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Phytochemical profiling of *Butea monosperma* (Lam). flower extracts

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Abstract
Butea monosperma (Lam) a medium sized deciduous tree, widely distributed throughout India, belongs to the Plant family Fabaceae. Popularly known as "Flame of Forest" in Marathi 'Palash', 'Karka' in Sanskrit. In the traditional system of medicine known as Ayurveda, Sushruta, Charaka and Unani has been used as therapeutics for treatment of a variety of ailments. The present research emphasizes on ethno pharmacological properties of *Butea monosperma* (Lam) with preliminary qualitative screening for phytochemicals studies on flowers. This analysis indicates presence of alkaloids, flavonoids, proteins, carbohydrates, phenols, saponins, steroids, tannins and terpenoids in different solvents. Among the different crude extracts, the Methanol extract showed the highest phenolic content (TPC) of 0.685 mg/ml. Total flavonoids content (TFC) were found to be highest in methanol extracts of 0.585 mg/ml.

Keywords: phytochemical, phenol, flavonoid, medicine, butea

Introduction
Butea monosperma (Lam) belong to the Fabaceae family comprising 630 genera and 18000 species, native to tropical region, a deciduous plant abundantly distributed in Maharashtra and south part of India. This beautiful plant known by various names 'Palash' in Marathi, 'Karka' in Sanskrit at regional level but most plant popularly known as "Flame of Forest" because its geographical distribution appearance in forest ecosystem during summer season [1]. *B. monosperma* is comprehensively mentioned in Ayurveda, Sushruta, Charaka, Homeopathy and Unani traditional system for its traditional therapeutic uses. Flowers of *B. monosperma* are used as Antioxidant, Antidepressant, Antigout, Antileprotic, Anti-inflammatory, Anticonvulsant, Diuretic, Antiulcer, Astringent, Antiestrogenic, Antihepatotoxic agent [2]. Aqueous extract of root used to cure night blindness, elephantiasis and also used antidote against venom of snake bite [3, 4] Stem and bark contains gallic acid, lupeol, pyrocatechin, lignin and betacyanin alanine, allophonic acid cyaniding, anthraquinone and Vitamin C [5]. Methanolic Leaf extract of *B. monosperma* shows antibacterial activity against *Bacillus pumilus* and *Salmonella typhi*. Aqueous extract of leaves possess anthelmintic property against *Pheretima phostuma* Chloroform extract of leaves possess antioxidant activity [6]. Ethanolic extract of *B. monosperma* possess TPC for stem is 64 ± 2.2 (GAE)/g DW for flower 34 ± 1.8 (GAE)/g DW and TFC for stem is (44 ± 2.4 rutin equivalent/g DW) and for flower is (22 ± 1.6 rutin equivalent/g DW) [7]. The total amount of flavonoid content in FEBM gels was calculated from equation of standard curve of quercetin. The flavonoid content was found to be 0.158, 0.226, and 0.328 % w/w, respectively in 0.5, 1.0 and 1.5 % gel [8]. *B.monosperma* leaf hydro-ethanolic extract contain 60% Ethanol 125.25±1.25a TPC (mg GAE/g DE) and 65.15±0.55a TFC (mg RE/g DE) [9]. Anti-cancer potential of aqueous extract of *Butea monosperma* flowers active potent in hepatic cell lines in culture and its chemopreventive effectiveness *In vivo* in a transgenic mouse model of hepatocellular carcinoma (HCC) and show that the extract has a strong promising effect on cancer both *In vitro* and *In vivo* [10].

Materials and Methods
Selection of Plant Material
To design current research, plant material, *Butea monosperma* (Lam) was selected. Reproductive floral part i.e. flowers were collected from Bhokar forest Nanded (MS) during flowering period April and authenticated with the help of reference of Flora of Marathwada by Naik V. N. [21].

Preparation of Plant extract
The collected flowers from fieldwork were brought to the research lab and laid on a clean paper sheet and shade air dried, avoiding a humid environment and contamination for getting disease free material. Flowers were allowed to dry enough to remove complete hydrated moisture from their floral parts. The dried biomass of the flower was grinded into small particles using a mill. The flowers were milled twice, first using a coarse mill and a fine mill to generate a fine powder. The produced dried powdered form of flower was placed in airtight fumigated dry container to avoid further contamination and used during experimental use. For experiment polar solvent such as water and methanol, on the other hand non-polar solvent such as chloroform and n-hexane were used for extraction. The 15 gm. of flower powder extracted with 250 ml of respective solvent by using Soxhlet extractor apparatus for consecutive 3 cycles. All the extracts were measured and lyophilized and stored in a deep freezer at 4°C.

Percentage Yield
The obtained yield of extract of flower in different solvent by using Soxhlet Extractor was measured. The percentage of the yield extracts of flower in different solvent were determined with the formula percentage yield= final weight of extract/initial dry weight of sample X 100 (Table 1)

Phytochemical screening

The flower extracts of plants in different polar solvent water and methanol and non-polar solvents chloroform and hexane were screened for various bioactive phytochemicals. The preliminary qualitative screening of phytochemicals were carried out by using standard methods and slight modification [11, 12, 13]

Quantitative estimation of plant extracts

Total phenolic content

The different flower extracts were estimated for total phenol content by using Folin - Ciocalteu reagent with Gallic acid as standard. The total phenol content was estimated according to the method of [14]. The oxidizing agent Phosphomolybdate present in the reagent Folin-Ciocalteu reacted with the substrate present in the flower extracts of different solvent to produce a blue colour complex. The reaction mixture contained 1 ml of extract of plant sample, 2 ml of distilled water, and 0.5 ml of Folin -ciocalteu reagent and 2 ml of sodium carbonate (20%). Allow reaction mixture for incubation for 1 min. in boiling bath and after cooling, the absorbance was measured at 660 nm by using UV-Vis Spectrophotometer against blank reagent. The total no. of phenols was calculated by preparing a calibration curve using different dilutions of Gallic acid (0.5 mg/ml) and the unknown amount of phenolics were estimated as mg/gm of plant samples. The result was determined from the standard curve and was expressed as Gallic acid equivalent (GAE) (mg/g of the extracted compound) [15].

Estimation of total flavonoid content

The individual flower extracts was estimated for total flavonoid content by using the $AlCl_3$ method previously described by [16]. The reaction mixture contained 1 ml of flower extracts, 3 ml methanol, 0.2 ml of 10% of $AlCl_3$, 0.2 ml (1M) of potassium acetate, and 5.6 ml of distilled water was added. The reaction mixture was incubated for half an hour at r.t. and absorbance was measured at 415 nm by using UV-Vis Spectrophotometer. Quercetin (0.1mg/ml) was used for preparing the standard curve with serial dilution. The total concentration of flavonoids in individual flower extract was calculated using standard curve and the amount was expressed in quercetin equivalent mg/gm [15].

Observations and Results

Extraction and Percentage of the yield of plant samples:

The 15 gm of powder form of flower taken for extraction in 250 ml of respective solvent in Soxhlet apparatus. The extraction method carried out for each solvent separately. Extraction yield was measured and it had been seen among all extracts, the percentage of yield in decreasing order 18.33%, 15%, 9.46%, 9.40% in methanol, water, chloroform and hexane respectively [17]. (Table No.1)

Table 1: Percentage yield of flower in polar and non-polar solvents.

Sr. No.	Name of Plants	Solvents	Yield of Extract (mg/gm)	Percentage of Yield*
1	*Butea monosperma* Lam.	Water	2.25	15.00
		Methanol	2.75	18.33
		Chloroform	1.41	09.40
		Hexane	1.42	09.46

*The results summarized are mean values of n= 3.

Preliminary Phytochemical Qualitative screening of plant extracts

The flower was screened for phytochemical content by using different chemicals and reagent test for each solvent extract, methanol extracts indicates presence of most of phytochemicals in great concentration except phlobatannins, there was negligible concentration of steroids in methanol extract whereas almost absent in rest of solvents. From overall inference obtained through phytochemical analysis in polar and non-polar solvents there were presence of alkaloids, flavonoides, tannins, terpenoids, glycosides, carbohydrates in *Butea monosperma* flower [18].

Table 2: Phytochemical Analysis of Different Solvent Systems of *Butea monosperma* Lam. flower.

Phytochemicals	Test	Solvents			
		Water	Methanol	Chloroform	Hexane
Flavonoids	Sodium hydroxide	+	+++	++	++
	Lead acetate	-	+++	++	+
	Ferric chloride	-	++	+	+
Saponins	Frothing	++	++	++	-
Tannins	Ferric chloride	++	++	++	-
	Chlorogenic	++	+++	++	-
	Formaldehyde	+++	+++	+	-
Terpenoids	Salkowski	--	+++	+++	+++
Glycosides	General Test	-	+++	++	+
Steroids	Salkowski's	-	+	-	-
	Lieberman- Buchard's	-	-	-	-
Carbohydrates	Free reducing sugar	++	+++	++	++
	Molisch's	++	++	++	+
Phlobatannins	Precipitate test	-	-	-	-
Alkaloids	Mayer's	-	+	++	-
	Dragendorff's	-	+++	++	++

*- Absent, + Present in low concentration, ++ Present in moderate concentration, +++ Present in high concentration.

Estimation of total phenol

The quantitative estimation of total phenol of flower extract showed great concentration 0.685 mg/ml in methanol as compared with that off standard Gallic acid 0.995 mg/ml whereas water extract estimation indicates less concentration of total phenolic 0.335 mg/m

Table 3: Estimation of Total Phenolic Contents

Sr. No.	Name of Plants	Solvents	Total phenol content (mg/ml)*
1	*Butea monosperma* Lam.	Water	0.335 mg/ml
		Methanol	0.685 mg/ml
		Chloroform	0.496 mg/ml
		Hexane	0.427 mg/ml
2	Standard Gallic acid	Distilled water	0.995 mg/ml

*The results summarized are mean values of n= 3.

Estimation of total Flavonoids:

The quantitative estimation of total flavonoids of flower extract showed great concentration 0.585 mg/ml in methanol as compared with that off standard Quercetin 0.755 mg/ml whereas water extract estimation indicates less concentration of flavonoids 0.235 mg/ml

Table 4: Estimation of total Flavonoids Contents

Sr. No.	Name of Plants	Solvents	Total flavonoids content (mg/ml)*
1	*Butea monosperma* Lam.	Water	0.235 mg/ml
		Methanol	0.585 mg/ml
		Chloroform	0.456 mg/ml
		Hexane	0.328 mg/ml
2	Quercetin	Distilled water	0.755 mg/ml

*The results summarized are mean values of n= 3.

Discussion

Plant material (flowers) 15 gm. was weighed and placed in soxhlet for extraction in different polar and non-polar solvents separately at room temperature. After completing 3-4 cycles extracts was filtered and concentrated in oven, percentage yield of flower extracts in respective solvent was measured, it observed that percentage yield in methanol extract of flower has maximum among rest of solvent 18.33% whereas rest of solvent percentage in decreasing order for water 15.00%, hexane 9.46%, chloroform 9.40% respectively. Preliminary qualitative phytochemical analysis of the different successive crude extracts of flower of. *Butea monosperma* Lam. revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, phenols, saponins, steroids, tannins and terpenoids. These secondary metabolites derived from primary metabolites are reported to have many active biological compounds and essential therapeutic properties, so this species is expected to have many medicinal uses [19, 22].

The total phenolic content was estimated according to the method of [14]. Among the different crude extracts, the Methanol extract showed highest phenolic content of 0.685 mg/ml, followed by chloroform extracts of 0.496mg/ml, which is then followed by hexane extracts of 0.427mg/ml and the last one with aqueous showed less content which is 0.335mg/ml respectively. They have been reported to have many biological effects on the plants as well as other living organisms. The total flavonoids content was estimated according to the method of [15, 16]. Total flavonoids content were found to be highest in methanol extracts of, 0.585 mg/ml followed by chloroform 0.456 mg/ml, followed by hexanes extract 0.328 mg/ml, the least flavonoids content was found in aqueous extract 0.235 mg/ml. Flavonoids contents in plant responsible for production of natural lacquer [20]. These Phytochemical compounds are derivative of plant primary and secondary metabolic processes, these are reported to have many biological role of primary metabolite in growth and development process of plant whereas, there is rare role of secondary metabolite for plant, considering this the current research emphasizes to exposes phytochemicals for medicinal therapeutic uses and can be further studied for the production of pharmaceutical drugs.

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