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Research Article

DOSE-DEPENDENT DIURETIC POTENTIAL EVALUATION OF *ABUTILON PANNOSUM* LEAF EXTRACT EMPLOYING THE LIPSCHITZ MODEL IN WISTAR ALBINO RATS

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ABSTRACT

Objectives: Given the growing interest in plant-based alternatives to synthetic diuretics, this investigation aimed to assess the diuretic activity of *Abutilon 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, namely, 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 *A. 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.

Keywords: *Abutilon pannosum*, Herbal diuretic potential, Lipschitz model, Natriuretic index, Saluretic index.

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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 [3-5]. 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 edema [8,9]. While synthetic diuretics, namely, furosemide [10,11] and hydrochlorothiazide [12,13], 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 [14]. Herbal medicines, owing to their phytoconstituent [15,16] 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 [17,18]. However, systematic scientific validation of these traditional claims remains limited for many medicinal plants [19,20].

Abutilon pannosum [21] (Family: Malvaceae) [22,23] is a herbaceous to sub-shrubby plant widely distributed across tropical and subtropical regions [24]. Conventionally, different parts of this plant have been employed in folk medicine to mitigate inflammatory conditions, urinary disorders, and general weakness [25,26]. Taxanomical classification of the studied plant is represented in Table 1. Despite its ethnomedicinal relevance, pharmacological data are scarce regarding its diuretic potential.

This gap in knowledge underscores the need for systematic evaluation. This study was therefore designed to scientifically evaluate the diuretic activity [27] of *A. 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.

METHODS

Chemicals

Methanol was obtained from (Lobachemie Pvt. Ltd), normal saline (Amanta Ltd.), and frusemide (Sanofi India Pvt. Ltd.). All the chemicals used were of analytical grade.

Instruments

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

Collection and preparation of plant material and extract [28-30]

The research utilized fresh foliage from *A. pannosum*; the leaves of the plants were collected in October to December from the local area of Sangli, Maharashtra. Plants were authenticated under the aegis of the Botanical Survey of India, Pune. (BSI/WRC/Iden. Cer./2023/1207230004649) as well as herbarium specimens VVN: 01 were deposited at the 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 into powder by virtue of pulverization through an electric grinding machine. The powdered material was defatted using a Soxhlet apparatus for 24 h before subsequent extraction with methanol. At the end of each respective extraction, the extract was filtered by virtue of Whatman number 1. Filtrate was concentrated below reduced pressure in a vacuum using a rotary evaporator. Extracts were preserved in an airtight pack at 4°C for the following investigation.

Pharmacognostical evaluation

Standardization of plant material

The assessment of physicochemical parameters was carried out for dried *A. pannosum* powder for percentage yield, moisture content, total Ash value, acid-insoluble ash, water-soluble ash, alcohol soluble extractive, and water-soluble extractive. Phytochemical screening was also performed.

Maintenance of animals and their feeding

Female albino Wistar rats (180–210 g) were obtained from the animal house of Appasaheb Birnale College of Pharmacy, Sangli. Rats were housed in standard polypropylene cages (6 animals per cage) and were maintained under standard hygienic conditions at 25–28°C with a 12 h light/dark cycle, as well as provided with standard pellets. Animals were given unlimited access to food and drink. The animals were also cared for and maintained as per the approved guidelines of the "Committee for the Purpose of Control and 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) [32-34].

Preparation of doses and treatments

Female rats were selected to avoid the potential influence of male sex hormones on diuretic response and for consistency with common practice in preliminary diuretic screening. Normal saline 25 mL/kg orally was given to the control group. Standard Furosemide at a dose of 10 mg/kg body weight was given orally. 3 test groups received suspension of extract of *A. pannosum* in normal saline at a dose of 150 mg/kg. 300 mg/kg, 450 mg/kg orally, respectively [34].

Acute toxicity study

The acute oral toxicity study was conducted in accordance with the Organization for Economic Co-operation and Development revised draft guidelines, following the 423 method [31]. Non-pregnant female rats were utilized for the study. Animals were weighed and kept on an overnight fast, after which they were divided into groups of three. The animals were then administered increasing oral doses of methanolic *A. 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 behavioral changes over a 3-h period. Subsequent observations were conducted every half an hour for the next 5 h. Mortality rates were then assessed daily for 3 days.

Evaluation of diuretic potential

Lipschitz model

Treatment groups: 30 female Albino Wistar rats, each weighing 180–210 g, were randomly assigned to one of the 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 before the experiment, but had unrestricted access to water. They were then placed in metabolic cages for a 2-h acclimation period.

- Group 1: Served as control and received normal saline 25 mL/kg body weight
- Group 2: Served as standard and received furosemide 10 mg/kg body weight orally
- Group 3: Animals were administered the 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 7 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. The following formula was used for computing [50]:

$$Diuretic\ index = \frac{Mean\ urine\ volume\ of\ test\ group}{Mean\ urine\ voulme\ of\ control\ group} \quad (1)$$

$$Lipschitz\ value = \frac{Mean\ urine\ volume\ of\ test\ group}{Mean\ urine\ voulme\ of\ standard\ group} \quad (2)$$

$$Natriuretic\ index = \frac{Concentration\ of\ sodium\ excretion}{Concentration\ of\ potassium\ excretion\ \ (same\ group)} \quad (3)$$

(*Saluretic index = Sum of concentration of sodium excretion and concentration of chloride excretion* [*same group*]) (4)

Statistical analysis

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

RESULTS

Phytochemical screening

The presence of phenols, flavonoids, alkaloids, saponins, triterpenoids, steroids, carbohydrates, glycosides, and alkaloids was observed in the methanolic extract.

Pharmacognostical evaluation

Standardization of plant material

Physicochemical parameters of *A. pannosum* were evaluated to assess its quality, purity, and suitability for pharmaceutical use, and the results are shown in Table 2.

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.

Diuretic parameter

Electrolyte excretion

Electrolyte excretion in urine was measured 24 h post-administration, and the results are summarized in Table 3. Compared to the control

Table 1: Taxonomic classification [42]

Kingdom	Plantae
Subkingdom	Viridiplantae (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)

Table 2: Pharmacognostical evaluation of *Abutilon pannosum*

S. 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

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[a]	87.27±0.4[a]	84.53±0.3[a]
APME 300	124.8±0.6[a]	102.2±0.27[a]	114.8±0.4[a]
APME 450	140.5±0.3[a]	112.2±0.3[a]	120.4±0.2[a]

All the data were expressed as (Mean±SEM), n=6, [a]p<0.05 statistically significant when compared to control. SEM: Standard error of mean, APME: *Abutilon pannosum* methanolic leaf extract

group, sodium excretion for *A. 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. Fig. 1 depicts the comparative electrolyte excretion profile of the treatment groups.

Urine volume and pH

After 24 h, 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 a significant increase in urine volumes of 4.10 mL, 8.20 mL, and 14.1 mL, respectively. When test doses of APME were administered, the pH values were 7.6, 7.9, and 8.2. As the doses increase, the pH goes on the alkaline side. In contrast, the standard pH is 8.27, and the control is 6.5, as shown in Table 4. Fig. 2 represent changes in pH and urinary volume of treatment groups.

Diuretic action, natriuretic, saluretic action

The diuretic potency of the agent is assessed employing the Lipschitz value. APME at a dose of 450 mg/kg body weight demonstrates strong diuretic activity, with a Lipschitz value of 3.42. A 300 mg/kg dose of APME continued to show a positive diuretic effect, achieving a Lipschitz value of 1. Results are summarized in Table 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[a]	7.6[a]
APME 300	8.20[a]	7.9[a]
APME 450	14.1[a]	8.2[a]

All the data were expressed as (Mean±SEM), n=6, [a]p<0.05 statistically significant when compared to control. SEM: Standard error of mean, APME: *Abutilon pannosum* methanolic leaf extract

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

APME: *Abutilon pannosum* methanolic leaf extract

In terms of natriuretic action, a natriuretic index >1 is considered indicative of a favorable 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 favorable natriuretic response with potential potassium-sparing activity.

DISCUSSION

The findings of this study confirm the diuretic potential of APME, attributable to its diverse phytoconstituents, including alkaloids and flavonoids. These compounds appear to act synergistically to promote both diuresis and saluresis, as evidenced by increased urinary volume and enhanced excretion of sodium and chloride.

When compared with other medicinal plants, the diuretic potency of APME is more pronounced. Although *Tribulus terrestris* and *Hibiscus sabdariffa* have been reported to increase sodium and chloride excretion, their effects are generally less robust and lack the clear dose-dependent pattern observed with *A. pannosum*. This comparison highlights the relative strength of APME as a natural diuretic candidate.

Mechanistically, the saluretic effect, particularly the enhanced sodium and chloride excretion, points toward interference with the Na(+)-K(+)-2Cl(-) cotransporter in the thick ascending limb of the loop of Henle. This mechanism is analogs to that of loop diuretics such as furosemide, which block reabsorption of these ions and thereby increase urine output. Flavonoids, known modulators of ion transporters, may directly inhibit this cotransporter, while alkaloids could contribute by improving renal blood flow and filtration efficiency, amplifying the overall diuretic response.

An additional noteworthy observation was the dose-dependent alkaline shift in urine pH. This suggests that APME may alter acid-base handling in the nephron, potentially by reducing hydrogen ion secretion or enhancing bicarbonate excretion. Such changes could have implications for systemic electrolyte balance and warrant further mechanistic exploration.

Despite these promising results, certain limitations must be acknowledged. The study employed a crude extract, which makes it difficult to attribute the observed effects to specific phytoconstituents. Isolation and characterization of individual compounds would be

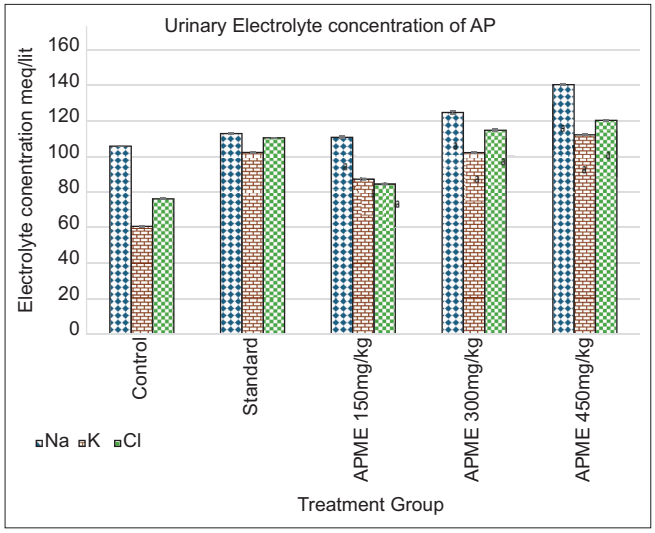


Fig. 1: Urinary electrolyte excretion effect of *Abutilon pannosum*. All the data were expressed as (Mean±Standard error of mean), n=6, [a]p<0.05 statistically significant as when compared to control

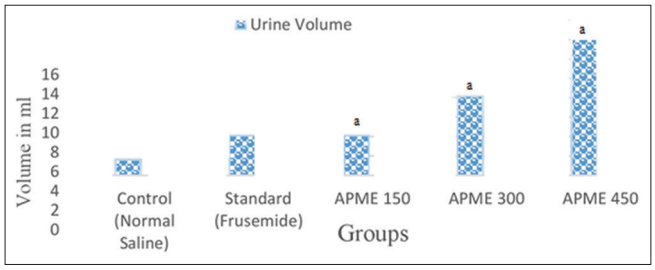


Fig. 2: Urine volume and P[H] effect of *Abutilon pannosum*. The figure represents the changes in urine output and urinary pH following treatment with *A. pannosum* in experimental animals. All the data were expressed as (Mean±Standard error of mean), n=6, [a]p<0.05 statistically significant as when compared to control

necessary to confirm their precise roles and to establish reproducible pharmacological profiles.

CONCLUSION

This study highlights the substantial diuretic potential of APME. The 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 the standard drug, signifying the presence of active phytoconstituents attributing to renal modulation and fluid balance. Owing to its natural origin and satisfying results, it could be employed as an alternative to synthetic diuretics. 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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