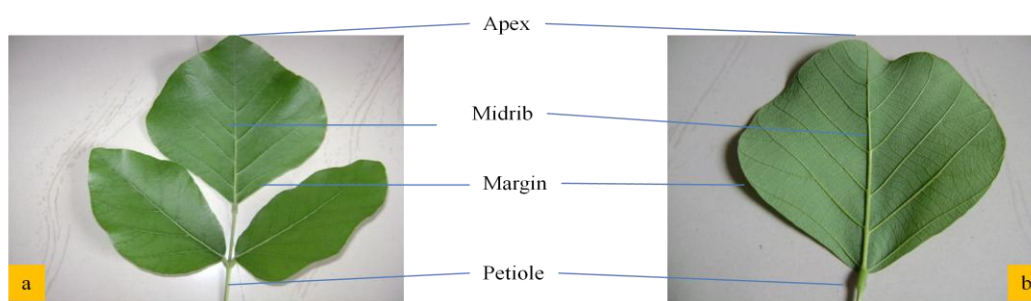
Slurry of silica gel G was prepared in distilled water and poured over a glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at 100-120°C (30min) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (1cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase, and allowed to run 3/4th of the height of the prepared plate²⁰. The plates were air dried and number of spots were noted and Rf value were calculated. Spots were visualized by respective spraying agents. Numbers of solvents systems were tried but the maximum resolution was shown in Toluene: ethyl acetate: Formic acid and n butanol: acetic acid: water for the ethanolic and aqueous extract respectively.

3. Results

3.1 Macroscopical characteristics

The leaves of Dhak tree are compound. Each has three leaflets. Mature leaves are dark green while Young leaves are light green in colour. They are odourless and tasteless. Lateral leaflets are obliquely ovate, obtuse, round at apex. The size varies from 15 cm to 20 cm by 10 cm x 15 cm with entire margin. Its texture is fairly tough; they are more or less leathery, with the surface glabrescent above and hairy silken beneath. Leaves are petiolate, it is about 7.5-20 cm long with small stipules. (Fig: 2)

Fig:2 Morphology of *Butea frondosa* leaf (a)Dorsal (b)Ventral surface



3.2 Microscopic characters

3.2.1 Midrib Region

Microscopical evaluation showed, transverse section passing through midrib. Single layered upper and lower rectangular shaped epidermal cells were observed. Epidermis was covered with thick cuticle. Double layered palisade cells were present below the upper epidermis and single layered of it was present above the lower epidermis. Collenchymatous cells were present in cluster above the lower epidermis in the midrib region. Xylem and phloem were arranged in ring. Xylem ring present towards the center and is surrounded by phloem ring. Vascular bundles are of collateral type. Calcium oxalate crystal are scattered throughout the cells. (Fig: 3)

3.2.2 Petiole region

Transverse section of petiole shows single layered flattened epidermal cells, covered with thick walled cuticle. Under the epidermis 2-5 layered circular shaped collenchymatous and 2-6 layered circular, thin walled, chlorenchymatous cells were present. Intracellular spaces were observed in between these cells. Bicolateral vascular bundles were arranged in a single ring. Some bundles were capped by one or two layered, thick walled, polygonal sclerenchymatous cells. Towards the centre pith was observed which was composed of large parenchymatous cells. (Fig: 4)

Fig. 3: T.S. of *Butea frondosa* leaf through midrib with lamina

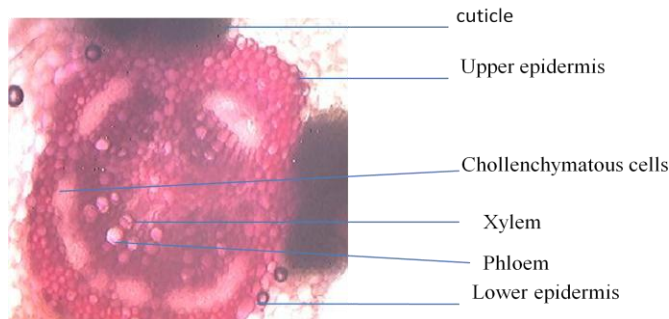
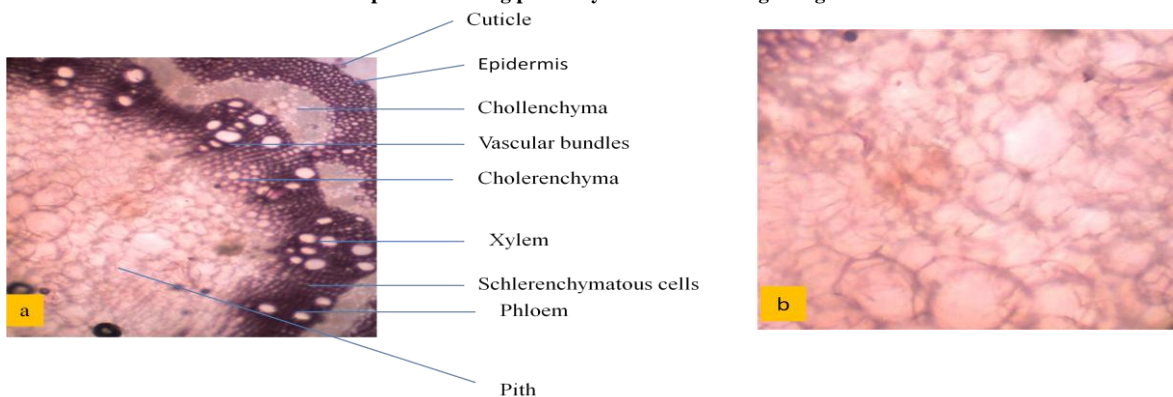


Fig:4 (a): T.S of petiole showing Cuticle, Collenchymatous cells, Vascular bundles, Cholerenchymatous and Schlerenchymatous cells (b) T.S. of petiole showing parenchymatous cells in high magnification.



3.3 Physicochemical parameters

The physico-chemical characters of powdered drug of leaves of *Butea frondosa* such as petroleum ether, chloroform, alcohol, and water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying, and foreign matter are presented in Table 1.

The fluorescence analysis of the powdered drug of *Butea frondosa* in various solvents was performed under normal and Ultra Violet (254nm and 366nm) light and powdered drug reaction with different reagents were evaluated in Table 2 and Table 3 respectively. The pH of 1% and 10% aqueous solution of powdered drug of *Butea frondosa* are noted in Table 4.

Table 1: Physico chemical parameters of leaves of *B. frondosa*

Quantitative parameter	Values obtained (%) w/w
Petroleum ether extractive	2.94
Chloroform extractive	3.08
Alcohol soluble extractive	5.06
Water soluble extractive	10.61
Total ash	10.16
Acid insoluble ash	2.83
Water – soluble ash	5.16
Loss on drying	6.20
Foreign matter	0.73

Table 2: Fluorescence analysis of powdered leaves of *B. frondosa*

Solvents Used	Observation		
	Day Light	UV 254nm	UV 366nm
Drug powder as such	Green	Light green	Dark green
Petroleum ether	Pale green	Greenish brown	Black
Chloroform	green	Black	Brownish black
Ethyl acetate	Pale green	greenish yellow	Greenish black
Toluene	green	Dark Green	Pale green
Acetone	Light green	Yellow	Dark yellow
Ethanol	Pale green	Dark green	black
Distilled Water	brownish green	dark green	violet green
Conc. H ₂ SO ₄	Dark green	Green	Black
Conc. HNO ₃	Greenish brown	Dark brown	Chocolate brown
NaOH in methanol	Dark green	greenish brown	Dark brown
FeCl ₃	Greenish orange	Light green	dark green
Picric acid	Yellowish green	Green	Black

Table 3: Powdered drug reaction with different reagents

Treatment	Observation
Powder as such	Light green
Conc. HCL	Pale green
Conc. HNO ₃	Yellowish brown
Conc. H ₂ SO ₄	Chocolaty brown
Glacial Acetic acid	Green
Benzene	Light green
NaOH in Methanol	Dark green

Table 4: Determination of pH of the drug

Sample	pH
pH of 1% solution	6.06
pH of 10% solution	5.76

3.4 Preliminary phytochemical screening

The preliminary phytochemical investigation of the petroleum ether, chloroform, ethanol and aqueous extracts of *Butea frondosa* showed the presence of sterols, tannins, flavonoids, amino acids, glycosides, phenolic compounds, carbohydrates, saponins and alkaloids are present. (Table 5)

Table 5: Qualitative analysis of phytochemicals in *Butea frondosa*

Phytochemicals	Petroleum ether	Chloroform	Ethanol	Aqueous
Sterols	++	++	+	-
Tannins	-	-	-	++
Flavonoids	-	-	++	+++
Proteins	-	-	-	-
Amino acids	-	-	+	+++
Glycosides	-	-	++	+++
Phenolic compounds	-	-	+	++
Carbohydrates	-	+	++	+++
Saponins	-	+	++	+++
Alkaloids	-	++	+	-

+++ = Good; ++ = Average; + = Poor; - = Nil

3.5 Thin layer chromatography

Thin layer chromatography of the aqueous and ethanolic extracts were carried out separately using toluene: ethyl acetate:formic acid (5:3.5:1.5) for the ethanolic extract and n butanol:acetic acid: water(5:3:2) for the aqueous extract as mobile phase respectively and the R_f values were recorded and depicted in Table 6. The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots. (Fig: 5)

Table 6: Thin layer chromatography of *Butea frondosa*

Test extracts	Solvent system	Number of spots	R _f value
Ethanolic extract	toluene:ethyl acetate:formic acid (5:3.5:1.5)	4	0.20, 0.30, 0.57,0.85
Aqueous extract	Butanol:acetic acid:water(5:3:2)	3	0.52,0.65,0.77

Fig: 5 (a)Ethanolic extract**(b) Aqueous extract**

4. Discussion and Conclusion

In the part of standardization study, the macroscopical examination of the leaves of *Butea frondosa* was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and organoleptic characters of the drugs. The microscopical characters determine the histological profile of the leaf and can serve as diagnostic parameters. The extractive value, ash value, loss on drying and fluorescent analysis of the leaf extracts have been carried out. Percentages of the extractive values were calculated with reference to air-dried drug. The extractive values in different solvents indicate the amount and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug of the leaves of *Butea frondosa* in various solvents was performed under normal and UV light to detect the fluorescent compounds. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents.

After present investigation it can be concluded that the standardization and preliminary phytochemical investigation of *Butea frondosa* yielded a set of standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf, to establish as the drug.

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