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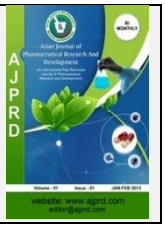

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Research Article

Phytochemical Standardization and Pharmacological Evaluation of Diuretic Activity of Flowers of *Butea Monosperma*

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A B S T R A C T

The historical usage of the seeds as a diuretic has not been confirmed pharmacologically. Due to the fact that diuretics are very effective in the treatment of chronic conditions such as hypertension, edema, renal abnormalities, and urinary tract infections, the present study was conducted to explore the aforementioned action with evidence. In the most ancient Indian traditional system of medicine (Ayurveda) diuretics are called as Muttra- virechanya dravya. These agents were widely explored in Indian ancient system of medicine. Diuretics cause increase in the rate of urine flow rate thus employed in numerous disorders like hypertension, anxiety, cardiovascular disorders, diabetes mellitus and liver degeneration diseases. In the light of the above-mentioned study, we can report that the methanol extract of *Butea monosperma* flowers is an effective diuretic and also resulted in increased sodium, potassium, and chloride ions in urine; which correlates well with the traditional use of the plant as a diuretic. Nevertheless, it can be suggested that the range of polar phenolic compounds such as flavonoids and tannins in combination with alkaloids might be responsible for the apparent diuretic activity of the plant. The observations showed *Butea monosperma* flowers had a diuretic spectrum similar to that of furosemide.

Keywords: Phytochemical Standardization, Pharmacological Study, Diuretic Activity, *Butea Monosperma*, Flower

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INTRODUCTION:

Diuretics are the drug that increases the rate of urine formation together with natriuresis. Diuretics are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis [1]. The British National Formulary (BNF) currently lists individual diuretics available for use in the UK but the original one, chlorothiazide, is not among them, although its derivatives, hydrochlorothiazide and benzothiazide, do feature in combination preparations. They are grouped into familiar categories – thiazides, loop diuretics (also known as high ceiling diuretics), potassium- sparing, osmotic and carbonic anhydrase inhibitors [2]. The most common adverse effect for any diuretic is mild hypovolemia, which can lead to transient dehydration and increased thirst. In general, adverse effects are dose-dependent and are higher with loop diuretics as they have the most significant diuretic effect [3]. Diuretics are powerful agents that impair sodium reabsorption in renal tubules. Their ability to alter long-term sodium balance induces important hemodynamic changes that result in a reduction in peripheral resistance and sustained reduction in blood pressure. A pharmacologically diverse group of drugs, they remain a mainstay in the therapy of hypertension [4]. The synthetic diuretics exhibit various adverse effects such as electrolyte imbalance and metabolic alterations etc due to these side effects we prefer natural diuretics. The crud drug being always available easily in abundance, comparatively cheaper with negligible side

effects [5]. Plants have been traditionally used as treatments for various diseases, among them several pathologies identified as pre-, intra-, and post-renal factors. The medicinal characteristics of plants have been attributed to their secondary metabolites, which can protect against pathogens or have important physiological benefits to prevent some diseases [6]. Plants provide a wide range of bioactive compounds which act as antioxidants, anti-inflammatory, diuretic, anticancer, and antimicrobial. Further, nephroprotective agents from plants mitigate processes such as interstitial nephritis, altered intraglomerular hemodynamics, tubular necrosis, or glomerulonephritis. However, they usually focused on intrinsic damage such as nephrotoxicity, omitting the pre-renal and post-renal factors, and to our knowledge, no classification of nephroprotective plants according to pre-, intra-, and post-renal diseases have been reported [7]. Butea monosperma is commonly known as Flame of forest, belonging to the family Fabaceae. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts. The pods should be collected and shown before the commencement of rains, root suckers are freely produced and help in vegetative propagation. The genus Butea includes Butea monosperma parviflora, Butea minor and Butea superba widely distributed throughout India [8]. Butea monosperma is extensibly used in Ayurveda, Unani and Homeopathicmedicine and has become a cynosure of modern medicine. The plants of this genus are wellknown for their colouring matters. Commonly Butea monosperma is used as tonic, astringent, aphrodisiac and diuretics. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours. It is reported to possess antifertility, aphrodisiac and analgesic activities [9]. *Butea monosperma* is used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The plants of this genus are well known for their colouring matters. Commonly *Butea monosperma* is used as tonic, astringent, aphrodisiac and diuretics. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours. It is reported to possess antifertility, aphrodisiac and analgesic activities. Flowers are useful in diarrhoea, astringent, diuretic, depurative, tonic, leprosy, skin diseases, gout, thirst, burning sensation. The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. Besides medicinal uses it is also having the economic use such as flowers are used for making platters, cups and bowls. On the basis of traditional uses, the present study was aimed to evaluate *Butea monosperma* for diuretic activity. The flowers of *Butea monosperma* have been shown to be used as a diuretic to treat high blood pressure in the past. The goal of this study was to first look at the plant from a pharmaceutical perspective.

MATERIAL AND METHODS

Collection, Authentication of plant and Preparation of samples: The fresh flowers of *Butea monosperma* was collected from Kanpur, India. The authentication of *Butea monosperma* flowers was performed by Prof. V. K. Khare, head of the botany department. A herbarium file was prepared and submitted in the department with voucher specimen no. CC/2025/MG/03. The flowers were dried under shade and powdered. They were screened with 60# sieves separately for pharmacognostic study. All powdered flowers were stored in airtight containers and used for phytochemical and pharmacological studies.

Pharmacognostic studies:

Macroscopical study: The macroscopical explanation of all parts of *Butea monosperma* plant were studied as parameters include size; shape; nature of outer and inner surfaces; types of fracture; and organoleptic characters like color; odour; taste etc. were studied.

Physicochemical analysis: The physicochemical analysis was investigated for dried crude drugs. The dried form of crude drugs was used to avoid batch-to-batch variation and also to evaluator with their quality. Their studies also provide the information on the nature of phytoconstituents present. The physicochemical analysis of *Butea monosperma* flowers were carried out using methods prescribed in the Ayurvedic pharmacopoeias of India with the prescribed following parameters [10].

a) Total Ash value
b) Acid insoluble ash value
c) Water soluble ash value
d) Alcohol soluble extractive value
e) Water soluble extractive value
f) Loss of moisture content
g) Swelling index

Preliminary Phyto-profiles: Extraction includes partition of bioactive segment of the plant tissues from the latent moiety by utilizing specific solvents in standard extraction systems. Plant herbs were extracted successively with hexane, and methanol utilizing maceration method of extraction. The totally dried flowers of *Butea monosperma* was coarsely powdered and afterward extracted with non-polar solvent petroleum ether for defatting of plant material. Flowers powder (200g) was stuffed in vessel and kept with hexane for 24 hours and procedure was repeated till complete extraction. The plant material then kept with ethanol for 24 hours and procedure was repeated till complete extraction. The obtained ethanol extract was filtered and concentrated on rotary evaporator to get ethanol extract.

Chemical Tests: The extracts were then used for the various qualitative test to identified the presence of various phytoconstituents i.e. alkaloids; glycosides; flavonoids;

carbohydrates; aminoacids; saponins; sterols and terpenoids; cardiac glycosides; coumarins; carotenoids; tannins; phenolic compounds; fixed oils and fats etc [11].

In-vivo pharmacological screening of diuretic activity by Lipschitz model: Thirty rats were deprived of water but not food for 18 hours. Their bladders were emptied by pulling on their tails and gently compressing the pelvic region. To impose a homogeneous water load, each of these rats was given 15 ml of isotonic saline (NaCl, 0.9 % w/v) orally. After 45 minutes, the rats were randomly divided into four groups (n = 6 per group) and given the following oral treatment. One ml of pure water, 100 mg/kg BM of extract, 200 mg/kg of BM extract, and 250 mg/kg of BM extract were used in the first three groups, while Group 5 received 13.5 mg/kg of Furosemide as standard.

Table 1: Treatment groups for diuretic activity

Group 1	Normal control (distilled water)
Group 2	Extract 100 mg/kg
Group 3	Extract 200 mg/kg
Group 4	Extract 250 mg/kg
Group 5	Standard drug (Furosemide-13.5 mg/kg)

For adaptation, each animal was isolated in metabolic cages for 24 hours prior to the start of the experiment, and then starved overnight with free access to water. Urine samples were taken at 6 hours, 12 hours, and 24 hours following the previous dosage. For electrolyte analysis, the urine samples were filtered and then kept at -20ºC [12].

Urine Parameters Measurements: For all rats, cumulative urine output was assessed after 6, 12, and 24 hours. A digital pH meter and a conductivity meter were used to test the pH and conductivity of fresh urine samples. The colour of urine was also noted. The entire urine output samples (24 hours). were then diluted (1:1000 in deionized water) to measure the total electrolytes (sodium, potassium, and chloride ions) concentrations in urine using a Flame Photometer.

Kidney Homogenate Preparation: At the termination of the experiment, the animals were decapitated and slaughtered. Arteovenous blood was drawn and centrifuged in heparinized tubes. The obtained plasma was maintained at 20°C for biochemical examination. The kidney was removed, defatted, weighed, and kept at 20°C for further biochemical examination [13].

Results and Discussion

Pharmacognostic study: Flowers: The macroscopical description of different parts of *Butea monosperma* flowers plant include size; shape; Butea monosperma, commonly known as Palash or Flame of the Forest, has compound, trifoliate flowers. Each flowers consists of three flowerslets, which are leathery and dark green, with the terminal flowerslet being rhomboid-obovate and the lateral ones obliquely ovate. The flowers grow alternately on the stem and are often pubescent (hairy) on both sides above as shown in Figure 1.

Figure 1: *Butea monosperma* flowers

Physicochemical analysis: Physicochemical analysis of powdered flowers of *Butea monosperma* was carried out in various parameters. The results reveal total ash values of flowers (5.6% W/W); acid insoluble ash was found (1.8 % W/W) and water-soluble ash was found (3.7 %W/W). Water soluble extractives were higher than alcohol soluble extractives in Flowers (18.9; 5.6% W/W). Moisture content was found highest in flowers (78.3 %W/W)

Preliminary phytoprofiles: Results exposed that %w/w yield in successive extraction of Butea monosperma flowers in petroleum ether; and ethanol were 11.5; and 21.5 respectively. Colour of extract of *Butea monosperma* flowers was dark green in petroleum ether; and yellowish green in ethanol. Consistencies of the flower's extracts were sticky and semisolid in petroleum ether while non sticky and solid in ethanol extracts. Dissimilar result of %w/w yield; colour and consistency of successive extracts of Butea monosperma flowers helps in identification of plant

Chemical test: Qualitative phytochemical examination of successive extracts indicated that the alkaloids were present in ethanol extracts of *Butea monosperma* flowers. The chemical constituent flavonoid was absent in ethanol extracts of *Butea monosperma* flowers. Ethanol extracts of contained carbohydrates. Phytosterols were found in petroleum ether. Cardiac glycosides and coumarins were absent in petroleum ether. Anthraquinone glycosides were present in ethanol extracts. Dissimilar result of phytoconstituents in successive extracts helps in identification of plant.

In-Vivo Diuretic activity by Lipschitz model:

Effect on Urine Output and Diuretic Activity: Table 1 specify the urine volume, diuretic effect, and diuretic activity. The total urine volume was assessed for the *Butea monosperma* flowers methanolic extracts (100, 200, and 250 mg/kg), standard diuretic (furosemide), and normal control

during periods of 6, 12, and 24 hours. According to the findings, *Butea monosperma* flowers extract displayed diuretic action at all doses tested, including furosemide at 6, 12, and 24 hours, and the impact was dose dependent. When compared to control rats, furosemide and *Butea monosperma* substantially enhanced urine flow at 6, 12, and 24 hours (p< 0.001). The high dosage voided more than twice as much urine as that of control.

Table 1: Effect of methanolic extract of *Butea monosperma* on volume of cumulative urinary excretion after 6, 12 and 24 hrs

Groups	After 12 hrs			After 24 hrs		
	Urine Volume (ml)	Diuretic action[a]	Diuretic activity[b]	Urine Volume (ml)	Diuretic action[a]	Diuretic activity[b]
Control	165 ± 2.4	1	0.57	244 ± 4.9	1	0.55
Extract 100 mg/kg	187 ±2.2	1.13	0.65	284 ±4.1	1.16393	0.64
Extract 200 mg/kg	210 ±1.53	1.27	0.73	318 ±3.96	1.30328	0.72
Extract 250 mg/kg	248 ±1.21	1.5	0.86	388 ±2.63	1.59016	0.88
Standard drug (Furosemide-13.5mg/kg)	288 ±1.47	1.75	1	443 ±2.79	1.81557	1

All Values are expressed as mean±SEM where n=6.
a Diuretic action=urine volume of test/urine volume of control.
b Diuretic activity=urine volume of test /urine volume of Standard (Furosemide).
***Significant difference at p< 0.001 as compared with control group.

Effects on urine pH and conductivity: The pH and conductivity of urine were tested after 24 hours. Control rats had a urine pH of 6.11±0.02. At 24 hours following administration of *Butea monosperma* flowers extract at dosages of 100, 200, and 250 mg/kg body weight, the urine pH was 6.73±0.13, 7.25±0.09, and 7.89±0.16 respectively. Furosemide made the urine significantly alkaline by raising the pH to 8.08±0.21 (P<0.001). At 24 hours, the conductivity of control rats was observed to be 12.57±0.31. The urine conductivity of furosemide-treated rats increased considerably to 19.27±0.19. The urine conductivity of rats treated with *Butea monosperma* flowers extract was 14.32±1.09, 17.44±0.89, and 18.87± 0.65 at dosages of 100, 200, and 250 mg/kg, respectively.

Effects on electrolyte excretion: As compared to normal control rats, the diuretic responses of the methanolic *Butea monosperma* flowers extract with its electrolyte excretion potency were very moderate at all dosages. The *Butea monosperma* flowers extract at doses of 200 and 250 mg/kg showed a significant increase in Na+, K+, and Cl- excretion. The results of urinary electrolyte excretion after treatment of QL extract were comparable to the furosemide group also (Table 2). The effects of methanolic extract of *Butea monosperma* on Na+, K+, and Cl- excretion at 6, 12, and 24 hrs

Table 2: Effect of methanolic extract of Butea monosperma flowers on electrolyte excretion after 24 hrs

Groups	Na+ excretion[a] (mEq/kg/24hrs)	Na+Index[b]	K+ excretion[a] (mEq/kg/24hrs)	K+Index[b]	Cl- excretion[a] (mEq/kg/24hrs)	Cl-Indexb
Control	18±1.00***	1	19±0.82***	1	15±2.10***	1
Extract 100 mg/kg	52 ± 2.12***	2.9	37±1.41***	1.9	50±2.13***	3.3
Extract 200 mg/kg	102±1.25***	5.7	100±0.71***	5.3	100±1.03***	6.7
Extract 250 mg/kg	150±1.78***	8.3	47±2.35***	2.5	150±1.42***	10
Standard drug (Furosemide-13.5mg/kg)	92±5.96***	5.1	34±2.62***	1.8	90±6.21***	6

a. All Values are expressed as mean±SEM where n=6.
b Index=Excetion of test/excretion of control.
b Diuretic activity=urine volume of test /urine volume of Standard (Furosemide).
***Significant difference at P< 0.001 as compared with control group.

Effects on natriuretic, saliuretic and carbonic anhydrase inhibition: Table 3 depicts the results for natriuretic, saliuretic, and carbonic anhydrase inhibition. The furosemide (13.5 mg/ kg) and methanolic extract of *Butea monosperma* at doses (200 and 250 mg/kg) showed potent natriuretic and saliuretic activity as compared to normal control. In our study, the methanolic extract of *Butea monosperma* showed less effect on carbonic anhydrase inhibition.

Table 3: Effect of ethanolic extract of *Butea monosperma* flowers on Saluretic, Natriuretic and carbonic anhydrase inhibition after 24 hrs

Groups	Saluretic Effect (Na++ Cl-)a	Natriuretic Effect (Na^+/K^+)[a]	CAI Cl (Na++ K+)a	Saluretic index[b]	Natriureti c index[b]	CAI indexb
Control	33.2 ± 3.1	0.9±1.8	0.4± 0.65*	1	1	1
Extract 100 mg/kg	101.9± 4.18***	1.4± 1.30**	0.6± 0.4*	3.1	1.5	1.4
Extract 200 mg/kg	202.5± 2.19***	1.9± 1.65**	0.6± 0.33*	6.1	2	1.6
Extract 250 mg/kg	300.1± 3.32***	3.3± 70***	0.8± 0.32**	9	3.5	1.9
Standard drug (Furosemide-13.5mg/kg)	182.8±12.57***	2.6± 2.35***	0.7± 0.39**	5.5	2.8	1.8

The utilization of herbal medicines and phytonutrients / nutraceuticals continues to rise as a result of increased acceptance and public interest in both developed and developing nations. Herbs and natural plant products are particularly gaining popularity for the management of cardiovascular diseases and associated disorders. The soaring interest in traditional medicine is attributed to the failure of modern medicine to alleviate many chronic illnesses. *The Butea monosperma* flowers are highly venerated for their nutritional values and medicinal properties. The seeds of the plant are used as an astringent and diuretic in the treatment of urinary disorders.

This study examined the diuretic potential of *Butea monosperma* flowers using a methenolic extract and showed an increase and acceleration in the elimination of fluid with urinary hypoosmolarity and a moderate increase in natriuretic activity. These results demonstrate that the methanolic extract of the *Butea monosperma* flowers has a moderate diuretic activity. The increase in natriuresis in response to acute treatment by extract may partly explain the increase in diuresis. It showed that the extract's action was time and dose dependent. This can be explained by kinetic differences in the active principle's presence in the extracts and the increment in the urine output in rats might result from the high potassium content in the plant extract. The pH values were also alkaline as compared with control. *Butea monosperma* flowers contains a flavonoid known as quercetin as a secondary metabolite, and flavonoids act as diuretics by playing an important role in the RAS system.

SUMMARY AND CONCLUSION:

The pharmacognostic evaluation of *Butea monosperma* flowers extract was contains a flavonoid known as quercetin as a secondary metabolite, and flavonoids act as diuretics by playing an important role in the RAS system. The proposed study is evident that the ethanolic extract of flowers of Butea monosperma flowers have potent and dose-response diuretic properties in experimental animal model. In view of all these various uses associated with these compounds found in *Butea monosperma* flowers extract, further research on this plant flowers to quantify the concentration of these compounds per known amount for industrial use. The present study has confirmed the ethnopharmacological use of the extract of *Butea monosperma* flowers as a diuretic agent, but further studies are necessary to evaluate the mechanisms involved in its biological activity and safety following repeated exposure.

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