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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF DIURETIC ACTIVITY OF PHYLA NODIFLORA IN RATS

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ABSTRACT: Phyla nodiflora (L.) Greene, a medicinal herb belonging to the family Verbenaceae, has been traditionally used for the treatment of urinary disorders, edema, kidney diseases, and inflammatory conditions. The present study was undertaken to evaluate the phytochemical constituents and diuretic activity of the hydroalcoholic extract of Phyla nod flora in saline-loaded Wistar rats. The whole plant was extracted using a hydroalcoholic solvent, and preliminary phytochemical screening was performed using standard qualitative methods. The screening revealed the presence of flavonoids, phenolic compounds, tannins, saponins, alkaloids, terpenoids, glycosides, and steroids, indicating the presence of biologically active secondary metabolites. Healthy Wistar albino rats were divided into five groups comprising a normal control, a standard group treated with furosemide (10 mg/kg, p.o.), and three test groups receiving the hydroalcoholic extract of Phyla nod flora at doses of 200, 400, and 800 mg/kg, respectively. Following oral saline loading (0.9% NaCl, 25 mL/kg), urine samples were collected for six hours and evaluated for urine volume, sodium, potassium, and chloride excretion. Diuretic activity was assessed by determining the diuretic index, Lipschitz value, saluretic index, natriuretic index, and carbonic anhydrase inhibition index. The extract produced a significant and dose-dependent increase in urine output and electrolyte excretion compared with the control group. The highest dose (800 mg/kg) demonstrated a diuretic index of 2.28 and a Lipschitz value of 0.90, indicating activity comparable to the standard drug. The extract also significantly increased the saluretic and natriuretic indices, suggesting enhanced sodium and chloride excretion with relatively lower potassium loss, while a reduction in the carbonic anhydrase inhibition index indicated partial involvement of carbonic anhydrase inhibition in its mechanism of action. The observed diuretic activity is likely attributable to the synergistic effects of flavonoids, phenolic compounds, saponins, tannins, and other phytoconstituents present in the extract. In conclusion, the hydroalcoholic extract of Phyla nodiflora possesses significant dose-dependent diuretic activity and provides scientific evidence supporting its traditional use as a natural diuretic. These findings suggest that the plant may serve as a promising herbal therapeutic agent for the management of edema, hypertension, and other disorders associated with fluid retention, although further pharmacological and clinical studies are warranted to establish its safety and therapeutic efficacy.

Keywords: Phyla nodiflora, Hydroalcoholic extract, Diuretic activity, Saluretic index, Natriuretic index, Lipschitz value, Carbonic anhydrase inhibition index.

INTRODUCTION The maintenance of water and electrolyte homeostasis is essential for normal physiological function and survival. One of the most important mechanisms involved in this process is antidiuretic activity, which refers to the

reduction in urine formation by enhancing water reabsorption in the kidneys. This process is primarily regulated by antidiuretic hormone (ADH), also known as arginine vasopressin (AVP), a nonapeptide hormone synthesized in the hypothalamus and released from the posterior pituitary gland. ADH plays a crucial role in maintaining plasma osmolality, extracellular fluid volume, and arterial blood pressure by controlling renal water conservation. Disturbances in antidiuretic function can lead to several pathological conditions, including diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion (SIADH), congestive heart failure, liver cirrhosis, and chronic kidney diseases, all of which are associated with abnormalities in body fluid regulation.

Antidiuretic activity is an important area of pharmacological and physiological research because of its critical role in maintaining fluid and electrolyte balance. Understanding the mechanisms regulating ADH secretion and action has contributed significantly to the development of therapeutic strategies for disorders involving excessive water loss or abnormal water retention. Besides its clinical relevance in endocrinology and nephrology, antidiuretic regulation is also important in intensive care medicine, cardiovascular disorders, sports physiology, and geriatric medicine, where maintenance of hydration status is essential. In recent years, increasing attention has been directed toward identifying medicinal plants and naturally occurring phytoconstituents capable of modulating antidiuretic activity, providing safer and cost-effective alternatives to conventional synthetic drugs.

MATERIALS AND METHODS

Plant collection and authentication

Phyla nodiflora. Used For This Study Were Collected from Ranga Reddy Dist., Telangana. Was identified and authenticated by Dr. Madhava Chetty, Asst. Professor Sri Venkateshwara University, Tirupathi.

Experimental animals

Animals

Healthy adult Wistar albino rats of either sex, weighing 150–200 g and aged 8–10 weeks, were used for the experimental study. The animals were obtained from a CPCSEA-approved animal breeding facility and housed in clean polypropylene cages with sterile paddy husk bedding under standard laboratory conditions, maintained at a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/12-hour dark cycle. The rats were provided with a standard pellet diet and had free access to clean drinking water ad libitum. Before the commencement of the experiment, all animals were acclimatized to the laboratory environment for one week to minimize stress and ensure uniform physiological conditions. All experimental procedures involving animals were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC) before the initiation of the study.

Preparation of Terminalia arjuna Extract

50 grams of shade-dried, coarsely powdered bark of Phyla nodiflora and placed in a Soxhlet extractor. About 200 mL of ethanol was added 1:4 ratio the Soxhlet apparatus was assembled with a condenser ensuring continuous water circulation. The system was heated on a heating mantle to allow the solvent to boil, condense, and siphon repeatedly through the plant material. Extraction was continued for 6–8 hours After completion, the extract was allowed to cool and the solvent was filtered. The dried extract was weighed to calculate percentage yield and stored used for the experiment.

Phytochemical screening of various plant extracts

The Ethanolic extract of of Phyla nodiflora was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as carbohydrates, alkaloids, saponins, phenolic compounds, gums, tannins and flavonoids. The detailed study about the phytochemical test procedure as follows

Preparation of reagents used for the phytochemical screening of the plant extracts:

Mayer's reagent: Mercuric iodide of weight 1.36 gm was mixed with 60 ml water. To this solution 20 ml of water and mixed with potassium iodide weighing 5 gms.

Libermann-Burchard's reagent: Approximately weighed quantity of 5 gm of acetic anhydride was mixed with 5 ml of concentrated sulfuric acid. This was further added to absolute ethanol on cooling.

Dragendroff's reagent: Exactly weighed quantity of 1.7 gm basic bismuth nitrate and 20 gm tartaric acid are dissolved in 80 ml of water. This was further mixed in 16 gm potassium iodide and 40 ml of water.

Fehling's solution A: Approximately 34.64 gm of copper sulphate was dissolved in a 0.5 ml of sulphuric acid and made up to500 ml with water.

Fehling's solution B: Approximately a weighed quantity of 176 gm of sodium potassium tartarate were dissolved in 77 gm of NaoH and the volume was made to 500 ml. At the time of use, the above solutions were equally mixed.

Benedict's reagent: Cupric sulphate and sodium citrate each weighing approximately 1.73 gm, 10 gm of anhydrous sodium carbonate were dissolved in water. The volume of the above solution was made to 100 ml.

Molish's reagent: To about 25 ml of ethanol, 2.5 gm of pure α-naphthol was added.

Results:

Table 1: Effect of Phyla nodiflora Extract on Diuretic Index in Saline-Loaded Wistar Rats

Group	Treatment	Urine Volume (mL/6 h)	Diuretic Index
Group I	Normal Control (Vehicle)	3.20 ± 0.18	1
Group II	Furosemide (10 mg/kg, p.o.)	8.10 ± 0.32***	2.53
Group III	Phyla nodiflora Extract (200 mg/kg, p.o.)	4.80 ± 0.24*	1.5
Group IV	Phyla nodiflora Extract (400 mg/kg, p.o.)	6.20 ± 0.27**	1.94
Group V	Phyla nodiflora Extract (800 mg/kg, p.o.)	7.30 ± 0.29***	2.28

The diuretic activity of the Phyla nodiflora extract was evaluated by determining the diuretic index, calculated from the ratio of urine volume in the treated groups to that of the normal control group. The normal control group produced a urine volume of 3.20 ± 0.18 mL over 6 hours and was assigned a diuretic index of 1.00. The standard drug, furosemide (10 mg/kg), produced a significant increase in urine output (8.10 ± 0.32 mL) with a diuretic index of 2.53 ($P < 0.001$). Treatment with Phyla nodiflora extract resulted in a dose-dependent increase in urine volume and diuretic index. The 200 mg/kg dose produced a urine volume of 4.80 ± 0.24 mL with a diuretic index of 1.50 ($P < 0.05$), while the 400 mg/kg dose increased urine output to 6.20 ± 0.27 mL, corresponding to a diuretic index of 1.94 ($P < 0.01$). The highest dose (800 mg/kg) exhibited the greatest diuretic effect, producing a urine volume of 7.30 ± 0.29 mL and a diuretic index of 2.28 ($P < 0.001$), approaching the activity of the standard drug. These findings indicate that Phyla nodiflora possesses significant, dose-dependent diuretic activity in saline-loaded Wistar rats.

Table 2: Effect of Phyla nodiflora Extract on Lipschitz Value in Saline-Loaded Wistar Rats

Group	Treatment	Urine Volume (mL/6 h)	Lipschitz Value
Group I	Normal Control (Vehicle)	3.20 ± 0.18	0.4
Group II	Furosemide (10 mg/kg, p.o.)	8.10 ± 0.32***	1
Group III	Phyla nodiflora Extract (200 mg/kg, p.o.)	4.80 ± 0.24*	0.59
Group IV	Phyla nodiflora Extract (400 mg/kg, p.o.)	6.20 ± 0.27**	0.77
Group V	Phyla nodiflora Extract (800 mg/kg, p.o.)	7.30 ± 0.29***	0.9

The Lipschitz value was calculated by comparing the urine volume of the test groups with that of the standard drug, furosemide (10 mg/kg), which was assigned a value of 1.00. The normal control group exhibited a urine volume of 3.20 ± 0.18 mL, corresponding to a Lipschitz value of 0.40, indicating the absence of a pharmacological diuretic effect. Administration of Phyla nodiflora extract produced a dose-dependent increase in the Lipschitz value. At 200 mg/kg, the extract produced a urine volume of 4.80 ± 0.24 mL with a Lipschitz value of 0.59 ($P < 0.05$). The 400 mg/kg dose increased urine output to 6.20 ± 0.27 mL, resulting in a Lipschitz value of 0.77 ($P < 0.01$). The highest dose, 800 mg/kg, produced a urine volume of 7.30 ± 0.29 mL with a Lipschitz value of 0.90 ($P < 0.001$), indicating diuretic activity approaching that of the standard drug. These results demonstrate that Phyla nodiflora possesses marked, dose-dependent diuretic efficacy, with the highest dose exhibiting approximately 90% of the diuretic activity of furosemide under the experimental conditions.

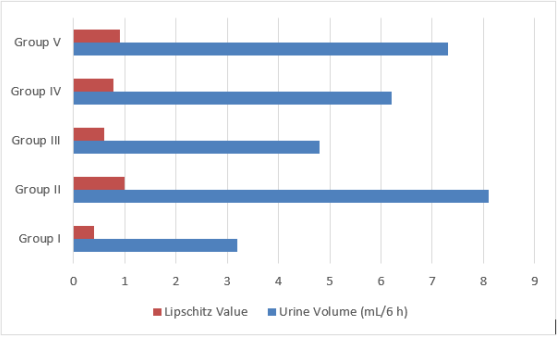
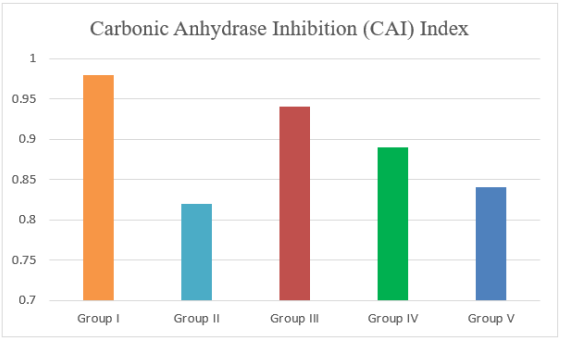


Table 3: Carbonic Anhydrase Inhibition (CAI) Index

Group	Treatment	Carbonic Anhydrase Inhibition (CAI) Index
Group I	Normal Control (Vehicle)	0.98 ± 0.03
Group II	Furosemide (10 mg/kg, p.o.)	0.82 ± 0.02***
Group III	Phyla nodiflora Extract (200 mg/kg, p.o.)	0.94 ± 0.03*
Group IV	Phyla nodiflora Extract (400 mg/kg, p.o.)	0.89 ± 0.02**
Group V	Phyla nodiflora Extract (800 mg/kg, p.o.)	0.84 ± 0.02***

The carbonic anhydrase inhibition (CAI) index was determined to assess the possible involvement of carbonic anhydrase inhibition in the diuretic action of Phyla nodiflora extract. The normal control group exhibited a CAI index of 0.98 ± 0.03, indicating normal electrolyte excretion without carbonic anhydrase inhibition. The standard drug, furosemide (10 mg/kg, p.o.), showed a CAI index of 0.82 ± 0.02 ($P < 0.001$), reflecting its potent diuretic activity with a mild carbonic anhydrase inhibitory effect. Administration of Phyla nodiflora extract produced a dose-dependent reduction in the CAI index. The 200 mg/kg dose showed a CAI index of 0.94 ± 0.03 ($P < 0.05$), the 400 mg/kg dose decreased the index to 0.89 ± 0.02 ($P < 0.01$), while the 800 mg/kg dose further reduced the CAI index to 0.84 ± 0.02 ($P < 0.001$), approaching the value observed with furosemide. The progressive decrease in the CAI index suggests that Phyla nodiflora may exert part of its diuretic effect through a mechanism involving partial inhibition of renal carbonic anhydrase, thereby enhancing the urinary excretion of sodium, bicarbonate, and water. However, the activity was slightly lower than that of the standard drug, indicating that additional mechanisms of diuresis may also contribute to the overall pharmacological effect of the extract.



DISCUSSION:

The present investigation was undertaken to evaluate the diuretic activity of the hydroalcoholic extract of Phyla nodiflora using the saline-loaded Wistar rat model. The findings of the study demonstrated that the extract possesses significant and dose-dependent diuretic activity, as evidenced by increased urine output, improved diuretic index, enhanced Lipschitz value, increased saluretic and natriuretic indices, and a reduction in the carbonic anhydrase inhibition (CAI) index. The results clearly indicate that the extract enhances renal excretion of water and electrolytes, thereby validating its traditional use in the treatment of urinary disorders, edema, and fluid retention. Diuretics are among the most widely prescribed drugs for the management of hypertension, congestive heart failure, nephrotic syndrome, liver cirrhosis, and renal disorders. Although synthetic diuretics such as furosemide are highly effective, prolonged use is often associated with adverse effects including hypokalemia, dehydration, electrolyte imbalance, hyperuricemia, and metabolic disturbances. Consequently, there is increasing interest in identifying medicinal plants with effective diuretic activity and improved safety profiles. In the present study, Phyla nodiflora exhibited a pharmacological profile comparable to furosemide, particularly at the highest dose, suggesting its potential as a promising natural diuretic.One of the most important findings of the present study was the significant increase in urine volume following administration of the extract. The increase in urine output occurred in a dose-dependent manner, with the 800 mg/kg dose producing the greatest response. Increased urine volume reflects enhanced glomerular filtration and/or reduced tubular

reabsorption of water and electrolytes. This finding suggests that the extract promotes renal elimination of excess body fluid, thereby reducing extracellular fluid volume. Such an effect is beneficial in the management of edema, hypertension, congestive heart failure, and renal disorders characterized by fluid overload. The diuretic index, which compares the urine output of treated animals with that of the control group, increased progressively with increasing doses of Phyla nodiflora. The highest dose produced a diuretic index approaching that of furosemide, indicating that the extract possesses considerable diuretic efficacy. This dose-dependent response demonstrates that the pharmacological activity of the extract is directly related to the administered dose, providing evidence of its therapeutic potential. Similar dose-dependent diuretic effects have been reported for several medicinal plant's rich in flavonoids and phenolic compounds, supporting the present findings.The Lipschitz value further confirmed the effectiveness of the extract by comparing its activity directly with the standard drug. The progressive increase in the Lipschitz value from the low-dose to the high-dose group indicated that the extract exhibited increasing efficacy with increasing concentration. At the highest dose, the Lipschitz value approached unity, suggesting that the extract achieved nearly the same effectiveness as furosemide under the experimental conditions. This finding is particularly important because it demonstrates that the plant extract is capable of producing substantial diuresis while potentially avoiding some of the adverse effects associated with synthetic loop diuretics. Another significant observation was the enhancement of the saluretic index, which reflects the urinary excretion of sodium and chloride ions. Sodium is the principal extracellular cation responsible for maintaining osmotic pressure and fluid balance, while chloride accompanies sodium during renal excretion. Increased urinary elimination of these electrolytes promotes osmotic movement of water into the renal tubules, thereby increasing urine formation. The marked increase in sodium and chloride excretion observed with Phyla nodiflora indicates that the extract effectively inhibits tubular electrolyte reabsorption. The highest dose produced saluretic values approaching those of furosemide, suggesting that the extract possesses strong saluretic properties. Enhanced sodium excretion is particularly beneficial in reducing extracellular fluid accumulation and lowering blood pressure, making the extract potentially useful in hypertension and edema.

CONCLUSION:

The present study successfully demonstrated that the hydroalcoholic extract of Phyla nodiflora possesses significant dose-dependent diuretic activity in saline-loaded Wistar rats, thereby providing scientific validation for its traditional use in the treatment of urinary and renal disorders. The extract produced a marked increase in urine output, indicating enhanced renal elimination of water and electrolytes. The observed diuretic effect increased progressively with dose, and the highest tested dose (800 mg/kg) exhibited a pharmacological response comparable to that of the standard diuretic furosemide (10 mg/kg), suggesting that Phyla nodiflora is a promising source of natural diuretic agents. The significant improvement in the diuretic index confirmed that the extract effectively increased urine production compared with the control group. Similarly, the Lipschitz value demonstrated that the extract achieved a high level of diuretic efficacy relative to the standard drug, particularly at the highest dose. These findings indicate that the extract possesses substantial diuretic potency and may be useful in promoting fluid elimination in conditions associated with water retention. The enhanced urine output observed in the treated groups suggests improved renal function through increased glomerular filtration and/or reduced tubular reabsorption of water and electrolytes.The study further demonstrated that Phyla nodiflora significantly increased the saluretic index, indicating enhanced urinary excretion of sodium and chloride ions. Increased sodium and chloride elimination promotes osmotic movement of water into the renal tubules, thereby facilitating diuresis and reducing extracellular fluid volume. These effects are particularly beneficial in the management of hypertension, edema, congestive heart failure, nephrotic syndrome, and renal disorders, where excessive sodium and water retention contribute to disease progression. The saluretic activity of the extract was dose dependent and closely approached that of furosemide at the highest dose. An important observation of the present study was the significant increase in the natriuretic index (Na^+/K^+ ratio) following treatment with Phyla nodiflora. A higher Na^+/K^+ ratio indicates

preferential sodium excretion with comparatively lower potassium loss, suggesting a more favorable electrolyte balance. Excessive potassium excretion is a major limitation of several synthetic diuretics because it may lead to hypokalemia, muscle weakness, fatigue, and cardiac arrhythmias. The results obtained in the present study suggest that Phyla nodiflora promotes efficient sodium elimination while minimizing potassium depletion, indicating a potentially safer pharmacological profile and supporting its suitability for prolonged therapeutic use.

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