

Evaluation Of Diuretic Activity Of Gum Of Butea Monosperma

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Abstract

The traditional systems of medicine together with homoeopathy and folklore medicine continue to play a significant role largely in the health care system of the population. Butea monosperma(palas) belonging to the family leguminosae grown wildly in many parts of India. Kidney, as excretory organ of our body serves important function of excretion of waste products, regulation of fluid volume and electrolyte content etc. Damage to kidney can lead to severe life threatening complications. Diuretics are drugs capable of increasing levels of urine. The plant parts are used in the form of extract, juice, infusion, powder and gum. Gum of Butea monosperma is used to treat diuretic activity of kidney in folk medicine. Present study shows that the methanol extract of Butea monosperma gum possess good diuretic activity.

Keywords : Butea monosperma, Herbal medicine, Kidney, Urine, Diuretic activity.

Introduction

Herbal medicine (also herbalism) is the study of pharmacognosy and the use of medicinal plants, which are a basis of traditional medicine.[1] There is limited scientific evidence for the safety and efficacy of plants used in 21st century herbalism, which generally does not provide standards for purity or dosage.[2] The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care.[3]

With tremendous expansion in the interest in and use of traditional medicines worldwide, two main areas of concern arise that bring major challenges. These are international diversity and national policies regarding the regulation of the production and use of herbs (and other

complementary medicines) and their quality, safety, and scientific evidence in relation to health claims. [4,5]

Urinary system

The urinary system, also known as the urinary tract or renal system, consists of the kidneys, ureters, bladder, and the urethra. The purpose of the urinary system is to eliminate waste from the body, regulate blood volume and blood pressure, control levels of electrolytes and metabolites, and regulate blood pH. The urinary tract is the body's drainage system for the eventual removal of urine.[6] The kidneys have an extensive blood supply via the renal arteries which leave the kidneys via the renal vein. Each kidney consists of functional units called nephrons. Following filtration of blood and further processing, wastes (in the form of urine) exit the kidney via the ureters, tubes made of

smooth muscle fibres that propel urine towards the urinary bladder, where it is stored and subsequently expelled from the body by urination (voiding). The female and male urinary system are very similar, differing only in the length of the urethra.[7]

Structure of kidney

In humans, the kidneys are located high in the abdominal cavity, one on each side of the spine, and lie in a retroperitoneal position at a slightly oblique angle.[8] The asymmetry within the abdominal cavity, caused by the position of the liver, typically results in the right kidney being slightly lower and smaller than the left, and being placed slightly more to the middle than the left kidney. [9,10]

The human kidney is a bean-shaped structure with a convex and a concave border. A recessed area on the concave border is the renal hilum, where the renal artery enters the kidney and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perirenal fat, renal fascia, and pararenal fat. The anterior (front) surface of these tissues is the peritoneum, while the posterior (rear) surface is the transversalis fascia.[11]

Role of the kidneys

The kidneys are the major functional units of the urinary system. They are responsible for the production of urine as well as several other functions, including balance of electrolytes such as sodium, potassium, chloride and phosphate levels. The kidneys also have a role in maintaining blood pH because of their ability to excrete hydrogen and conserve bicarbonate. They also regulate blood volume by conserving or eliminating water in the urine, which has a direct effect on blood pressure. In addition, they produce the enzyme renin, which allows control of blood pressure.[12]

Production of urine

Filtration

Once fluid, and a range of dissolved substances, has moved through the filtration barrier, it enters the glomerular capsule and is known as filtrate. Filtrate is similar to blood plasma, but it contains almost no proteins because proteins do not cross the filtration barrier in normal circumstances. The filtration barrier only allows certain substances to move through, and filtration is dependent on three main principles. Inside the glomerulus, a hydrostatic pressure of 55mmHg in the plasma promotes the movement of water and dissolved substances through the filtration barrier. This pressure is opposed by the filtrate hydrostatic pressure inside the glomerular capsule, which is 15mmHg. The pressure is also opposed by the osmotic pressure of the blood.[13,14]

Reabsorption

With an average of 7,500mL of filtrate produced per hour, reabsorption is vital to ensure the maintenance of fluid balance. Reabsorption is a delicate balance of returning essential nutrients to the circulation, return of controlled amounts of electrolytes to the circulation and reabsorption of the majority of the fluid. Reabsorption involves the normal processes of solute transport across cell membranes, for example water and urea are passively reabsorbed while other substances such as glucose, amino acids, sodium and potassium are reabsorbed via active transport mechanisms. Only 1mL of the filtrate forms urine, meaning that 124mL per minute are reabsorbed. Overall, this leads to a normal production level for urine of around 60mL per hour. In practice, it is common to use the average of 30mL per hour when measuring hourly urine volumes in patients with acute conditions. The proximal convoluted tubule is highly permeable to water so there is rapid osmotic movement of water and reabsorption of 65-80% of electrolytes such as sodium, potassium, chloride and bicarbonate.[15]

Secretion

Secretion is the movement of substances from the blood into the tubule of the nephron and is an important renal process that regulates the level of several substances. It occurs mainly in the distal convoluted tubule and the collecting ducts. Although creatinine is freely filtered, it is also secreted increasing the total amount in filtrate by about 20%. Other substances such as hydrogen, potassium and ammonia, and some drugs are also secreted, for example morphine, aspirin and penicillin. The collecting ducts unite to form larger ducts, ultimately leading the filtrate to the pelvis of the kidney where the filtrate exits the kidneys via the ureters, and is known as urine. Normal urine consists of 95% water (with an average adult producing around 1-2L of urine per day), urea, creatinine, potassium, ammonia, uric acid, sodium, chloride, magnesium, sulphate, phosphate and calcium.[16]

Excretion

The last step in the processing of the ultrafiltrate is excretion: the ultrafiltrate passes out of the nephron and travels through a tube called the collecting duct, which is part of the collecting duct system, and then to the ureters where it is renamed urine. In addition to transporting the ultrafiltrate, the collecting duct also takes part in reabsorption.[17]

Diuretic activity

A diuretic is any substance that promotes diuresis, the increased production of urine. This includes forced diuresis. A diuretic tablet is sometimes colloquially called a water tablet. There are several categories of diuretics. All diuretics increase the excretion of water from the body, through the kidneys. There exist several classes of diuretic, and each works in a distinct way. Alternatively, an antidiuretic, such as vasopressin (antidiuretic hormone), is an agent or drug which reduces the excretion of water in urine. Diuretic are commonly defined as drugs that increase the amount of urine output by the kidneys. These agents augment the renal excretion of sodium and either

chloride or bicarbonate primarily, and water excretion secondarily.[18]

Therapeutic Uses of Diuretic Agents

Diuretics are a medication used in the management and treatment of edematous and other non-edematous disease conditions. Diuretics are a class of drugs.[19]

Diuretics are drugs that pharmacologically tilt the renal fluid regulation in favor of the excretion of water and electrolytes. Thus, diuretics are substances that increase the production and volume of urine. This class of drugs achieves this objective primarily by suppressing receptors that aid in the reabsorption of Na⁺, the most abundant extracellular cation, from the renal tubules, thereby increasing the osmolality of the renal tubules and consequently suppressing water reabsorption. Osmotic diuretics cause a direct increase in luminal hyperosmolarity in the renal tubules without affecting electrolyte balance, whereas aquaretics are substances that act directly by only affecting the excretion of water.[20]

Pharmacological Activity

Antimicrobial, diuretic, antifertility, anticonvulsive, antihelminthic, antidiarrhoeal, antimicrobial, wound healing, antigiardiasis, and hepatoprotective, antihypertensive, antitumor, antidiabetic, anti-inflammatory, free radical scavenging activity are among the biological and pharmacological activities of *Butea monosperma*. [21]

Material and method

Collection of Plant Material

For the preparation of herbal formulation plant material was collected from the nearby botanical garden. The plant material was fresh and the unwanted material was removed from it.

The plants of **Butea Monosperma Gum** was then washed with water to remove physical impurities like soil and dirt, dried at room

temperature and subjected to botanical evaluation with different parameters. The parameters that were used for evaluation were nature, odour, colour, taste, size, shape.

Authentication of Plant Material

The collected plant material were identified by Botanist, Department of Botany. A specimen file was also prepared and submitted in the department.

Soxhelt Apparatus

The shade dried whole plants material of *Butea Monosperma* were reduced to coarse powder and around 200 g of powdered plant material was subjected to successively hot continuous extraction (Soxhlet extractor) with petroleum ether (only for the purpose of defatting), chloroform and Ethanol. Each time before extracting with the next solvent, the powdered material was dried in hot air oven below 50 °C. Finally, the marc was macerated with distilled water and few drops of chloroform was added as a preservative for more than 24 hours to obtain the aqueous extract. Each extract was then distilled to dryness under reduced pressure using Buchi Rota evaporator. The extract obtained with each solvent was weighed and its percentage in terms of the air-dried weight of the plant material was calculated. And also the colour and consistency of the extract was noted.

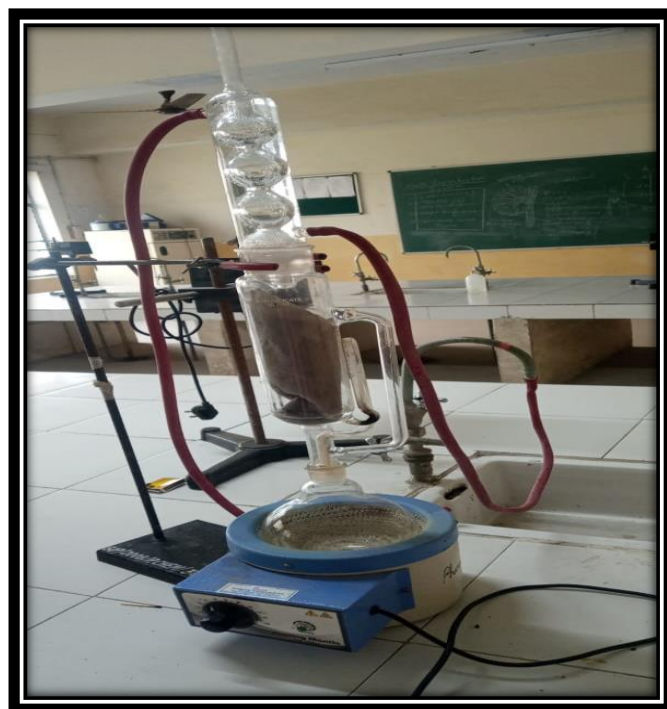


Figure 1 Soxhelt Apparatus

The % Yield in different solvents plant extracts were calculated by using the following formula.

$$\% \text{ Yield} = [\text{Net weight of powder in gram after extraction} / \text{Total weight of powder in gram taken for extraction}] \times 100$$

Extractive Values:

Determination of water-soluble extractive value:

Procedure: About 5 g of the powdered drug was weighed in a weighing bottle and transferred to a dry 250 ml conical flask. A 100 ml graduated flask was filled to the delivery mark with water. The weighing bottle was washed and the washings were poured out together with the remainder of the solvent into the conical flask. The flask was corked and set aside for 24 h, shaking frequently (Maceration) and filtered into a 50 ml cylinder. When sufficient filtrate had been collected, 25 ml of the filtrate was transferred to a weighed, thin porcelain dish, as used for the ash values determinations. It was then evaporated to dryness on a water-bath and the drying was then completed in an oven at 100 °C. Further it was cooled in a desiccator and weighed. The percentage w/w of extractive

with reference to the air-dried drug was calculated.

Table 1 Calculation of water-soluble extractive value

25 ml of water extract gives	“x” g of residue
100 ml of methanolic extract gives	“4x” g of residue
5 g of air-dried drug gives	4x g of water Soluble residue
100 g of air-dried drug gives	80x g of methanolic Soluble residue
Methanol-soluble Extractive Value of the sample	80x %

Moisture content (loss on drying):

Procedure: About 1.5 g of the powdered drug was taken in porcelain dish and dried in an

oven at 105 °C and allowed to cool in a desiccator. The loss in weight was recorded as moisture content.

Table 2 Calculation of Moisture content (loss on drying)

Weight of powdered drug + china dish	“x” g
Weight of powdered drug + china dish	“y” g
(After 2 h at 105 °C in the oven)	
Loss in weight	Z = X-Y g

Ash Content:

Determination of Total Ash Value:

Procedure: A tarred silica crucible was weighed and ignited. About 2 g of the powdered drug was weighed into the crucible. The crucible was supported on a pipe-clay triangle placed on a ring of retort stand. It was heated with a burner, using a flame about 2 cm high and supporting

the dish about 7 cm above the flame, it was heated till vapors almost ceased to be evolved; the crucible was lowered and heated more strongly until all the carbon was burnt off and it was allowed to cool in a desiccator. Weight of ash and calculation of the percentage of total ash with reference to the air-dried sample of the crude drug was performed.

Table 3 Determination of Total Ash Value

Weight of the empty dish	“x” g
Weight of the drug taken	“y” g
Weight of the dish + ash	“z” g
(After complete incineration)	
“y” g of the crude drug gives	(Z - X) g of the ash
100 g of the crude gives	[100 × (z - x) g of the ash]/y
Total ash value of the sample	[100 × (z - x)]/y %

Determination of Acid-insoluble Ash Value:

Procedure: As per the steps mentioned in the procedure for determination of total ash value of crude drug, the process was proceeded. Further: Using 25 ml. of dilute hydrochloric acid, the ash from the dish used for total ash was washed into 100 ml beaker. A wire gauge over a Bunsen burner was placed and boiled for five

minutes. Then it was filtered through an ‘ashless’ filter paper; the residue was washed twice with hot water. The filter paper and residue were put together into the crucible; heated gently until vapors ceased to evolve and then more strongly until all carbon had been removed. Weighing of the residue and calculation of acidinsoluble ash of the crude drug with reference to the air-dried sample of the crude drug was done.

Table 4 Determination of Acid-insoluble Ash Value

Weight of the empty crucible	“x” g
Weight of the drug taken	“y” g
Weight of the crude drug+ ash	“z” g
(After complete incineration)	
Weight of the residue	“a” g
(Acid-insoluble ash)	
“y” g of the crude drug gives	‘a’ g of acid - insoluble ash
100 g of the air-dried drug gives	{[100 × (a) g of the acid] - insoluble ash}/y
Acid-insoluble ash value of the sample	[100 × (a)]/y %

Determination of Water – soluble Ash Value:

Procedure: The Procedure was same as above wherein water was used, in place of dilute hydrochloric acid.

Diuretic activity:

Animals were divided in total of four groups (n = 3 in each group). All animals were deprived of food and water 18 h prior to the experiment. On the day of experiment, the dosing were scheduled as follows:

- **Group I: Normal saline**
- **Group II: Furosemide 10 mg/kg p.o. as reference diuretic drug.**
- **Group III: Ethanol extract 100 mg/kg p.o.**

- **Group IV: Ethanol extract 200 mg/kg p.o.**

Immediately after the dosing, animals were placed in metabolic cages and urine was collected up to 5 h after dosing. Room temperature was maintained up to 25 ± 0.5 °C.

During this period no water or food was made available to the animals. Diuretic activity was assessed by measuring the following parameters:

- **Total urine volume**
- **Urine concentration of Na⁺, K⁺ and Cl⁻.**

Results

Extractive Values

Table 5 Extractive Values

Solvent used	Amount (ml)	Temperature(degree centigrade)	Power drug required	%yield
Petroleum ether	500ml	40	100gm	3.2
Ethyl acetate	500ml	55	100gm	4.5
Ethanol	500ml	60	200gm	4.8
Aqueous	500ml	80	75gm	3.8

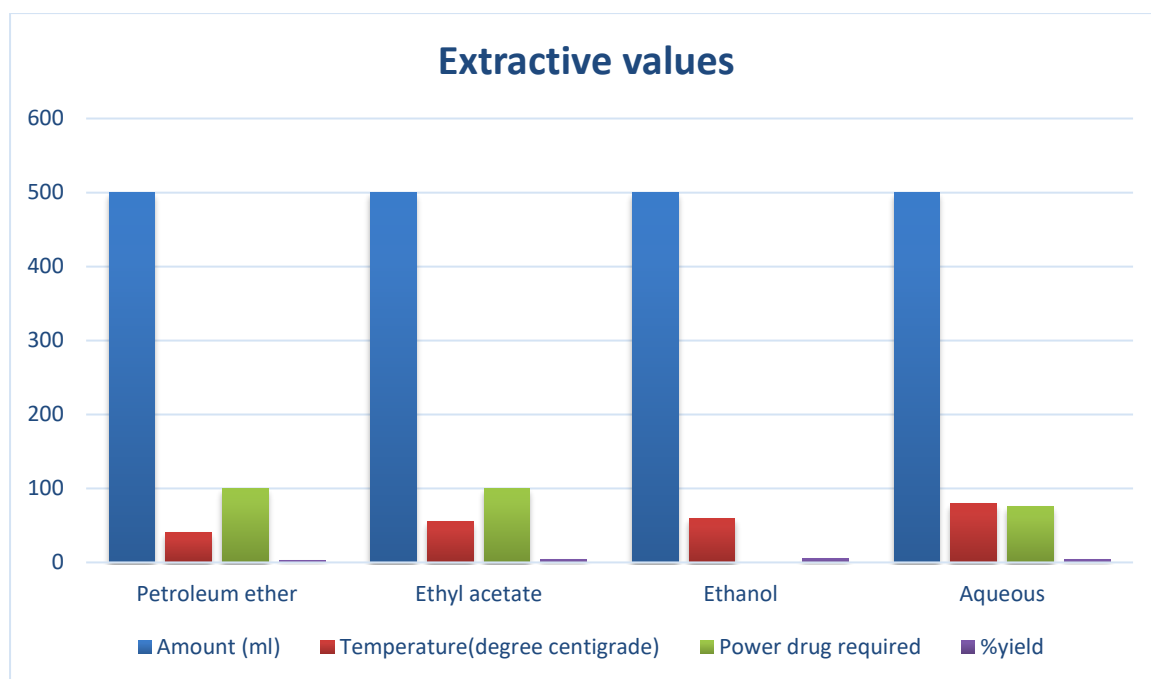


Figure 2 Graph of extractive values



Figure 3 Extractive in different solvent used:

Loss on Drying And Foreign Organic Matter

Table 6 Loss on Drying And Foreign Organic Matter

Crude drugs	Loss on drying (%w/w)*	Foreign matter(%w)*
Butea monosperma Gum Extract	14.36	2.25

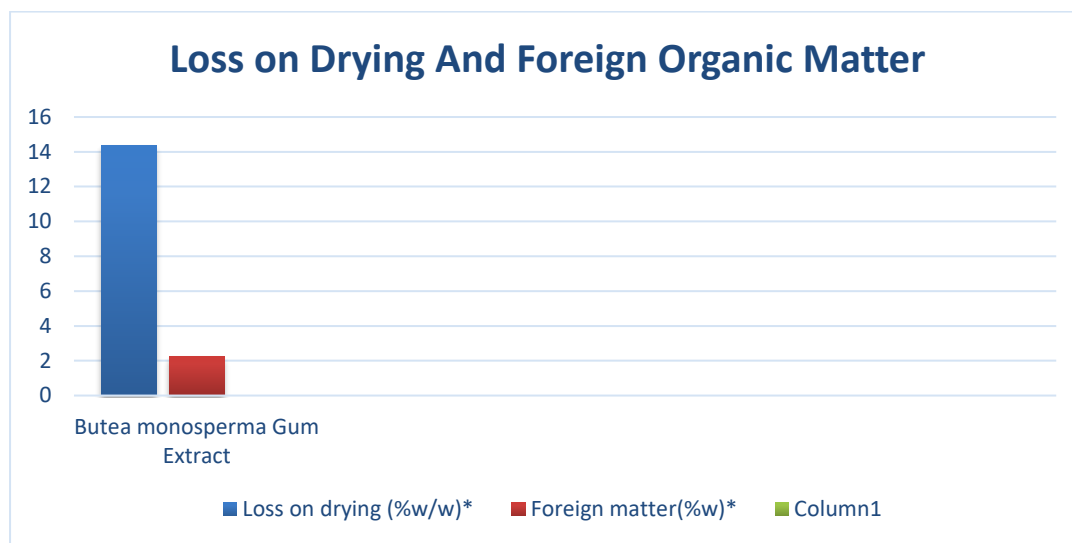


Figure 4 Graph of Loss on Drying And Foreign Organic Matter

Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Table 7 Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Crude drugs	Total ash value % w/w	Water soluble ash % w/w	Acid insoluble ash value % w/w
Butea monosperma Gum extract	7.5	9.6	4.7

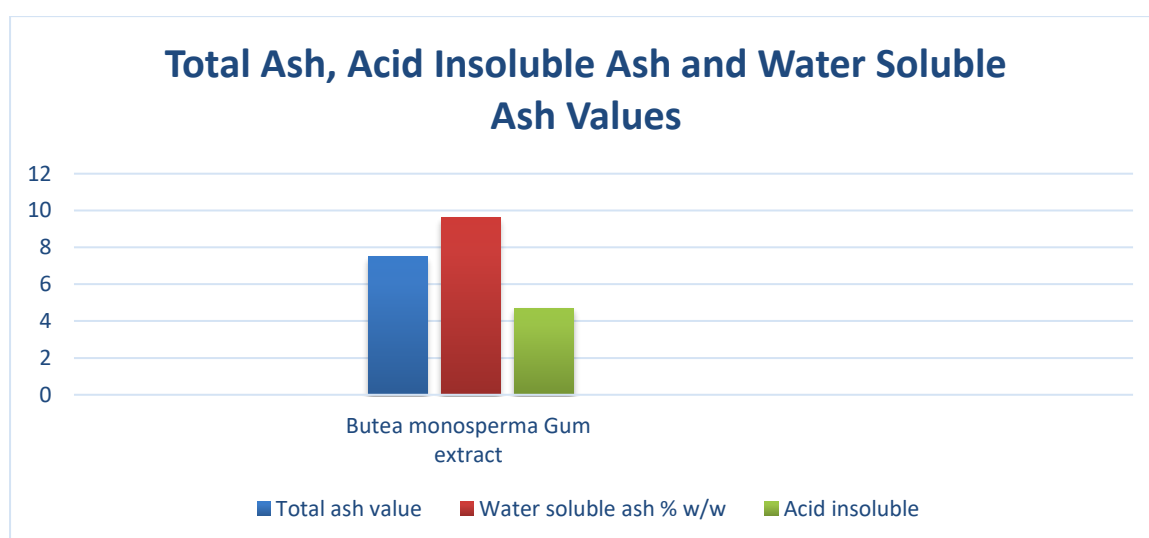


Figure 5 Graph of Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Phytochemistry of Butea monosperma Gum extract:

Phytochemical analysis of the powdered extract (prepared using Soxhlet extraction) was done in accordance with standard methods. The

methods are described briefly in the following sections. All analyses were done using different solvents:

Table 8 Phytochemical analysis

S.No	Active Constituent	Ethenol extract	Petroleum Ether	Water	Ethyl acetate
1	Alkaloid	-	-	+	-
2	Saponin	-	-	-	-
3	Titrpene	+			
4	Glycoside	+	-	-	-
5	Protein	-	-	+	-
6	Carbohydrate	-			
7	Phenolic	+			
8	Tanin	+	-	-	-
9	Sterol	-	-	-	-

Where:

[-] Means Absent

[+] Means Present

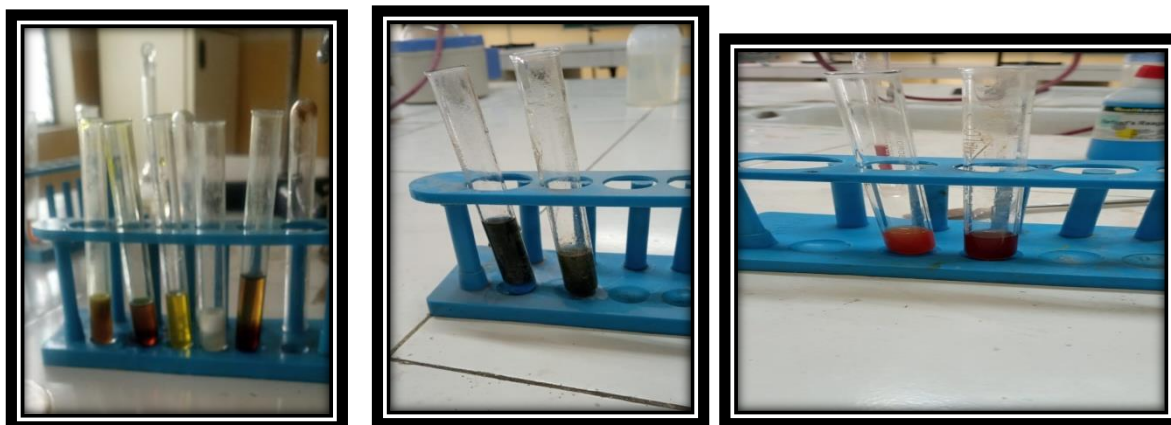


Figure 6 Solutions of Identification Tests

Physicochemical Evaluation of Formulation

Standard procedures were used to investigate various physicochemical assessment parameters of formulations of ointments, and the findings are displayed in the table below.

Table 9 Physicochemical Evaluation

S.no	Parameters	Observation of formulation
1.	Colour	White
2.	Odour	Characteristic
3.	Consistency	Homogenous
4.	Ph	~ 6.5

Urinary parameters

The concentration of Na⁺ and K⁺ ions in the fresh urine samples was estimated using calibrated flame photometer and was expressed in parts per million (ppm). Before estimating the electrolyte levels, the samples were filtered to remove debris and shedding. A calibrated pH meter was used to measure the pH of the fresh urine samples.

Calculation of diuretic index, Lipschitz value, saliuretic index and Na⁺ / K⁺ ratio The following formulas were used for the calculation of different urinary parameters:

Diuretic index = Mean urine volume of the test group / Mean urine volume of the control group

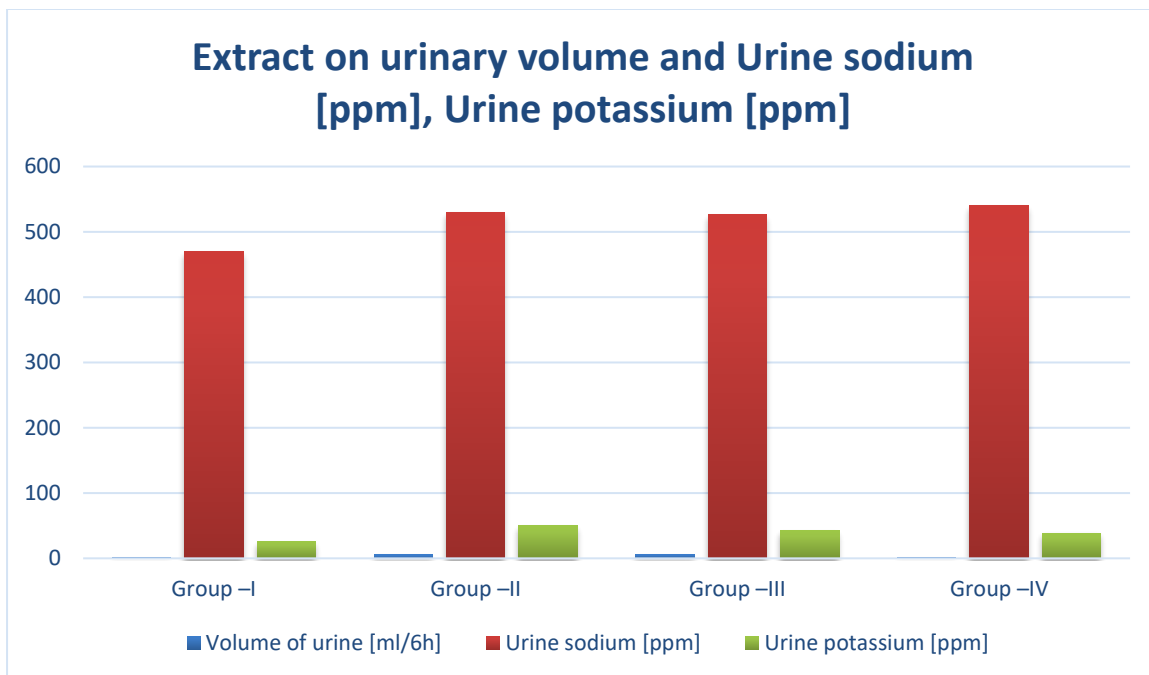
Lipschitz value = Mean urine volume of the test group / Mean urine volume of the reference group

Saliuretic index = Concentration of electrolyte in urine of the test group / Concentration of electrolyte in urine of the control group.

Na⁺ / K⁺ ratio = Concentration of Na⁺ in urine of a group / Concentration of K⁺ in urine of the same group

Table 10 Effect of Butea monosperma Gum extract on urinary volume

S.no	Group	Volume of urine [ml/6h]	Urine sodium [ppm]	Urine potassium [ppm]
1.	Group –I	1.5	470	25.5
2.	Group –II	5.5	530	50.3
3.	Group –III	6.0	526	42.45
4.	Group –IV	1.5	540	38.75

**Figure 7 Graph of extract on urinary volume and Urine sodium [ppm], Urine potassium [ppm]****Table 11 Effect of Butea monosperma Gum extract on Lipschitz value, Diuretic index, Saliuretic index**

S.no	Group	Lipschitz value	Diuretic index	Saliuretic index	
				Na	K
1.	Group –I	-	1.5	-	-

2.	Group –II	-	5.5	1.16	1.17
3.	Group –III	0.25	2.1	1.08	1.5
4.	Group –IV	0.42	4.2	1.1	1.82

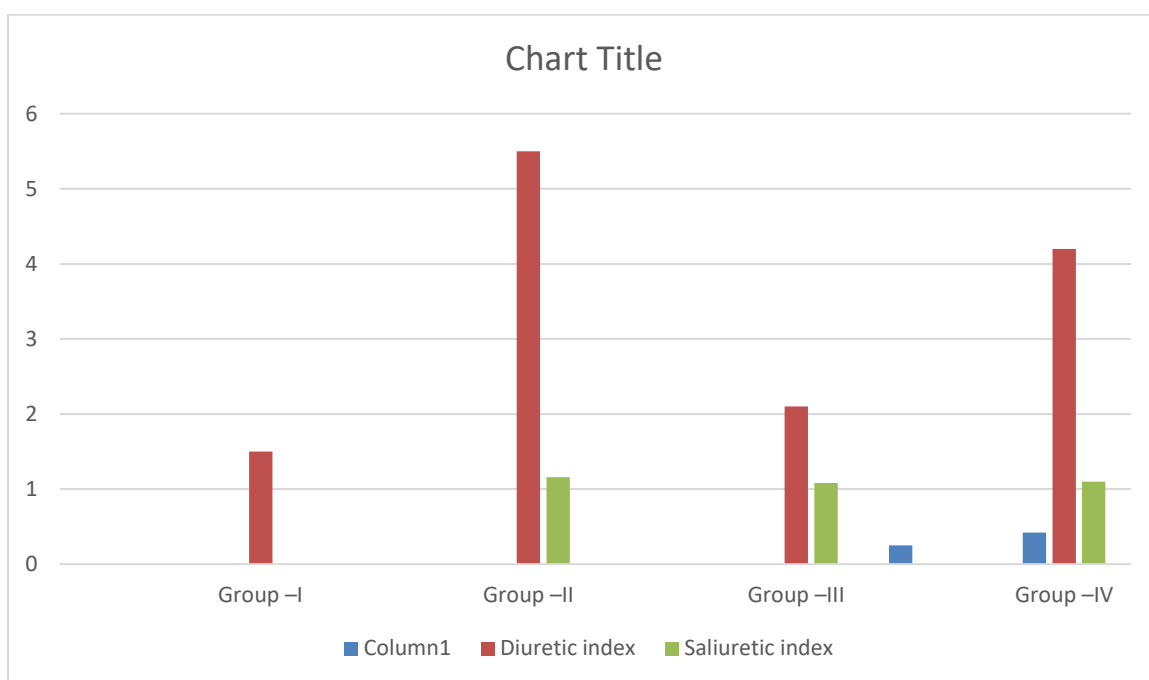


Table 8 Effect of Butea monosperma Gum extract on pH, Na⁺/K⁺

S.no	Group	Na ⁺ /K ⁺
		pH
1.	Group –I	17.65
		6.8
2.	Group –II	12.21
		7.9
3.	Group –III	11.12
		6.5

4. Group –IV

10.87

6.9

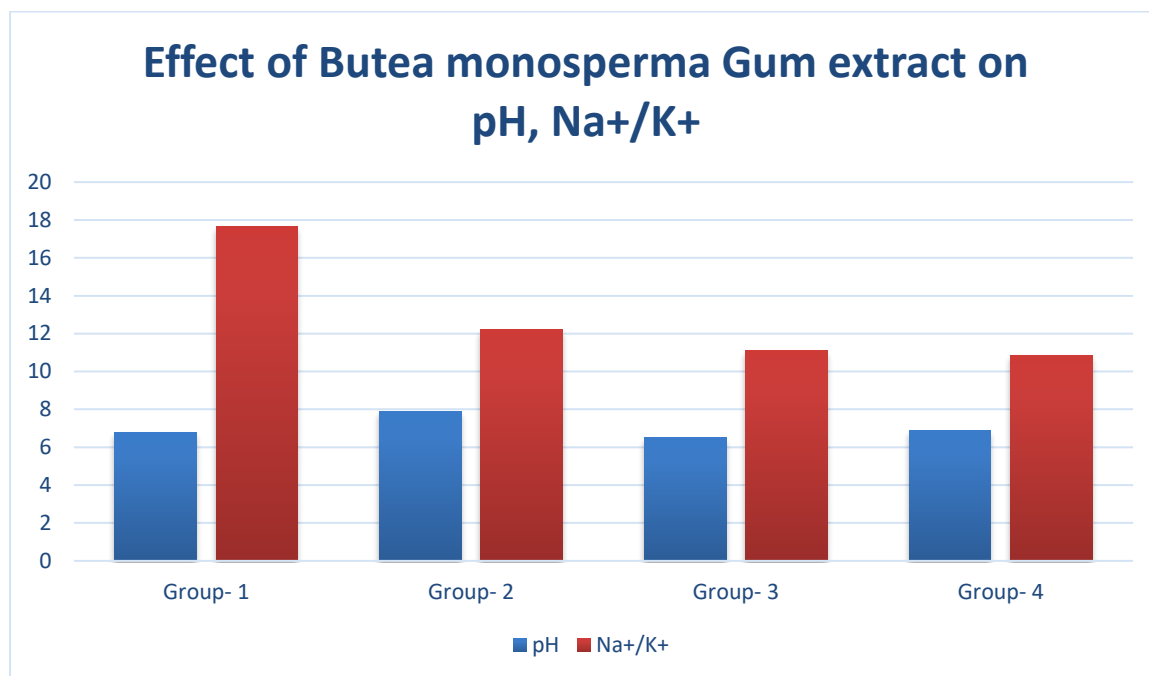


Figure 9 Graph showing effect of Butea monosperma Gum extract on pH, Na+/K+

Conclusion

All herbal plants are used as natural medicines to restore the normal physiological system after it has been altered by outside organisms or by any bodily dysfunction. All herbal plants are used as natural medicines to restore the normal physiological system after it has been altered by outside organisms or by any bodily dysfunction. The preliminary phytochemical screening of the ethanolic fraction showed the presence of Titrpene, Glycoside, tannins, Phenolic and flavonoids. The isolated polymer was found to be sweet and Reddish-brown in colour with a Pleasant odour. The Butea Monosperma Gum extract fracture and texture were found to be harsh and uneven. It was discovered to have Extractive Values in different solvents Petroleum ether, Ethyl acetate, Ethanol, Aqueous] were found to be 3.2%, 4.5%, 4.8%, 3.8% respectively for which Power drug required was gm. Ash values were

also calculated to characterize the Extract. Total ash, acid insoluble ash and water-soluble ash were found to be 7.5%, 4.7% and 9.6% respectively. The i.p. administration of Butea monosperma gum increased the urinary flow in a dose-dependent manner. When compared with the control group, 2.8 and 3.5 fold increase in urine output was observed in the group III and IV respectively. The diuretic index values of the test groups (group III and IV) were 2.5 and 3.6, respectively, which indicated a good diuretic activity at the dose of 50 mg/kg. The Lipschitz values demonstrated that, at the doses of Butea monosperma gum showed 25% and 42% of diuretic activity. Further studies are required to assess the medicinal value of Butea monosperma gum as a potential diuretic agent.

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Conflict of interest

The Authors declare no conflict of interest.

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