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Research Article

Pharmacognostic and Phytochemical Evaluation of the Flowers of *Butea monosperma* (Flame of Forest)

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ABSTRACT

There has been worldwide renewal of interest in herbal system of medicines. Side effects of allopathic drugs have scared people all over the world and there is a consorted effort to find an alternative therapeutic method. In Indian traditional system like Ayurveda various herbs are used for the treating various alignments. Ayurveda is well recorded and documented system of medicine. Due to commercialization in production there is need of standardization of herbal medicine.standardization and quality evaluation of plants and herbs steel remains the challenging task. *Butea monosperma* (Lam.) (*Fabaceae*) popularly known as 'flame of the forest' has been widely used in the traditional Indian medical system of 'Ayurveda' for the treatment of a variety of ailments including liver disorders. In this article pharmacognosy and phytochemistry of the flowers of *Butea monosperma* is studied.

Keywords: *Butea monosperma*, pharmacognosy and phytochemistry

INTRODUCTION

There has been worldwide renewal of interest in herbal system of medicines. Side effects of allopathic drugs have scared people all over the world and there is a consorted effort to find an alternative therapeutic method. In Indian traditional system like Ayurveda various herbs are used for the treating various alignments. Ayurveda is well recorded and documented system of medicine. Due to commercialization in production there is need of standardization of herbal medicine. Global market share of herbal medicine is increasing and hence guidelines for their quality assessment and quality control are required. Several Pharmacopoeias like British Herbal Pharmacopoeia, Indian Herbal Pharmacopoeia, British Herbal Compendium (BHC),Ayurvedic Pharmacopoeia of India lay down monographs for herbs to maintain their quality in their respective nations. For quality control of herbal medicines a different approach is required than that of synthetic medicine. Standardization and quality evaluation of plants and herbs steel remains the challenging task. [1, 2]

Butea monosperma (Lam.) (*Fabaceae*) popularly known as 'flame of the forest' has been widely used in the traditional Indian medical system of 'Ayurveda' for the treatment of a variety of ailments including liver disorders. In this article pharmacognosy and phytochemistry of the flowers of *Butea monosperma* is studied. The attempt is made to establish the authenticity of *Butea monosperma* flowers.

MATERIALS AND METHOD

Procurement, Identification and Preparation of sample: Fresh bright orange red coloured *Butea monosperma* flowers were collected from the forest area of India (Badlapur, Maharashtra), in the month of February and March. Flowers were cleaned and other plant materials like stem, leaves were removed.

The crude drug material was authenticated at Blatter Herbarium, St.Xavier's College, Mumbai, India. (Herbarium Sheet No. 1219) Flowers were dried at 50^0c ($\pm$ 1^0 c). Flowers were then powdered in the mixer and sieved through 40# sieve.

Morphological evaluation: Morphography is the study of an object while morphology is the description of that form. In this study the macroscopic appearances of the drug like shape, size, colour, odour, taste and external markings are studied to establish specific identity of a particular species. [9]

The macroscopical evaluation for the flowers of *Butea monosperma* is given in the Table No. 1

Figure No. 1 The flowers of *Butea monosperma*

Microscopical Evaluation: Plant drugs are commonly used in the powdered form, where macromorphology is destroyed, and hence microscopical evaluation is essential. The cytomorphological characters can help in identification and authentification. Microscopical evaluation in the present study includes the study of histology and the study of powders to find distinguishable characters. [10]

Materials and methods: The flowers of *Butea monosperma* were soaked in chloral hydrate for clearing. The sections were stained with phloroglucinol and hydrochloric acid.

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Table no. 1: Macroscopy of the flowers of *Butea monosperma*

Characters	Observations
Colour	Bright orange in colour, densely clustered, Stalk dark brown
Taste	Bitter
Odour	Odourless
Outer surface	Velvety in touch
Inner surface	Velvety in touch
Corolla	1 to 2 inches long, Papilionaceous,
Calyx	Half inch long, teeth, short, deltoid.Dark olive green in colour, Velvetty outside.
Stamens	Didelphus

Figure No.1 The flowers of Butea monosperma

Figure No. 2 depict the histological characters the floral petals of Butea monosperma

Powder characteristics: The sample powdered drug was cleared with chloral hydrate.

Microscopical examination was carried out as follows:

- Powder was examined as such without staining
- Powder was stained with Iodine (N/50) to identify the presence of starch granules.
- Powder was stained with phoroglucinol (1% w/v in 90% methanol) and concentrated hydrochloric acid to identify the lignified tissues.

Figure no. 3 shows powder characteristics of the flowers of Butea monosperma

Qualitative Phytochemical estimation: Extraction is the process of the seperation of medicinally active component of the plant tissues from the inert or inactive components by using selective solvents and standard extractive procedures. [12, 18] The sample powdered drug ie *Butea monosperma* was subjected to hot extraction method using soxhlet apparatus with 80% methanol solvent. Also Successive extraction was carried out using solvents in order of increasing polarity. The successive extractive values were determined to lay down standards for raw material. Successive extracts of petroleum ether, benzene, chloroform, acetone, methanol and water were prepared. The extractive values and preliminary phytochemical studies were carried out on these extracts. Water soluble extractive value and alcohol soluble extractive value were determined according to standard pharmacopoeial procedures. [16, 17] The qualitative phytochemical screening of successive extracts of *Butea monosperma* flowers done using standard reagents to detect various plant constituents. The results of qualitative phytochemical screening of successive extracts of *Butea monosperma* flowers are shown in the table no. 4

Quantitative Phytochemical estimation: The quantitative phytochemical evaluation was done using standards procedures. Total glycosides, total tannins, total resins, free reducing sugar, total sugar, combined reducuing sugar values were determined. Results are mentioned in the table no. 5.

Fluorescence analysis: Many substances when suitably illuminated emit light of a different wavelength from that of the incident light. The emitted light (fluorescence) ceases when the existing light is removed. Powdered plant material(sample) exhibit fluorescence when exposed to radiation in the UV range and frequently the fluorescence is sufficiently characteristic and is a useful tool in analysis.[19] the characteristic fluorescent properties can be valuable aid in the identification of plant material (sample).

Butea monosperma flower powder, 80% methanolic extract of *Butea monosperma* flowers was used. All observations were made under UV 366 nm and UV 254 nm.

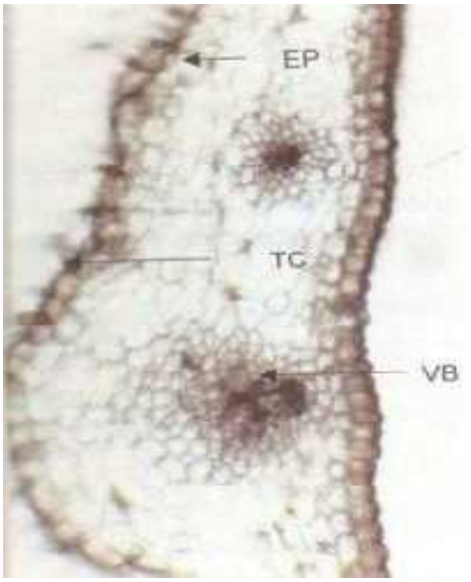
Following test were performed and the results are mentioned in the table no. 6

Drug mounted in nitrocellulose in amyl acetate. A sample of dry powdered drug was placed on a glass slide, affixed with nitrocellulose, allowed to dry and observed under the ultraviolet light.

Drug treated with 1N sodium hydroxide in methanol.

Drug treated with 1N sodium hydroxide in methanol, dried and mounted in nitrocellulose in amyl acetate.

Drug treated with 1N hydrochloric acid.



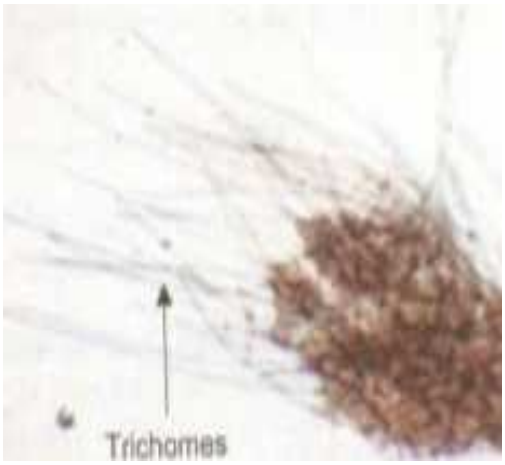


Figure no. 2: T.S. of Corolla of the flowers of Butea monosperma
EP: Epidermis, VB: Vascular bundles, TC: Trichomes

Figure No. 3 Trichomes of flower of Butea monosperma

Table no. 2 Total extractive values for flowers of *Butea monosperma*

Extract	Extractive Value* (%w/w)	Colour of extract
Water soluble extractive	12.64	Yellow
Alcohol soluble extractive	10.8	Fresh yellow

*-----*Average of the three readings.*

Table no. 3 : The successive extractive values for flowers of Butea monosperma

Extract	Extractive Value* (%w/w)	Colour of extract
Petroleum ether	2.33	Very light yellow
Benzene	2	Slight yellow tint
Chloroform	1	Light yellow
Acetone	4.33	Dark yellow
Methanol	12	Very dark yellow
Water	10.66	Brown

*-----*Average of the three readings.*

Drug treated with 1N hydrochloric acid dried and mounted in nitrocellulose in amyl acetate.
Drug treated with 1N sodium hydroxide in water.
Drug treated with 1N sodium hydroxide in water, dried and mounted in nitrocellulose in amyl acetate.
Drug treated with nitric acid diluted with an equal volume of water.
Drug treated with sulfuric acid diluted with an equal volume of water.
High performance thin layer chromatography: For authenticity and standardization of plant material estimation of the active ingredients is essential. High performance thin layer chromatography (HPTLC) serves as precise procedure for the analysis of medicinal plants. HPTLC scanning HPTLC scanning of each extract produces a characteristic peak pattern. The present study was undertaken to develop chemoprofiles of the extracts of dried flowers of *Butea monosperma.*
Preparation of extracts: Definite weighed amount of sample was subjected to aqueous and methanolic extraction using soxhlet extraction. The extracts were filtered and used.
Sample application: the water extract, methanol extract were band spotted by means of Hamilton microsyringe on precoated silica gel F plates with the help of spotter-Linomat IV (CAMAG)
Chromatographic conditions for total water extract:: Application mode: CAMAG Linomat IV,Development mode: CAMAG twin trough chamber, plate material : HPTLC silica gel 60 F 254 (Merk), solvent system used: ethyl acetate: glacial acetic acid: formic acid: water, Chamber saturation time: 75min,development distance:55mm,development time :15min,scanner:CAMAG IIV3.14,Detection: 254nm,Integrator:CATS V4.06 Software
Chromatographic conditions for total methanol extract: Application mode: CAMAG Linomat IV,Development mode: CAMAG twin trough chamber, plate material : HPTLC silica gel 60 F 254 (Merk), solvent system used: ethyl acetate: glacial acetic acid: formic acid: water, Chamber saturation time: 75min,development

distance:63mm,development time :15min,scanner:CAMAG IIV3.14,Detection: 254nm,Integrator:CATS V4.06 Software
Figure no. 4 and 5 shows HPTLC fingerprint chromatogram of the total aqueous extract and methanolic extract of Butea monosperma.

RESULTS

Morphological evaluation: shown in Table no. 1: Macroscopy of the flowers of *Butea monosperma.*

Microscopical Evaluation: It consisted of a single layer of epidermis. Epidermal cells were closely placed. Numbers of unicellular and multicellular long trichomes were seen embedded in epidermal cells.

Powder characteristics: The microscopic examination of powder of flowers of *Butea monosperma* showed the presence of number trichomes as shown in the figure no. 3.

Qualitative phytochemical estimation: The preliminary phytochemical screening indicated the presence of glycosides, tannins, phenolic compounds, phytosterols, proteins and amino acids. Extracts did not show the presence of alkaloids.

Quantitave phytochemical estimation: Quantitave phytochemical estimation of total glycosides, total tannins. total resins, free reducing sugar, total sugar, combined reducing sugar values were done. Results are mentioned in the table no. 5.

Table No. 4 The Qualitative phytochemical screening of successive extracts of the *Butea monosperma* flowers

Test and Reagent	Extract Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Water
Alkaloid						
Dragendroff's test	-ve	-ve	-ve	-ve	-ve	-ve
Mayer's test	-ve	-ve	-ve	-ve	-ve	-ve
Wagner's test	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrates						
Molish	-ve	-ve	-ve	+ve	+ve	+ve
Fehling's	-ve	-ve	-ve	+ve	+ve	+ve
Borford's	-ve	-ve	-ve	+ve	+ve	+ve
Flavonoids						
Shinoda test	-ve	-ve	+ve	+ve	+ve	+ve
Glycosides						
Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
Legal test	-ve	-ve	-ve	-ve	-ve	-ve
Kellar Killani	-ve	-ve	-ve	-ve	-ve	-ve
Proteins and amono acid						
Ninhydrin test	-ve	-ve	-ve	+ve	+ve	+ve
Phytosteols						
Liberman burchad test	+ve	-ve	-ve	+ve	+ve	-ve
Phenolic compounds and tannins						
Ferric chloride test	-ve	-ve	-ve	+ve	+ve	+ve
Lead test	-ve	-ve	-ve	+ve	+ve	+ve
Saponin test						
Foam test	-ve	-ve	-ve	-ve	-ve	-ve
Fixed oils and fats						
Spot test	-ve	-ve	-ve	-ve	-ve	-ve

Table No. 5 Quantitave Phtochemical Estimation

Phytoconstituents	% w/w
Glycosides	5.6%
Tannins	1.2%
Resins	0.9%
Free reducing sugars	3.86%
Combined reducing sugars	6.00%
Total sugards	2.14%

Table No. 6 Fluorescence analysis at UV 254 nm and UV 366 nm

Method	Drug			
	Butea monosperma flower powder		*Butea monosperma* flower 80% methanolic extract	
	254 nm	366 nm	254 nm	366 nm
	-	Greenish yellow	-	Greenish yellow
	Dark brown	Yellowish brown	Light brown	Brownish yellow
	-	Yellowish brown	-	Brownish yellow
	Dark brown	Brown	Brown	Yellowish brown
	-	Greenish yellow	-	Brown
	Light brown	Yellowish brown	Brown	Yellowish brown
	-	Yellowish orange	-	Yellowish brown
	Dark brown	Brownish green	Dark brown	Brownish green
	Dark brown	Yellowish brown	Dark brown	Greenish yellow

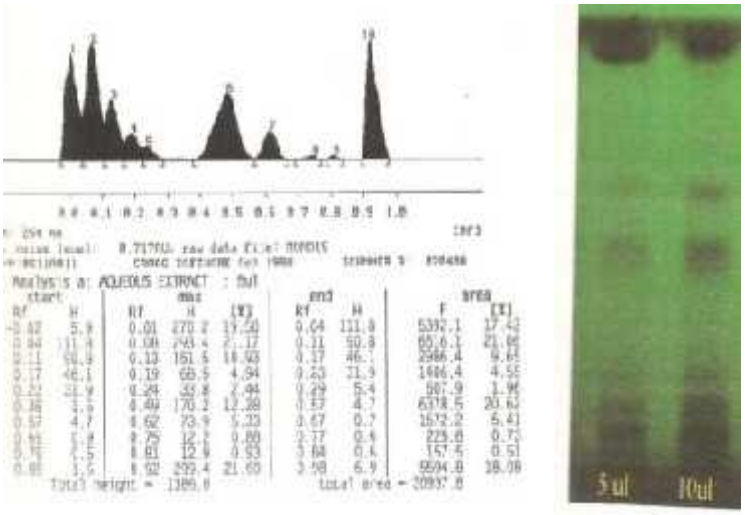


Figure no. 4 shows HPTLC fingerprint chromatogram of the total aqueous extract extract of Butea monosperma under 254nm.

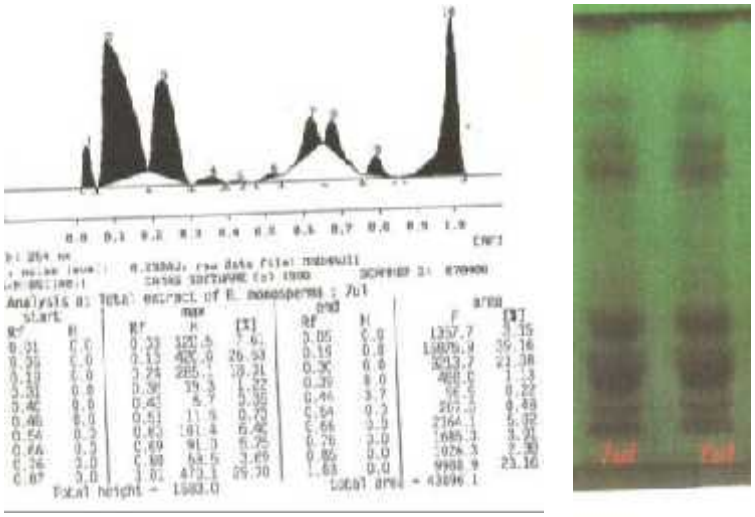


Figure no. 5 shows HPTLC fingerprint chromatogram of the methanol extract of Butea monosperma under 254nm.

Fluorescence analysis: For *Butea monosperma* flower powder and its 80% methanolic extract observations under UV 254 nm and UV 366 nm are given in the table no. 6

High performance thin layer chromatography: The fingerprinting of the chromatograms (Figure No. 4 and 5) of the methanol and aqueous extract of *Butea monosperma* showed not much of the difference in the chemical constituents. Both the plates were treated with ferric chloride and ammonia showed positive result stating presence of flavonoids and phenolic acompounds. Figure no. 4 and 5 shows HPTLC fingerprint chromatogram of the total aqueous extract and methanolic extract of Butea monosperma.

DISCUSSION AND CONCLUSION

Flowers of *Butea monosperma* (Lam) commonly known as 'Flame of forest' are known to possess a yellow colouring matter and are traditionally used during 'Holi' festival in India. The bright colour of the flowers is attributed to the presence of chalkones and aurones. The isobutrin, a chalkone was found to be true colouring matter of this plant. The pharmacognostical and phytochermical experiments were undertaken to authenticate the material collected and to further evaluate the quality parameters and to lay down standards.

Macroscopic examination of flowers showed the flowers were odolrless, velvety to touch and dark orange colour. Microscopic examination of flowers the transverse section of the corolla of the flower petal showed the single layer of epidermis and number of unicellular and multicellular trichomes embedded. Also in powder evaluation presence of trichomes seen.

Water soluble and alcohol soluble extractive values were determined for the flowers of *Butea monosperma.* The successive extractive values were also determined. Preliminary photochemical screening of extracts of flowers of *Butea monosperma* indicated the presence of glycosides, tannins, resins, sterols and sugars. The values for total glycosides, tannins, resins, free reducing sugars and total reducing sugars were also determined. Fluorescence analysis showed characteristic colours at 254nm and 366nm. The fingerprinting of the chromatograms of the methanol and aqueous extracts of *Butea monosperma* showed similar presence of chemical constituents. Understanding the importance of herbal medicines further experiments can be undertaken to explore more characteristic of flowers of *Butea monosperma* and also to standardize the botanicals.

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