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### Research Article

## Uncovering the Diuretic Potential of *Abutilon hirtum* Leaves Extract in Wistar Albino Rats

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### ABSTRACT

*Abutilon hirtum*, traditionally recognized for its alleged diuretic effects, underwent examination to assess its viability as a natural substitute for synthetic diuretics. The methanolic extract of *A. hirtum* leaves (AHME) was methodically prepared using the soxhlet extraction technique and assessed for its diuretic activity in Wistar albino rats. Five different groups were created from the animals: a control group, a standard group that received furosemide, and three test groups that received 150, 300, and 450 mg/kg of body weight of AHME. The evaluation of diuretic activity was conducted through the Lipschitz test, which involved measuring various parameters, including urine volume, pH, electrolyte excretion, diuretic effect and Lipschitz value. The highest dose (450 mg/kg) substantially raised urine volume, electrolyte excretion, and pH compared to the control, with a Lipschitz value of 2.35. These results corroborate the traditional use of *A. hirtum* leaves and provide a basis for subsequent pharmacological research by confirming their diuretic ability.

### INTRODUCTION

Diuretic boasts a Greek etymology. Diu' translates to 'through' while 'ourein' signifies 'to urinate'.<sup>[1]</sup> Diuretics are the medications that stimulate the kidneys to expel water and electrolytes.<sup>[2]</sup> They disrupt sodium reabsorption in the kidneys, affecting sodium levels and triggering significant changes in blood flow dynamics.<sup>[3]</sup> Traditional diuretics go beyond their reputation for treating fluid retention (edema). They are a workhorse medication for high blood pressure, heart failure, and certain kidney problems, but their benefits extend to managing issues like kidney stones (nephrolithiasis), high calcium levels (hypercalcemia), and electrolyte imbalances like high potassium (hyperkalemia) or low sodium (hyponatremia).<sup>[4]</sup> In contemporary times, herbal remedies derived from indigenous plants have become increasingly significant, not just in India but globally. For centuries, people have

turned to herbs for their diuretic properties. There's a rich tradition of numerous herbal preparations using single plants or combinations employed as natural diuretics.<sup>[5]</sup> As synthetic diuretics pose potential risks, exploring natural plant-based compounds with potent diuretic properties and fewer adverse effects has become imperative.

*Abutilon hirtum*, a notable member of the Malvaceae family, holds significant promise for medicinal applications. The shrub or perennial plant *A. hirtum* can grow up to 2.5 m in height. It has persistent, somewhat woody stalks. Its stems are persistent and have a woody texture. Sticky, velvety, and hairy are the stem, leaf stalks, and flower stalks. The leaves are lobed, alternating, and simple. Flowers are predominantly yellow, with a striking red center. It is indigenous to tropical and subtropical regions across America, Africa, Asia, and Australia.<sup>[6]</sup>

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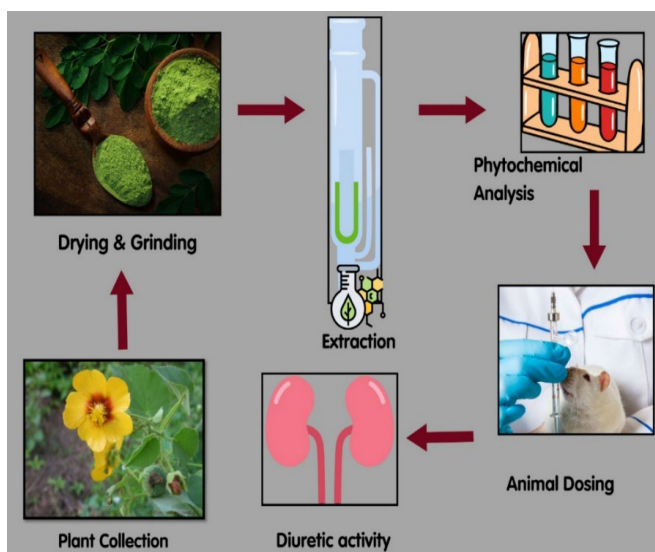


Fig. 1: Graphical Abstract

*A. hirtum* has a long history in traditional medicine, where it is used to treat conditions such as urinary disorders, diarrhea, coughs, pain, ulcers, toothaches, and abscesses. This wide range of applications is attributed to its rich phytochemical profile, which includes flavonoids, triterpenes, coumarins, sterols, phenolic acids, and ellagic acid. Such bioactive entities are claimed to have notable therapeutic properties, like anticancer, antioxidant, antibacterial, hepatoprotective, anti-inflammatory, antidiabetic, analgesic, antipyretic, and cytotoxic activities. Particularly, the presence of compounds that influence renal function strongly supports the plant's traditional use as a diuretic, promoting urinary health and fluid balance while aligning with its broader medicinal potential.<sup>[7-9]</sup> This study explores the diuretic activity of the leaves of *A. hirtum* from the Malvaceae family to uncover its untapped potential. Evaluating the diuretic activity of medicinal plants is crucial for advancing our understanding of plant-based therapies and their potential integration into modern medicine. The analysis of *A. hirtum*'s diuretic properties offers promising leads for developing cost-effective, plant-based diuretic drugs with enhanced efficacy. The visual summary of the key findings of this study is depicted in Fig. 1.

## MATERIALS AND METHOD

### Drug and Chemicals

Methanol (Loba Chemie Pvt. Ltd), normal saline (Amanta Ltd), frusemide (Sanofi India Pvt. Ltd.), distilled water. The chemicals that were utilized were all of analytical grade.

### Collection and Authentication

In the months of December and January, *A. hirtum* leaves were collected from the Aurangabad areas of Maharashtra. The specimen of the whole plant was authenticated from the Botanical Survey of India, Pune.

### Extraction

Collected leaves were rinsed under tap water, dried under a shed and then roughly powdered with a grinder first. The powdered *A. hirtum* leaf material (about 100 g) was soaked in pet ether for the whole night. After that, it was treated with methanol as the solvent in the soxhlet extraction procedure. The further extract was concentrated by using a rotary evaporator.<sup>[10]</sup>

### Phytochemical Analysis

Extracts of the plant *A. hirtum* were subjected to phytochemical evaluation for the presence or absence of various bioactive components.<sup>[11,12]</sup>

### Experimental Animal

Animal house of Appasaheb Birnale College of Pharmacy Sangli provided Wistar albino rats of either gender measuring 180 to 250 g. The animals were reared in cages, allowing four to six animals per group, by maintaining standard temperature and humidity conditions. The animals were freely provided with food and water at all times, but 18 hours prior to the experiment, access to both food and water was discontinued. The study approval from the Institutional Animal Ethics Committee was obtained with protocol no. IAEC/ABCP/20/2023-24.

### Acute Toxicity Study

The rats were deprived of food overnight and then separated into groups of three. They were given oral doses of methanolic extract of *A. hirtum* at increasing levels: 200, 500, 750, 1,000, 2,000, and 5,000 mg/kg of body weight. After administering this extract, the rats were monitored for notable behavioral changes during the first 3 hours and then every 30 minutes for the following 5 hours. Afterward, they were checked once every 24 hours over the next three days to track and mortality rates.<sup>[13]</sup>

### Animal Grouping

The animals were divided into five groups, each consisting of six members as shown in Table 1.

### Evaluation of Diuretic Activity

The diuretic potential was evaluated through the application of the modified Lipschitz protocol. Before the experiment, the animals underwent an overnight fast but had unlimited access to water. Additionally, they were given a two-hour acclimatization period in their metabolic cages. The rats received a hydration treatment of normal saline at a dose of 25 mL/kg before being given the test dose. By gently pressing on the pelvic region and moving the rats' tails downward, the rodents' bladders were emptied. Each group of animals received the treatment according to the specifications outlined in the grouping section continuously for seven days. Urine was collected on the last day and analyzed for total urine volume, pH, sodium, potassium, chloride

concentration, diuretic index, Lipschitz value, saluretic and natriuretic index was also calculated.<sup>[14, 15]</sup>

### Statistical Analysis

All findings were presented as the standard error of the mean and then subjected to Dunnett's multiple comparison test after a one-way ANOVA.

## RESULT

### Phytochemical

Plants showed the presence of various phytochemicals as represented in Table 2.

### Acute Toxicity

As there were no signs of toxicity observed in rats under acute toxicity study, the lethal dose for the AHME was considered to be above 4000 mg/kg of the body weight.

### Assessment of Electrolyte Excretion

The effect of AHME on electrolytes, i.e., sodium, potassium and chloride excretion in urine after 24 hours is presented in Table 3. All groups were compared to the control group. At the higher dose, AHME shows electrolyte excretion values of  $131.2 \pm 0.51$  for sodium (Na),  $119.5 \pm 0.31$  for potassium (K), and  $120.3 \pm 0.50$  for chloride (Cl).

**Table 1:** Animal grouping

Group	Treatment	Dose/kg body weight
1	Control	25
2	Standard	10
3	Methanolic extract of <i>A. hirtum</i> (MEAH)	150
4	MEAH	300
5	MEAH	450

Extract dissolved in normal saline.

**Table 2:** Phytochemical analysis

S. No.	Test	<i>A. hirtum</i>
1.	Alkaloid	++
2.	Glycoside	++
3.	Carbohydrate	++
4.	Triterpenoid	++
5.	Steroids	++
6.	Flavonoids	++
7.	Phenols	++
8.	Proteins	++
9.	Saponins	++

### Assessment of Urine volume and P<sup>H</sup>

At the end of activity i.e. after 24 hours. The volume of urine was measured for control, standard, and test groups. AHME 150, 300, and 450 doses result in 3.92, 5.15, and 9.70 mL of urine, respectively. The pH of urine at 24 hours for the control is 6.5 and the standard is 8.27. In the test groups, the pH for AHME at 100, 250, as well as 400 mg/kg of body weight is 7.52, 7.92, and 8.17 respectively as shown in Table 4.

### Assessment of Diuretic action, Lipschitz value

The Lipschitz value is calculated using a specific formula. If the value is 1, it indicates a positive effect. If the value is two or more, it is considered a potent diuretic. Test group 3 of AHME has a Lipschitz value of 2.35, indicating potent diuretic action.

### Natriuretic and Saluretic action

The natriuretic value is the proportion of sodium to potassium excretion, while the saluretic value is the combined value of sodium and potassium excretion. *A. hirtum* demonstrated notable saluretic action compared to the control group but did not exhibit a significant natriuretic effect as represented in Table 5.

## DISCUSSION

Numerous physiological functions in the human body depend on water, which makes up around 60% of the normal adult's body weight. The act of maintaining a balance between fluid intake and loss through various physiological mechanisms is a highly controlled aspect of the body's fluid balance. Renal urine excretion facilitates the removal of metabolic waste products, excess electrolytes, and water, thereby preserving fluid homeostasis. In order to encourage the excretion of water and electrolytes and consequently enhance urine output and volume, diuretics are medications that pharmacologically change renal fluid dynamics. Na<sup>+</sup> is the most common extracellular cation, and this class of medications mostly works by inhibiting receptors that allow it to be reabsorbed from the renal tubules.

**Table 3:** Urinary electrolyte excretion effect of *A. hirtum*

Group	Na+ mmol/eq.	K+ mmol/eq.	Cl mmol/eq.
Control (Normal Saline)	$105.8 \pm 0.12$	$60.61 \pm 0.34$	$76.19 \pm 0.20$
Standard (Frusemide)	$112.9 \pm 0.32$	$102.3 \pm 0.51$	$110.4 \pm 0.16$
AHME 150	$103.5 \pm 0.3$	$98.46 \pm 0.12$	$83.13 \pm 0.31$
AHME 300	$119.2 \pm 0.16$	$107.4 \pm 0.24$	$112.3 \pm 0.68$
AHME 450	$131.2 \pm 0.51$	$119.5 \pm 0.31$	$120.3 \pm 0.50$

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



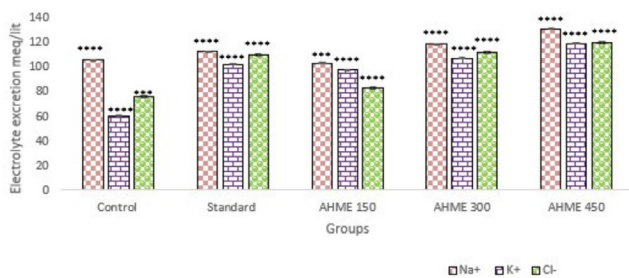


Fig. 2: Urinary electrolyte excretion effect of *A. hirtum*

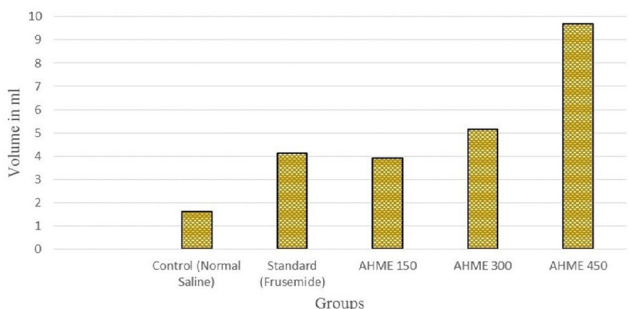


Fig. 3: *A. hirtum* effect on urine volume

Table 4: Urine volume and pH effect of *A. hirtum*

Group	Urine volume	pH
Control (Normal Saline)	1.61	6.5
Standard (Frusemide)	4.12	8.27
AHME 150	3.92	7.52
AHME 300	5.15	7.92
AHME 450	9.70	8.17

Table 5: Diuretic, Lipschitz, saluretic, natriuretic effect of *A. hirtum*

Group	Diuretic effect	Lipschitz value	Saluretic index	Natriuretic index
Control (Normal Saline)	-	0.39	181.99	1.74
Standard (Frusemide)	2.55	-	223.3	1.10
AHME 150	3.19	0.95	186.83	1.24
AHME 300	4.16	1.25	231.5	1.10
AHME 450	6.02	2.35	251.5	1.09

This increases the osmolality within the tubules, hence lowering water reabsorption. A study was conducted to assess the diuretic potential of *A. hirtum* by examining its impact on key electrolyte excretion in the urine. The study used Dunnett’s multiple comparison test after a one-way ANOVA to examine the data. Results indicated a dose-dependent increase in urinary electrolyte concentration following *A. hirtum* administration compared to a control

group. AHME (450 mg/kg) has a pronounced effect on sodium excretion. The elevated sodium excretion indicates enhanced natriuretic activity, which is a key characteristic of diuretic agents as it helps in the reduction of fluid retention and blood pressure. Potassium excretion also increased as compared to the control group. The chloride excretion was elevated at a higher dose of AHME as shown in Fig. 2. Chloride excretion is important as it helps to maintain osmotic balance and proper function of bodily fluids. Results of urine volume as shown in Fig. 3. measurement demonstrate a dose-dependent increase in urine volume. This enhanced urine output with AHME suggests a stronger diuretic effect, which is beneficial for reducing fluid retention and promoting renal function. The substantial increase in urine volume at the highest dose of AHME (9.70 mL) highlights its potential for therapeutic use in conditions requiring effective diuresis, such as hypertension, edema, and certain renal disorders. The increase in urine pH aligns with their diuretic effects, indicating a shift towards an alkaline urine environment. The greater efficacy of AHME could be attributed to its specific phytochemical composition. Test group 3 (450 mg/kg) of AHME demonstrated a Lipschitz value of 2.35, categorizing it as a potent diuretic. *A. hirtum* exhibited significant saluretic activity compared to the control group but did not show a pronounced natriuretic effect.

## CONCLUSION

The findings of this study unequivocally provide strong evidence supporting the diuretic effectiveness of *A. hirtum*. Its capacity to significantly boost the excretion of electrolytes in the urine implies a strong mechanism of action. Since the plant has shown a significant saluretic effect rather than a natriuretic one, its action may resemble that of loop diuretics. By obstructing the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> symporter in the thick ascending limb of the loop of Henle, these diuretics reduce electrolyte reabsorption. In light of the plant’s intricate phytochemical composition, more research is necessary to identify and define the precise substances that give rise to the diuretic effects of the plant.

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