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Dose-Dependent Diuretic Potential Evaluation of *Abutilon Pannosum* Leaf Extract Employing The Lipschitz Model in Wistar Albino Rats

Vidya V. Nalawade [1]*, Pramodkumar J. Shirote[2]

[1]Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India.416416.

[2]Department of Pharmaceutical Chemistry, Dr. Bapuji Salunkhe Institute of Pharmacy, Miraj, Maharashtra, India 416416.

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ABSTRACT

Background: *Abutilon pannosum*, a medicinally pivotal species from the diverse Malvaceae family, has been traditionally leveraged for various therapeutic facets but remains underexplored for its diuretic potential. Objectives: Given the growing interest in plant-based alternatives to synthetic diuretics, this investigation aimed to assess the diuretic activity of *A. pannosum* leaf extract in vivo. Methods: Leaves of *A. pannosum* were subjected to Soxhlet extraction employing methanol as solvent to obtain a concentrated plant extract rich in bioactive constituents. Preliminary phytochemical screening was performed. Pharmacological investigation entailed the assessment of key urinary parameters, encompassing total urine volume, pH, and electrolyte excretion (Na^+, K^+, Cl^-). Diuretic efficacy was quantified employing the Lipschitz value, while saluretic and natriuretic indices were computed to determine the extent of overall electrolyte and sodium elimination. Wistar albino rats were divided into five groups (n=6): control, standard (furosemide 10 mg/kg), and three test groups. Results: Preliminary phytochemical screening inferred the presence of secondary metabolites, viz., alkaloids, flavonoids, tannins, and saponins, compounds commonly associated with diuretic potential. The extract of *A. pannosum* exhibited significant diuretic activity in a dose- dependent manner. The highest dose (450 mg/kg) exhibited a marked increase in urine output, elevated pH, and boosted electrolyte excretion. A maximum Lipschitz value of 3.42 was recorded, reflecting robust diuretic activity similar to the benchmark compound. Conclusion: The findings suggest that *Abutilon pannosum* possesses promising dose- dependent diuretic attribute, owing to the presence of active phytoconstituents. These results support its potential as a natural alternative for diuretic therapy.

1. INTRODUCTION:

India has a long-standing heritage in traditional medicine, with systems such as Ayurveda, Siddha, and Unani utilizing a vast diversity of medicinal plants for therapeutic purposes.[1,2] The country's rich biodiversity, encompassing over 45,000 plant species—of which approximately 7,000 possess recognized medicinal properties—offers significant potential for pharmacological exploration.[3,4,5] Among these, numerous plants have been employed in the management of renal and urinary disorders, highlighting the continuing relevance of herbal remedies in maintaining fluid and electrolyte balance.[6,7]

Diuretics are pharmacological entities that enhance the excretion of excess water and electrolytes through urine, thereby exhibiting a pivotal role in the mitigation of conditions such as hypertension, congestive heart failure, renal ailments, and

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edema.[23,24] While synthetic diuretics, viz., furosemide [25,26] and hydrochlorothiazide,[27,28] are extensively used, their long-term use is often associated with adverse effects, including electrolyte imbalance, dehydration, and renal toxicity. These limitations have allured interest in the exploration of plant-based alternatives that may offer diuretic benefits with fewer side effects.[29] Herbal medicines, owing to their phytoconstituent [30,31] diversity and relatively lower toxicity, have been historically leveraged in various traditional systems of medicine for their diuretic potential. Several plant-derived compounds such as flavonoids, saponins, alkaloids, and tannins are recognized to modulate renal function and influence fluid and electrolyte balance.[32,33] However, systematic scientific validation of these traditional claims remains limited for many medicinal plants.[34,35]

Abutilon pannosum[36] (Family: Malvaceae) [37,38] is herbaceous to sub-shrubby plant widely distributed across tropical and subtropical regions.[39] Traditionally, different parts of this plant have been employed in folk medicine to mitigate inflammatory conditions, urinary disorders, and general weakness.[40,41] Despite its ethnomedicinal relevance, there is a scarcity of pharmacological data regarding its diuretic potential.

Table 1: Taxonomic Classification [42]

Kingdom	Plantae
Subkingdom	Viridaeplantae (green plants)
Infra kingdom	Streptophyta (land plants)
Division	Tracheophyta (vascular plants)
Subdivision	Spermatophytina (seed plants)
Infra division	Angiospermae (flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Dilleniidae
Super order	Rosanae
Order	Malvales
Family	Malvaceae (mallows)
Subfamily	Malvoideae
tribe	Abutilieae
Genus	*Abutilon Mill* (Indian mallow)

Thisgap in knowledge underscores the need for systematic evaluation. This study was therefore designed to scientifically evaluate the diuretic activity[43] of *Abutilon pannosum* leaf extract in a dose-dependent manner employing the well-established Lipschitz model. The study sought to investigate the impact of the extract on key urinary parameters such as urine volume, pH, and electrolyte excretion (Na^+, K^+, Cl^-), along with the calculation of Lipschitz value, saluretic index, and natriuretic index to quantify diuretic efficacy in comparison to a standard diuretic drug. The findings from this study could contribute to the pharmacological validation of *A. pannosum* as a potential natural diuretic agent.

2. MATERIALS AND METHODS

2.1 Chemicals: Methanol was obtained from (Loba chemie Pvt. Ltd), normal saline (Amanta Ltd), frusemide (Sanofi India Pvt. Ltd.). All the chemicals used were of analytical grade.

2.1.2 Instruments: Flame Photometer (Equiptronics FPM 128), Digital pH meter (Equiptronics EQ 610).

2.2 Collection and Preparation of Plant Material and Extract [44,45]

The research utilized fresh foliage from *Abutilon pannosum*, the leaves of the plants were collected in October to December from local area of Sangli, Maharashtra. Plants were authenticated under the aegis of Botanical Survey of India, Pune. (BSI/WRC/Iden.Cer./2023/1207230004649) as well as herbarium specimens VVN: 01 was deposited at same. Plants leaves were parted from stems as well as washed, shade dried at room temperature until it becomes utterly dry. After drying leaves were turned to powder by virtue of pulverization through electric grinding machine. The powder was defatted by soaking it in petroleum ether overnight. Coarsely powdered shade-dried leaves (1000 g) underwent extraction by virtue of Soxhlet employing solvent methanol according to the methodology described in the Indian Pharmacopoeia 1996. At end of each respective extraction, extract was filtered by virtue of Whatman number 1. Filtrate was concentrated below reduced pressure in vacuum using a rotary evaporator. Extracts were preserved in airtight pack at 4 °C for following investigation.

2.3 Pharmacognostical evaluation:

Standardization of plant material: Assessment of physicochemical parameters were carried out for dried *Abutilon pannosum* powder for percentage yield, moisture content, total Ash value, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive. Phytochemical screening was also performed. The plant extract was subjected to qualitative tests to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, and sterols. [46,47]

2.4 Maintenance of animals and their feeding:

Female albino wistar rats (180-210 g) were obtained from animal house of Appasaheb Birnale College of Pharmacy, Sangli. Rats were housed in standard polypropylene cages (6 animals per cage) furthermore were maintained under standard hygienic conditions at 25-28 °C with 12 hr light/dark cycle as well as provided with standard pellet. Animals were given unlimited access to food and drink. The animals were cared for also maintained as per the approved guidelines of the "Committee for the Purpose of Control and

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Supervision of Experiments on Animals" (CPCSEA, India) and the protocol was approved by the Institutional animal Ethical Committee, Appasaheb Birnale College of Pharmacy, Sangli. (BSI/WRC/Iden.Cer./2023/1207230004649).[48]

2.5 Preparation of doses and treatments

Normal saline 25 ml/kg orally was given to control group. Standard Furosemide at a dose of 10 mg/kg body weight was given orally. 3 test groups received suspension of extract of *Abutilon pannosum* in normal saline at a dose of 150 mg/kg. 300 mg/kg, 450 mg/kg orally respectively.

2.6 Acute toxicity study

The acute oral toxicity study was conducted in accordance with the OECD revised draft guidelines, following the 423 method.[49] Non-pregnant female rats were utilized for the study. Animals were weighed and were kept on overnight fast, after which they were divided into groups of three. The animals were then administered increasing oral doses of methanolic *Abutilon pannosum* extract, ranging from 200 to 5,000 mg/kg of body weight. This approach allowed for a thorough assessment of the extract's effects across a wide concentration range. Following extract administration, the rats were monitored for behavioural changes over a three- hour period. Subsequent observations were conducted every half hour for the next five hours. Mortality rates were then assessed daily for three days.

2.7 Evaluation of diuretic potential

Lipschitz model: Click or tap here to enter text.
Treatment groups: Thirty female Albino wistar rats, each weighing 180-210 g, were randomly assigned to one of five groups (six rats per group) for pharmacological activity evaluation.

A 25 ml/kg dosage of normal saline was used to hydrate the rats before the test dose was administered. The rats' urinary bladders were emptied by applying gentle pressure to the pelvic area and using a downward motion on their tails. The animals were fasted overnight prior to the experiment but had unrestricted access to water. They were then placed in metabolic cages for a two-hour acclimation period.
Group 1: served as control and received normal saline 25 ml/kg body weight.
Group 2: served as standard and received Frusemide 10 mg/kg body weight orally.
Group 3: animals were administered methanolic extract of *A. pannosum* (MEAP) at dose of 150 mg/kg body weight.
Group 4: animals were administered MEAP at dose of 300 mg/kg body weight.
Group 5: animals were administered MEAP at dose of 450 mg/kg body weight.
All the five groups received the same treatment continuously for seven days. Urine was collected and various parameters related to electrolytes were measured, including concentrations of sodium, potassium, and chloride, as well as urine volume, pH, diuretic index, Lipschitz value, and indices for saluretic and natriuretic effects. Following formula were used for computing [50]

$$Diuretic\ index = \frac{Mean\ urine\ volume\ of\ test\ group}{Mean\ urine\ voulme\ of\ control\ group} \quad \ldots\ldots\ldots\ldots \text{Equation 1}$$

$$Lipschitz\ value = \frac{Mean\ urine\ volume\ of\ test\ group}{Mean\ urine\ voulme\ of\ standard\ group} \quad \ldots\ldots\ldots\ldots \text{Equation 2}$$

$$Natriuretic\ index = \frac{Concentration\ of\ Sodium\ excretion}{Concentration\ of \text{potassium excretion (same group)}} \quad \ldots\ldots\ldots\ldots \text{Equation 3}$$

2.8 Statistical analysis:

The data are expressed as mean ± standard error of mean (mean ± SEM). Statistical analysis was performed by one way (ANOVA), followed by Dunnet's multiple comparison test to determine the significance between control and treatment group. Statistical significance was defined as $p<0.05$.

3 RESULT

3.1. Phytochemical screening

The presence of phenols, flavonoids, alkaloids, saponins, triterpenoids, steroids, carbohydrate, glycoside, and alkaloid were observed in methanolic extract.

3.2 Pharmacognostical evaluation Standardization of plant material:

Physicochemical parameters of *Abutilon pannosum* were evaluated to assess its quality, purity, and suitability for pharmaceutical use and results are as fallows.

Table 2: Pharmacognostical evaluation of *A. pannosum*

Sr. No.	Physicochemical parameters	*Abutilon pannosum* % w/v
1	Alcohol soluble extractive	5.2
2	Water soluble extractive	2.8
3	Total ash	6.15
4	Acid insoluble ash	2.03
5	Water soluble ash	3.15
6	Moisture Content	2.66

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3.3 Acute toxicity study:

The acute toxicity study demonstrated that the methanolic extract was safe at a dose of 4000 mg/kg body weight, with all animals remaining alive active and healthy throughout the observation period. Therefore, based on the safety profile, doses of 150, 300, and 450 mg/kg were selected for the diuretic study to ensure a wide safety margin.

3.4 Diuretic parameter:

3.4.1 Electrolyte excretion:

Electrolyte excretion in urine was measured 24 hours post-administration, and the results are summarized in Table 2. Compared to the control group, sodium excretion for *Abutilon pannosum* methanolic leaf extract (APME) was observed at 110.8±0.5 for 150 mg/kg,

124.8±0.6 for 300 mg/kg, and 140.5±0.3 for 450 mg/kg. Potassium excretion values at doses of 150, 300, and 450 mg/kg were 87.27±0.4, 102.2±0.27, and 112.2±0.3, respectively. Similarly, chloride excretion at these doses was 84.53±0.3, 114.8±0.4, and 120.4±0.2, respectively.

Table 3: Urinary electrolyte excretion effect of *Abutilon pannosum*

Group	Na+ mmol/eq.	K+ mmol/eq	Cl^- mmol/eq.
Control (Normal Saline)	105.8±0.1	60.61±0.3	76.19±0.2
Standard (Frusemide)	112.9±0.3	102.3±0.5	110.4±0.1
APME 150	110.8±0.5	87.27±0.4	84.53±0.3
APME 300	124.8±0.6	102.2±0.27	114.8±0.4
APME 450	140.5±0.3	112.2±0.3	120.4±0.2

All the data were expressed as (Mean ± SEM), n=6, P <0.05 statistically significant as when compared to control.

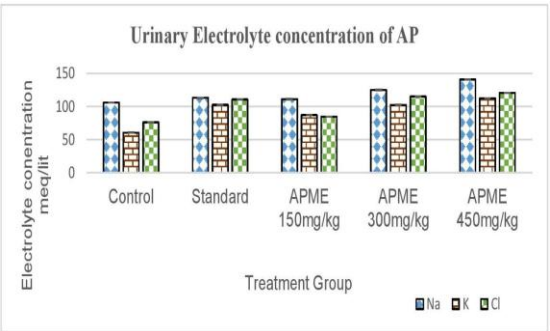

Fig. 1: Urinary electrolyte excretion effect of *Abutilon pannosum*

3.4.2 Urine volume and pH:

After 24 hours, the urine volumes were recorded for the control, standard, and test groups. The administration of APME at doses of 150 mg, 300 mg, and 450 mg resulted in significant increase in urine volumes as 4.10 ml, 8.20 ml, and 14.1 ml, respectively. When test doses of APME was administered, the pH values were 7.6, 7.9, and 8.2. As the doses increases the pH goes on alkaline side. In contrast, the standard pH is 8.27 and control is 6.5.

Table 4: Urine volume and pH effect of *Abutilon pannosum*

Group	Urine Volume	pH
Control (Normal Saline	1.61	6.5
Standard (Frusemide)	4.12	8.27
APME 150	4.10	7.6
APME 300	8.20	7.9
APME 450	14.1	8.2

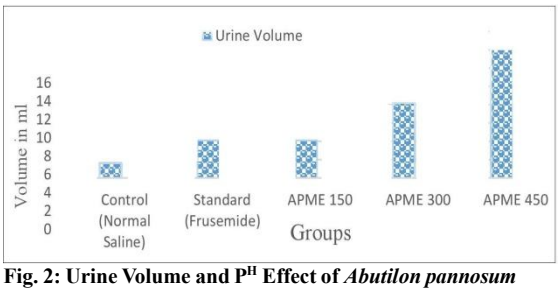

Fig. 2: Urine Volume and P^H Effect of *Abutilon pannosum*

3.4.3 Diuretic action, natriuretic, saluretic action

The diuretic potency of the agent is assessed employing Lipschitz value. APME at dose of 450 mg/kg body weight demonstrates strong diuretic activity, with a Lipschitz value of 3.42. 300 mg/kg dose of APME continued to show a positive diuretic effect, achieving a Lipschitz value of 1.

In terms of natriuretic action, a natriuretic index greater than 1 is considered indicative of a favourable diuretic effect, particularly of the potassium-sparing type. In the present study, APME at the highest tested dose (450 mg/kg) demonstrated a natriuretic index of 1.25, suggesting a mild but favourable natriuretic response with potential potassium-sparing activity.

Table 5: Diuretic, Lipschitz, Saluretic, Natriuretic effect of *Abutilon pannosum*

Group	Diuretic effect	Lipschitz value	Saluretic Index
Control (Normal Saline)	-	0.39	181.99
Standard (Frusemide)	2.55	-	223.3
APME 150	2.54	0.99	195.33
APME 300	5.09	1.99	239.6
APME 450	8.75	3.42	260.9

4 DISCUSSION:

Diuretic potential of the leaves of plant *Abutilon pannosum* was evaluated in this study. Plants were first discovered to contain a variety of bioactive substances, including steroids, glycosides, alkaloids, and flavonoids. Various investigations suggested that these chemicals are responsible for diuretic activity by different approaches. Electrolyte excretion, including sodium, potassium, and chloride, is the most direct marker of diuretic action. APME exhibits saluretic qualities as evidenced by its promotion of a notable rise in the excretion of certain electrolytes, specifically sodium and chloride. This effect is strongly linked to alkaloids and flavonoids, which have been demonstrated to increase the excretion of sodium and chloride by blocking their reabsorption in the renal tubules. In the nephron, most likely in the loop of Henle or the distal tubules, where handling of sodium, chloride, and potassium is crucial, APME may interfere with ion transport processes, as suggested by the diuretic impact through saluretic action. Flavonoids, which are well-known for controlling ion channels and transporters, may have something to do with this. On the other hand, by promoting blood flow and enhancing filtration efficiency, alkaloids may help to enhance renal excretory function more broadly. The capacity of APME to enhance urine volume serves as a direct measure of its diuretic effectiveness, with the highest dosages having the most noticeable effects. This shows that the concentration of active phytoconstituents in the extract corresponds with the dose-dependent nature of the diuretic action. The efficacy of APME in increasing urine production was confirmed by the Lipschitz value, which is a measure of diuretic potency. The diuretic and saluretic properties of APME are supported by the combined action of these phytochemicals, which makes it a viable option for natural diuretic treatments. Deeper understanding of these particular molecules' contributions to the effectiveness of diuretics might be possible with additional study aimed at isolating and characterizing them.

5 CONCLUSION:

This study highlights of substantial diuretic potential of *Abutilon pannosum* methanolic leaf extract. Extract demonstrates the presence of bioactive chemicals in APME. The extract notably ameliorated urine output and promoted electrolyte excretion, especially sodium and chloride ions, in a dose-dependent manner furthermore it could be attributed to phytochemicals that greatly boosted urine volume in a dose-dependent manner and encouraged greater excretion of electrolytes, particularly salts and chloride. The Lipschitz value further confirmed APME's diuretic potency compared to standard drug signifying the presence of active phytoconstituents attributing to renal modulation and fluid balance. Owing to natural origin and satisfying results it could be employed as alternative to synthetic diuretic. However, further exhaustive scholarly research is needed to identify and elucidate the roles of its various active components.

Authors contribution: Vidya V. Nalawade: contributed to formal analysis, experimental and writing original draft; **Pramodkumar J. Shirote:** contributed to supervision, review and editing.

Conflict of Interest: The authors declare no conflict of interest.

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