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Research Article

Phytochemical Screening and Evaluation of Diuretic Activity of *Setaria glauca*

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ABSTRACT

This study aims to research the diuretic properties of ethanolic extract of whole plant of *Setaria glauca* by Lipschitz model in albino rats. Ethanolic extract of *S. glauca* was administered to experimental albino rats orally at 2000 mg/kg p.o. The acute toxicity of the extract was evaluated as per OECD guideline 420–425. The LD_{50} of the extract was found to be between 2000 mg/kg p.o. male albino rats (n=6) were divided up into four groups. the control group was given saline as usual albino rats were divided into four groups to assess the diuretic activity of ethanolic extract of whole plant of *S. glauca* metabolic cages are used. group II contains furosemide (10 mg/Kg, p.o.) while groups III and IV receive saline as the control group's vehicle. (100 mg/kg), medium (200 mg/kg), dosing of ethanolic extract of whole plant of *S. glauca*, respectively. Immediately following the methanolic extract of *S. glauca*, treatment Three rats were placed in each metabolic cage and kept at 21°C ± 0.5°C while all the rats were hydrated with saline (10 mL/kg, p.o.). urine the volume was measured. Each hour until the conclusion of the 5th hour and of each creature was calculated. The diuretic action and diuretic activity were decided based on the urine yield. Moreover, concentration of urinary sodium, chloride, and potassium particles was decided. The urinary Na^+/K^+ proportion and carbonyl anhydrase movement ($Cl^-/(Na^+/K^+)$) where moreover surveyed. Animals were denied access to nutrient and water for a period of 5 hours. At the conclusion of the 5 hours, the total amount of urine accumulated with each metabolic cage has been measured. The volume of urine as well as the levels of the different ions, such as sodium, potassium, and chloride, were evaluated. Rats' urine volume and concentration of urinary electrolytes increase because of the aqueous crude extract and of *S. glauca*.

Introduction

Setaria glauca, commonly known as "*Panicum glaucum L.*, *yellow foxtail*", may be a tropical plant having a place in the *family Poaceae (Gramineae).* As these trees are tall in India and can reach up to 6–12 m in height. The plant's roots are widely used to treat parasites, and inflammation. The licorice root, emollient, digestive, and stomachic germinated seed of yellow-seeded cultivars are used in the treatment of impaired digestion, dyspepsia, and food stagnancy in the abdomen. White seeds are refrigerant and used in the treatment of fever and cholera. Green seeds are virility-strengthening and diuretic. [1,2] The creation of oil-water separation materials is now a crucial undertaking due to rising water pollution and health issues brought on by greasy wastewater. The ZnO/PANI/PAN fibrous membranes' micro-/nanosized pores furthermore ensure its excellent emulsion separation performance, including an ultrahigh surfactant-free emulsion permeate flux of 8597.40 L/(m^2 h), an ultrahigh surfactant-stabilized emulsion permeate flux of 2253.50 L/(m^2 h), and excellent separation efficiency (above 99%). Additionally, the composite membrane exhibits high biological safety, is

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unharmful to the aquatic environment, and maintains stable underwater superoleophobicity and hydrophilicity under challenging circumstances. The ZnO/PANI/PAN nanofibrous membranes have exceptional characteristics that greatly increase their potential for treating oily wastewater. [3]

Patients with tuberculosis (TB) are given numerous oral doses of the first-line medications (such as isoniazid) every day for at least 6 to 8 months. The aim of the current study were based on various studies concerning the diuretic activity of *S. glauca*, such investigation shows the plant's anti-inflammatory characteristics.[4-5] Another study found that the hydroalcoholic extract of *S. glauca* had potential diuretic activity. *S. glauca* may be a restorative plant that has been utilized for the treatment of diverse sicknesses such as cardiogenic shock, nephritis, toxaemia, oedema, diuretic-related high blood pressure, pre-menstrual tension, and gestation compound are very valuable in easing these conditions

Materials And Methods

Drug and Chemical

Furosemide, the standard drug was used from Pradhan Mantri Jan Aushadhi Kendra, Thatipur. The chemical compounds used in this study were all purchased by Shri Ram College of Pharmacy, Banmore, Morena were of quality standard and came from credible resources.

Plant Material Selection and Collection

Collection of Plant Material

The whole plant of *S. glauca* was collected from the banks of the river in the village of Vijaygarh, Morar, Gwalior, Madhya Pradesh.

Fresh *S. glauca* plants were shed dried and crushed by hand grinder weighing 500 g crushed plant grinding machine extraction with ethanol by soxhlet assembly for 2 hours at room temperature. The extract was filtered twice, once with cotton gauze and once with Whatman filter paper. The filtrate was solubilized and freeze-dried. The dried extract, amounting to 18% (w/w), was stored in a silicon desiccator until further use.

Preparation of Extract

Whole plants were washed in clean running water, dried in the shade, and dried crushing by using a hand grinder The preparation of ethanolic extract of *S. glauca* was carried out using soxhlation in the Pharmacology Department, Shri Ram College of Pharmacy, Banmore, Morena, Madhya Pradesh. About 200 g of *S. glauca* using the soxhlet apparatus, ethanol was used to extract the powder (95%). The extraction process took 18 to 20 hours to complete before a colorless solvent appeared in the side tube. The extracted material was dried using the evaporation of the

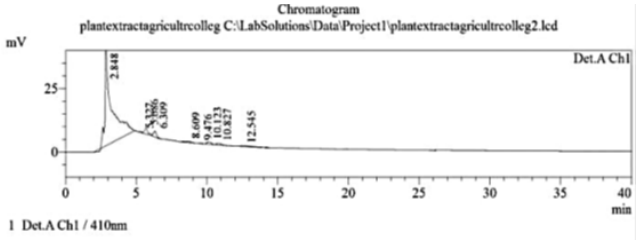

Fig. 1: HPLC graph for analysis of diuretic agents present in the extract of *S. glauca*.

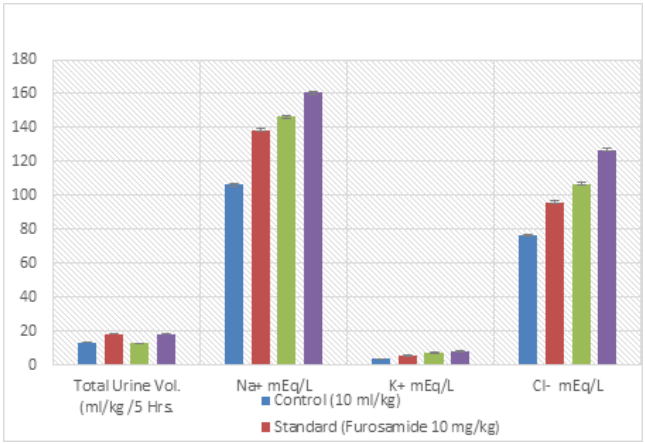

Fig. 2: Urine volume Na^+, Cl^-, K^+ ion after drug and extraction.

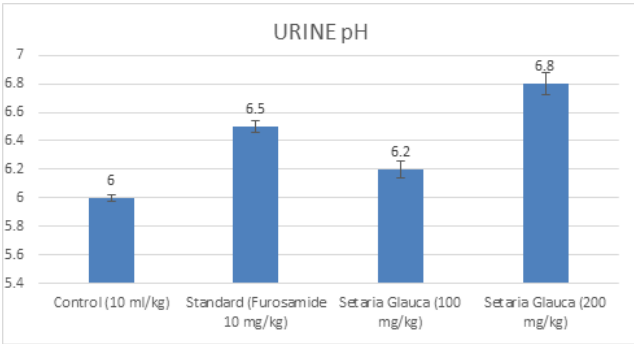

Fig. 3: The urine pH after the administration.

Table 1: Peak in HPLC graph for determination of diuretic agents in extract of *S. glauca.*

Peak S. N.	*Ret. Time*	*Area*	*Height*	*Area%*	*Heigh%*
	2.848	1375566	61204	89.931	89.217
	5.327	4579	341	0.299	0.497
	5.686	41727	2406	2.728	3.507
	6.309	34471	2206	2.254	3.216
	8.609	14875	396	.973	0.577
	9.476	3956	208	0.259	0.304
	10.123	17983	863	1.176	1.259
	10.827	15491	643	1.013	0.937
	12.545	20930	334	1.368	0.87

Table 2: Dosing of animals and output of urine and electrolytes.

Group	*Total urine vol. (mL/kg/5 hours*	*Na+ mEq/L*	*K+ mEq/L*	*Cl- mEq/L*
Control (10 mL/kg)	13.16 ± 0.06	106.35 ± 0.70	3.5 ± 0.14	76.58 ± 0.58
Standard (Furosamide 10 mg/kg)	18.25 ± 0.03	138.40 ± 0.90	5.2 ± 0.24	95.9 ± 0.77
S. glauca (100 mg/kg)	12.81 ± 0.03	146.3 ± 0.69	6.9 ± 0.31	106.7 ± 0.68
S. glauca (200 mg/kg)	18.26 ± 0.07	160.6 ± 0.95	7.9 ± 0.19	126.8 ± 0.82

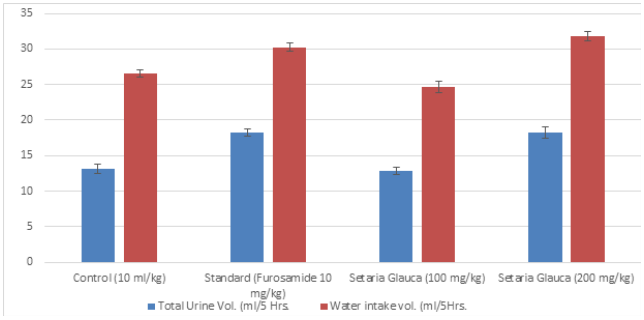

Fig. 4: Measurement of total urine volume and water intake volume.

Table 3: Determination of urine pH Value.

Group	*Urine pH*
Control (10 mL/kg)	6
Standard (Furosamide 10 mg/kg)	6.5
S. glauca (100 mg/kg)	6.2
S. glauca (200 mg/kg)	6.8

Table: 3 Determination of urine pH Value.

Group	*Total urine Vol. (mL/5 hours.*	*Water intake vol. (mL/5 hours.*
Control (10 mL/kg)	13.16	26.6
Standard (Furosamide 10 mg/kg)	18.25	30.25
S. glauca (100 mg/kg)	12.81	24.6
S. glauca (200 mg/kg)	18.26	31.8

solvent on a bath of water kept at 50°C. The yield of ethanol extracts as a percentage of the total amount of powder used for the extraction was noted.

Experimental Animals

Male Albino rats aged 4 to 6 weeks and weighing 160–175 g, were chosen for this research. The laboratory animal house facility of the university provided all the animals used in this study. Shri Ram College of Pharmacy (SRCP) Banmore, Morena, Madhya Pradesh, India. The rodents were given a week to adjust to the research facility conditions before being placed in a metabolic enclosure for 16 hours before the actual tests began. The creatures were kept in a constant 12/12 hours light/dark cycle of research facility conditions, with a typical ambient temperature of 25°C. The creatures were given standard food and non-essential water. This study has received moral support from the Committee for Control and Supervision of Experiments on Animals CPCSEA guidelines and was approved by the IAEC. Reg. No. SRCP/ M. Pharm/IAEC//66/19-20.[6] Throughout the study period, all animals used in this study were treated humanly following the International Standards for Laboratory Animal Rescue and Use.[7]

Acute Toxicity Study

Determination of LD_{50}

Wistar rats with either sex ranging from 160 to 175 g have been used to investigate the acute toxicity (if any) of *S. glauca* plant (whole plant) extracts. In a typical day and night picture period, the animal was housed in regular polypropylene cages at 22 ± 2°C and 60% relative humidity (12:12 hours). Just before the tests, the animals were given a 14 day acclimatization time. The experiment protocols were accepted by the Shri Ram College of Pharmacy's institutional animal ethics committee before the research. The OECD 420–425 recommendations were followed while conducting the acute oral toxicity analysis. The animal was held starving overnight but given unlimited access to clean drinking water. The fasting conditions mice were grouped into six classes, each with six mice.[8] Each solvent crude extract was given orally at a dosage of 10 mg/kg of body weight. Using regular saline water as a vehicle, each group's mortality was tracked for 14 days. if two out of three creatures perished, the dose given was classified as hazardous. If a single animal died, the same dosage was administered again to validate the toxic dose. If no morbidity was confirmed, the identical procedure was repeated in each group by each extract at larger concentrations of 100, 300, 600, 1000, and 2000 mg/kg B.W. A $1/10^{th}$ and $1/20^{th}$ of the maximal dosage (2000 mg/kg B.W) were chosen as the prescribed levels for the assessment of diuretic actions.[9,10]

Phytochemical Screening

The ethanolic extract and *S. glauca* plant extract and *S. glauca,* as well as negative controls, were tested for the presence of alkaloids, carbohydrate alkaloids, volatile oil, flavonoids, and tannins in the diuresis study using the methods described by Trease and Evans.[11]

Diuretic Activity

The animals will be cared for and maintained according to the committee's approval for monitoring and oversight of animal experiments, which will follow the rules

of the establishment's ethical committee on animal experimentation.

Grouping of Animals and Dosing

Animals were divided into 4 groups of 6 animals in each group-

Group I- Received normal saline as a control (n = 6).

Group II- Received the standard treatment. Furosemide 10 mg/kg (n= 6).

Group III- The test group received ethanolic extract of *S. glauca* plant 100 mg/kg (n=6).

Group IV- Test group received ethanolic extract *S. glauca* 200 mg/kg (n = 6).

Screening for Diuretic Activity

Lipchitz Model

Wistar rats ranging from 160 to 175 grams were used in this research. They were kept in metabolic cages with a chicken wire underside and a funnel for urine collection. The faces were retained in the nozzle, and there are stainless steel filtrations., enabling just water to pass down for storage and calculation. Fifteen hours before the exam, food and drink were withheld. In one metabolic cage, three animals were housed. The rats in each category were given drugs based on the information provided above. In addition, both rats were given 1-mL of regular saline solution per 100 g orally. After 5 hours, urinary excretion was measured, and the sodium and potassium content of the urine was calculated using a flame photometer. The system was calibrated using a common solution comprising specific Na^+ and K^+ concentrations. A conductometer was used to measure the conductivity of fresh urine samples. On a fresh urine sample, pH was utilized a digital pH meter.[12,13]

$$\text{Urinary excretion} = (Vo/Vi) \times 100$$

where Vo is the total urinary output and Vi is the total volume of fluid

$$\text{Diuretic index} = Vt/Vc$$

where *V*t is the mean urine volume of the test group and *Vc* is the mean urine volume of the control group:

$$\text{Diuretic activity} = Vt/Vr$$

where *V*t is the mean urine volume of the test group and *Vr* is the mean urine volume of the reference group:

$$\text{Saliuretic index} = Ct/Cc$$

where *C*t is the concentration of electrolyte in the urine of the test group and *Cc* is the concentration of electrolyte in the urine of the control group:

$$Na^+/K^+ \text{ ratio} = Cn/Ck$$

where *C*n is the concentration of Na+ in the urine of a group and *C*k is the concentration of K^+ in the urine of the same group:

$$\text{Carbonic anhydrase inhibition} = Cl^-/(Na^+/K^+)$$

where Cl^- is the urinary chloride concentration, Na+ is the urinary sodium concentration, and K^+ is the urinary potassium concentration.

Determination of Urinary Na^+, K^+, and Cl^-

Urinary Na^+, K^+, and Cl^- concentrations of the experimental, control, and standard groups were determined using ion selective electrode analysis of blood and urine by kidney function test (KFT), urine RM, and urine culture.

Determination of Urine pH

All groups' fresh samples of urine were tested for pH using a calibrated digital pH meter (Dolphin mexico).

Statistical Analysis

ANOVA was used for the statistical analysis, and a *p*-value worth of under 0.05 was critical. Results are given as the mean minus the standard deviation of the mean. Data analysis was conducted using SPSS version 16.[14]

HPLC Analysis for Detection of Agents responsible for Diuretic Activity

Chromatographic Conditions

Solvent A: Phosphate buffer is used in the mobile process. Dissolved anhydrous potassium dihydrogen orthophosphate (0.0136 g) in solvent A.

Requirements: Each sample was correctly measured and transferred to four separate 50 mL volumetric flasks. The solution was sonicated for about 5 minutes after adding 10 mL of methanol to make up the amount of methanol. To obtain the necessary volume, the solution was diluted. The solution was taken up to 0.5 mL of orthophosphoric acid, 1000 mL with water, and HPLC grade water (900 mL). The mixture was diluted through a 0.45 membrane and sonicator degassed for 3 minutes acetonitrile (solvent B) (100%).[15]

Standard and Sample Preparation

To distinguish fresh and dry 10 mL volumetric flasks, 1.0 mg of each norm was precisely weighed. Each of the volumetric flasks received 5 mL of methanol, which was softly heated on a water bath, cooled, and created to 10 mL with methanol. 1-mL of each normal solution was moved to separate 10 mL volumetric flasks, which were then filled with methanol to the desired depth (std. mix).

The test extract (defatted HALH) was correctly measured (500 mg) and moved to a 25 mL volumetric flask, which was then sonicated for 6 minutes with 15 mL of methanol. Then it was heated for the next 5 minutes in a water tank. Cooled above that the solution to ambient temperature and added methanol to make up the thickness. Filtered onto 0.2 membrane filter paper after being thoroughly mixed.

Determination of Total Flavonoids

$AlCl_3$ was used as a complexation reagent to create a complex with the flavonoids, and this complex formed a complex with flavonoids that have a maximum absorption at 410 nm as shown in Table 1. This method was described in the literature. The number of total flavonoids was

measured as the quercetin equivalent, and a calibration curve with a value of 0.9971 was created. The regression equation derived from the calibration curve was used to calculate the flavonoid content after the calibration curve was passed through zero. The *S. glauca* produced 0.324 mg/mL of total flavonoids in the final extract.

Result And Discussion

The results of the acute toxicity studies suggested that the extracts obtained from *S. glauca* were the plant was well tolerated up to the dose of 2000 mg/kg body weight. No toxic plant extract of *S. glauca.* A dose of 100 and 200 mg/kg body weight was selected for the present studies. During the acute toxicity study, the behavioral activities were observed and were found normal.

Administration of extract obtained from a *S. glauca* at dose of 2000 mg/kg did not produce any observable toxicity in experimental animals. The toxicity studies also suggest that the extract-treated rats have no significant changes in the relative weights. The LD_{50} was found greater than the tested dose (2000 mg/kg) as all animals survived. In the acute toxicity study, animals did not show any change in their behavioral pattern. No significant difference in body weights and food consumption were observed when compared to the vehicle-treated group. Also, no gross pathological changes were seen. Thus, it was concluded that the extracts are safe at 2000 mg/kg, which is consistent with Sharma *et al*., 2018 results.[19]

Diuretic Activity

As we know that the quantity of urine in diuretic activity increases with this. $K^{+,}$ Na^{+}, and Cl^{-} ion volume increases, which relieves many diseases such as CHF (congestive heart failure), edema, hypertension arthritis, inflammation, etc. For this activity, we first saw the Wistar rat and his normal urine output which was 1 to 2 mL for 5 hours. And with this, we saw that they drink 8 -10 mL of water a day. The extract was given once up to 2000 mg/kg body weight in the acute toxicity study, and neither an adverse reaction nor mortality was seen.

Impact on urine volume results is shown in Table 2. The ethanolic extract of the whole plant of *S. glauca* at a dose of 100 mg/kg and maximal dosage of 200 mg/kg diuresis in 5 hours of test. *S. glauca* standard (furosemide 10 mg/kg), urine volume 18.25 ± 0.03 mL, and water intake in 5 hours 30.25 mL ethanolic plant extract of *S. glauca* (100 mg/kg) urine volume 12.81 ± 0.03 mL and water intake volume 24.6 mL/5 hours, maximum dose of *S. glauca* (200 mg/kg), urine volume 18.26 ± 0.07 and water intake volume 31.8 mL, 5 hours as shown in Figs. 2-4. By the 3rd hour of ethanolic crude extract administration, the rats given the lower doses (100 mg/kg) had a critical ($p < 0.05$) volume increase in urine excretion *S. glauca*. As shown in Table 2 & 3 and Figs. 3 & 4 animals were given higher doses (200 mg/kg) had a critical ($p < 0.05$) increase in urine excretion by the 5th hour. A significant ($p < 0.05$) excretion of urine was discovered at all tested doses, the ethanolic crude leaf extract of *S. glauca* induced a significant ($p < 0.01$) increase in urine output and diuretic activity (Table 2). Comparable to the standard drug, the highest diuretic activity was observed at 100 mg/kg. However urinary electrolyte excretions of both doses were less when compared with standard drug furosemide. As a result, it became clear from the findings that the plant's methanolic extract had a sizable diuretic effect. The diuretic properties of *S. glauca's* methanolic extract, which have been highlighted, may be attributed to the plant's alkaloids, flavonoids, and tannins.

Determination of Urine pH

The pH of the fresh urine samples of all groups was measured with a calibrated digital pH meter including the negative and positive control groups produced slightly acidic urine. The average urine pH of the normal and standard controls was determined to be 6.0 and 6.5, respectively. The pH of urine from rats treated with the ethanolic group extract for the two doses 100 and 200 mg/kg (6.2 and 6.8, respectively), and a slight decrease in pH 6.19 (Table 2 and Fig. 3).

As we all know, a diuretic means an increase in the amount of urine that is treated for certain diseases such as edema, inflammation, arthritis, and arthritis, which is mostly used in hypertension. This is because it also removes Na^{+}, K^{+}, and Cl^{-} ion from the body along with urine, and urea and uric acid also get out of the body and get rid of a disease like arthritis. We had the same objective of studying this plant and people are lagging behind ayurvedic and herbal medicines today. We studied the *S. glauca* plant to see how chemical compound alkaloids, saponin, flavonoids, tannins, and steroids were found, all of which were found in this plant by phytochemical study. The results of this study provided a quantitative basis for analysing *S. glauca's* traditional folkloric use as a diuretic. Additionally, it is used to treat renal disease and hypertension. The extract's diuretic properties support its use as a diuretic in medicine. The utilization of the plant in the treatment of some cardiovascular diseases may explore this effect.

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