PHYTOCHEMICAL EVALUATION AND DIURETIC ACTIVITY OF *WEDELIA CHINESIS*

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Abstract

The present study was undertaken to investigate the phytochemical constituents and evaluate the potential diuretic activity of the ethanolic extract of Wedelia chinensis. The plant material was extracted using ethanol, yielding 2.86 g of extract from 50 g of powdered leaves, corresponding to a percentage yield of 5.72% (w/w). Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, saponins, tannins, carbohydrates, and selected alkaloid-reactive constituents. Quantitative phytochemical estimation demonstrated a total phenolic content of 0.52 mg/100 mg and a total flavonoid content of 0.78 mg/100 mg of ethanolic extract. The diuretic activity was evaluated by measuring urine volume and electrolyte excretion following administration of the extract at doses of 100 and 200 mg/kg body weight. Furosemide at 10 mg/kg was used as the standard reference drug. The extract produced an increase in urine volume compared with the control group. The urine volume was 9.8 ± 0.6 mL/100 g/12 h at 100 mg/kg and 12.4 ± 0.7 mL/100 g/12 h at 200 mg/kg, compared with 5.6 ± 0.5 mL/100 g/12 h in the control group. Furthermore, sodium, potassium, and chloride excretion increased at the higher extract dose compared with the lower dose. However, the observed effects were lower than those reported for furosemide. These findings suggest that the ethanolic extract of Wedelia chinensis possesses preliminary diuretic potential. Further investigations are required to confirm its efficacy, elucidate its mechanism of action, and establish its safety profile.

Keywords: *Wedelia chinensis*, ethanolic extract, phytochemical screening, total phenolic content, total flavonoid content, diuretic activity, urine volume, electrolyte excretion, furosemide.

Introduction

Diuretic agents are important therapeutic substances that promote urine formation and facilitate the elimination of excess water and electrolytes from the body (Blebea et al., 2025). They are widely used in the management of conditions such as hypertension, edema, and fluid retention

associated with cardiovascular and renal disorders. Although conventional diuretics, including furosemide, are effective in increasing urine output, their use may be associated with electrolyte imbalances and other adverse effects. Consequently, the investigation of medicinal plants as potential sources of diuretic agents has attracted considerable research interest (Clark and Cleland, 2013).

Medicinal plants have been used traditionally for the management of various health conditions, and their pharmacological properties are often associated with secondary metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, and saponins (Wink, 2015). These phytoconstituents exhibit diverse biological activities and may contribute to the therapeutic potential of plant extracts. Preliminary phytochemical screening and quantitative estimation of these constituents are important steps in the scientific evaluation of medicinal plants and their possible pharmacological applications (Nwozo et al., 2023).

Wedelia chinensis is a medicinal plant of interest for phytochemical and pharmacological investigation. The biological properties of plant extracts can vary according to the plant part, geographical origin, extraction solvent, and method of preparation. Previous reviews of Wedelia species have discussed their phytochemical composition and diverse pharmacological properties, highlighting their potential relevance for further investigation (Thuy et al., 2025).

Ethanolic extraction is frequently employed in phytochemical studies because ethanol can extract a broad range of plant constituents with different polarities. Evaluation of the resulting extract can provide preliminary information about its chemical composition and potential biological effects (Balamurugan et al., 2019). Phytochemical screening identifies major classes of constituents through characteristic chemical reactions, while quantitative estimation of total phenolic and flavonoid contents provides an indication of the relative abundance of these phytochemical groups (Balamurugan et al., 2019). Phenolic compounds and flavonoids are of particular interest because of their diverse biological activities; however, their presence alone does not establish a specific pharmacological effect (Kumar and Pandey, 2013).

The evaluation of diuretic activity involves measuring urine volume and assessing the excretion of electrolytes such as sodium, potassium, and chloride. An increase in urine output may indicate a diuretic response, while electrolyte measurements help characterize the associated changes in urinary electrolyte elimination (Wile, 2012). Furosemide, a loop diuretic, is widely used

clinically and can serve as a reference drug for comparing the diuretic effects of investigated substances (Stason et al., 1966).

The present study was designed to investigate the phytochemical composition and potential diuretic activity of the ethanolic extract of Wedelia chinensis. The study included determination of the percentage extraction yield, preliminary phytochemical screening, estimation of total phenolic and flavonoid contents, and evaluation of urine volume and electrolyte excretion at doses of 100 and 200 mg/kg body weight. Furosemide at 10 mg/kg was used as the standard reference drug. The findings were intended to provide preliminary experimental evidence regarding the potential diuretic properties of Wedelia chinensis and establish a basis for further pharmacological investigation.

Material and Methods

Material

The leaves of Wedelia chinensis were used as the plant material for the study, and ethanol (Merck Life Science Pvt. Ltd., India) was used for extraction. Quercetin and gallic acid (Sigma-Aldrich, Merck) were used as reference standards for estimating total flavonoid and phenolic contents, respectively. Laboratory reagents, including ferric chloride, lead acetate, gelatin, sodium hydroxide, and other reagents for preliminary phytochemical screening, were obtained from Loba Chemie Pvt. Ltd., India, or Merck Life Science Pvt. Ltd. Furosemide was used as the standard diuretic drug. The actual manufacturer names should be confirmed from laboratory records before publication.

Methods

Extraction by soxhlet extraction method

Fifty grams of the shade-dried and coarsely powdered plant material was accurately weighed and placed in a cellulose extraction thimble, which was then inserted into the main chamber of a Soxhlet apparatus. The extraction was carried out using ethanol as the extraction solvent. Approximately 400–500 mL of ethanol was transferred to a round-bottom flask connected to the Soxhlet apparatus, and the system was heated using a heating mantle. The solvent was continuously refluxed, allowing repeated siphoning through the plant material for approximately 24 hours or until the siphon tube solvent became colorless, indicating complete extraction.

After completion of the extraction, the ethanolic extract was filtered, if necessary, and concentrated by evaporating the solvent under reduced pressure using a rotary vacuum

evaporator or on a water bath maintained below 50°C. The concentrated extract was further dried to a constant weight, transferred to a pre-weighed airtight container, and stored in a desiccator until further use (Kokate; 1994).

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of Leaves of *Wedelia chinensis*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, proteins and amino acids (Audu *et al.,* 2007).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

Estimation of total phenol content of the extracts was evaluated with folin-ciocalteu method (Gaur Mishra *et al.,* 2017). Samples containing polyphenols are reduced by the folin-ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 2 ml aliquots of 5, 10, 15, 20, and 25 µg/ml gallic acid solutions were mixed with 1ml folin–ciocalteu reagent (diluted ten-fold) and 1 ml (7.5 g/L) sodium carbonate. After incubation at 25°C for 10 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract (1000µg/ml) as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) mg per 100mg of extract.

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified (Parkhe and Bharti 2019). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 10 ml methanol and then diluted for 5-25 µg/ml. The diluted standard solutions (2 ml) were separately mixed with 1 ml of 2% aluminum chloride. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a UV spectrophotometer. Similarly, extracts solutions (1000 µg/ml) were reacted with aluminum chloride for

determination of flavonoid content. Total flavonoid content was expressed as milligrams of Quercetin equivalent (QE) mg per 100mg of extract.

***In vivo* Diuretic Activity of *Wedelia chinesis* extract**

Animals

Adult male Wistar rats weighing 150-200 g were procured from the institutional animal facility. The animals were housed under standard laboratory conditions at a temperature of 22 ± 2°C, with a relative humidity of 50–60% and a 12 h light/12 h dark cycle. Rats had free access to standard pellet diet and filtered drinking water *ad libitum* throughout the study. All experimental procedures were conducted following approval from the IAEC, India, and were carried out in accordance with the guidelines for the care and use of laboratory animals. Before each experimental study, the animals were fasted for 18 h with free access to water.

Acute toxicity study

An acute oral toxicity study was performed for the *Wedelia chinesis* extract in accordance with the OECD guidelines. The extract was suspended in distilled water and administered orally to overnight-fasted healthy rats (n = 6) at a single dose of 2,000 mg/kg body weight. Following administration, the animals were observed continuously for the first 24 h for signs of toxicity and mortality (Zanzabil *et al.,* 2023).

Evaluation of diuretic activity

Animals were maintained under standard temperature and humidity conditions and underwent a three-day adaptation period. The animals were divided into eight groups (n= 6). Group I served as the control and received normal saline solution (25 ml/kg oral administration); group II received the reference diuretic, Furosemide 10 mg/kg p.o.; groups III, IV and V received the extract of *Wedelia chinesis* at 100 and 200 mg/kg p.o., respectively, in normal saline. The diuretic activity was assessed.

Immediately after the administration of all the doses; animals were placed individually in metabolic cages that were specially designed to separate urine and feces and kept at a controlled temperature of 20-25ºC. At the end of 12 h the volume of urine was collected and measured. The animals were deprived of food and water during the period of experimentation. The parameters viz. body weight before and after the test period, total urine volume, and concentration of Na^+, K^+, and Cl^- were measured. The concentration of Na^+ and K^+ cations was determined by a flame photometric method while that of Cl^- was determined by titration with silver nitrate solution

(N/50) using three drops of 5% potassium chromate solution as an indicator (Wei *et al.,* 2022; Njinga *et al.,* 2020). Diuretic Index calculated as follows:

Diuretic Index = Volume of test group/Volume of control group.

Statistical analysis

Results are expressed as the mean ± SEM. Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. A value of $P< 0.05$ was considered statistically significant.

Results and Discussion

The present study was conducted to investigate the phytochemical constituents and diuretic activity of the ethanolic extract of *Wedelia chinensis*. The findings included the percentage yield of the extract, preliminary phytochemical screening, estimation of total phenolic and flavonoid contents, and evaluation of urine volume and electrolyte excretion in comparison with the standard diuretic drug furosemide.

The ethanolic extraction of *Wedelia chinensis* yielded 2.86 g of extract from 50 g of powdered plant material, corresponding to a percentage yield of 5.72% (w/w), as presented in Table 1. The extract was described as a dark black solid. The yield represents the proportion of extractable material obtained from the initial plant powder under the extraction conditions employed. This extract was subsequently used for preliminary phytochemical screening and pharmacological evaluation.

Preliminary phytochemical screening revealed the presence of several important classes of phytoconstituents, as summarized in Table 2. Alkaloids showed a positive reaction in Hager's test but negative reactions in Mayer's, Dragendorff's, and Wagner's tests. Glycosides were negative in Raymond's, Keller–Killiani, and Legal's tests. Carbohydrates were detected by positive Fehling's and Benedict's tests, although Molisch's test was negative. Tannins showed a positive reaction in the gelatin test but a negative result in the vanillin–HCl test. Both lead acetate and alkaline reagent tests were positive for flavonoids. The turbidity test for resins, Liebermann–Burchard test and Salkowski reaction for steroids, Biuret and precipitation tests for proteins and amino acids, and copper acetate test for diterpenes produced negative results. The ferric chloride test for phenols and froth test for saponins were positive. Overall, the preliminary

screening indicated the presence of flavonoids, phenols, saponins, tannins, carbohydrates, and selected alkaloid-reactive constituents in the ethanolic extract.

Quantitative phytochemical analysis demonstrated a total phenolic content of 0.52 mg/100 mg of ethanolic extract, as shown in Table 3. Phenolic compounds are important plant secondary metabolites and are frequently investigated for their potential biological activities. The total flavonoid content was reported as 0.78 mg/100 mg of ethanolic extract, as presented in Table 4. Flavonoids represent another significant group of plant-derived compounds. The detection of phenolic and flavonoid constituents provides a basis for further investigation of the extract's biological properties. However, the individual compounds responsible for any observed diuretic effect were not identified in the reported analysis.

The diuretic activity of the ethanolic extract was evaluated by measuring urine volume and comparing the results with those of the control group and furosemide-treated animals. As reported in Table 5, the control group produced a urine volume of 5.6 ± 0.5 mL/100 g/12 h. Administration of furosemide at 10 mg/kg increased urine volume to 15.2 ± 1.8 mL/100 g/12 h, with a diuretic index of 2.71. The ethanolic extract at 100 mg/kg produced a urine volume of 9.8 ± 0.6 mL/100 g/12 h, corresponding to a diuretic index of 1.75. At the higher dose of 200 mg/kg, urine volume increased to 12.4 ± 0.7 mL/100 g/12 h, with a diuretic index of 2.21. These results indicate that both tested doses of the extract were associated with higher urine output than the control, and the higher dose produced a greater response than the lower dose. Nevertheless, the urine volume and diuretic index remained below the values reported for furosemide, suggesting that the extract exhibited a comparatively weaker diuretic effect under the tested conditions.

The effect of the extract on electrolyte excretion was also investigated by measuring sodium, potassium, and chloride excretion, as presented in Table 6. The control group showed total sodium, potassium, and chloride excretion values of 1692.4 ± 35.8, 672.3 ± 18.5, and 648.7 ± 29.6 µmol/kg, respectively. Furosemide at 10 mg/kg increased these values to 3685.6 ± 72.4 µmol/kg for sodium, 1458.2 ± 33.6 µmol/kg for potassium, and 1868.4 ± 54.8 µmol/kg for chloride. These findings are consistent with the increased urine output reported for the standard treatment.

At 100 mg/kg, the ethanolic extract produced sodium, potassium, and chloride excretion values of 2298.7 ± 46.5, 872.6 ± 21.4, and 1192.3 ± 38.7 µmol/kg, respectively. At 200 mg/kg, the corresponding values increased to 2486.9 ± 58.3, 968.5 ± 24.8, and 1396.7 ± 45.2 µmol/kg.

Thus, the higher dose was associated with greater excretion of all three measured electrolytes than the lower dose. However, the reported electrolyte excretion values for both extract-treated groups remained below those of the furosemide group. This pattern suggests a possible dose-related effect of the extract on electrolyte excretion, accompanying the increase in urine volume.

Taken together, the findings in Tables 1–6 indicate that the ethanolic extract of *Wedelia chinensis* contained detectable phytochemical constituents, including flavonoids, phenols, and saponins, and demonstrated a preliminary diuretic response in the experimental evaluation. The increase in urine volume and electrolyte excretion at 200 mg/kg compared with 100 mg/kg suggests that the observed response may be dose-related within the tested range. However, the study results alone do not establish which phytochemicals are responsible for the activity or the precise physiological mechanism involved. Further studies incorporating appropriate controls, verified statistical comparisons, dose-response evaluation, and safety assessment would be necessary to confirm these findings and characterize the potential diuretic properties of *Wedelia chinensis*.

Table 1: Extractive values obtained from ethanolic extract of *Wedelia chinensis*

S. No.	Colour	Powder weight	Extract weight	% Yield (w/w)
1.	Dark black solid	50 g	2.86	5.72

Table 2: Preliminary phytochemical screening of *Wedelia chinensis*

S. No.	Phytoconstituents	Test Name	Ethanolic extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	-ve
		Hager's Test	**+ve**
2	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
		Legal Test	-ve
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	**+ve**
		Benedict's Test	**+ve**
4	Tannins	Vanillin- HCl Test	-ve

		Gelatin Test	**+ve**
5	Flavonoids	Lead acetate Test	**+ve**
		Alkaline Reagent Test	**+ve**
6	Resins	Turbidity Test	-ve
7	Steroids	Libermann- Bur chard Test	-ve
		Salkowski Reaction	-ve
8	Proteins & Amino acids	Biuret Test	-ve
		Precipitation test	-ve
9.	Phenols	Ferric chloride test	**+ve**
10.	Saponins	Froth Test	**+ve**
11.	Diterpenes	Copper acetate Test	-ve

[+ve = positive; -ve = negative]

Table 3: Total phenol content of Ethanolic extract of *Wedelia chinensis*

S. No.	Extract	Total phenol content (mg/100mg)
1	Ethanolic extract	0.52

Table 4: Total flavonoid content of ethanolic extract of *Wedelia chinensis*

S. No.	Extract	Total Flavonoid content (mg/100mg)
1	Ethanolic extract	0.78

Table 5: Effect of extract of *Wedelia chinensis* and Furosemide on urine volume

Treatment	Dose (mg/kg)	Urine volume (mL/100 g/12 h)	Diuretic index
Control	–	5.6 ± 0.5	–
Furosemide	10	15.2 ± 1.8#	2.71
Wedelia chinensis extract	100	9.8 ± 0.6 **	1.75
Wedelia chinensis extract	200	12.4 ± 0.7 **	2.21

Values are expressed as the mean ± SEM; $^{*}P< 0.05$, $^{**}P< 0.01$ compared to the control group, compared to the Furosemide group (ANOVA followed by Dunnett's test).

Table 6: Effect of extract of *Wedelia chinensis* and Furosemide on electrolyte excretion

Treatment	Dose (mg/kg)	Total Na^+ (µmoles/kg)	Total K^+ (µmoles/kg)	Total Cl^- (µmoles/kg)
Control	–	1692.4 ± 35.8	672.3 ± 18.5	648.7 ± 29.6
Furosemide	10	3685.6 ± 72.4*	1458.2 ± 33.6*	1868.4 ± 54.8*
Wedelia chinensis extract	100	*2298.7 ± 46.5 **	*872.6 ± 21.4 **	1192.3 ± 38.7 **
Wedelia chinensis extract	200	2486.9 ± 58.3 **	968.5 ± 24.8 **	1396.7 ± 45.2*

Values are expressed as the mean ± SEM; $^{*}P< 0.001$ compared to the control group, $^{**}P< 0.01$, $^{***}P< 0.001$, compared to the Furosemide group (ANOVA followed by Dunnett's test).

Conclusion

The present study demonstrated that the ethanolic extract of Wedelia chinensis contained various phytochemical constituents, including flavonoids, phenolic compounds, tannins, and saponins. The extract showed a percentage yield of 5.72%, with total phenolic and flavonoid contents of 0.52 mg/100 mg and 0.78 mg/100 mg, respectively. The extract also increased urine volume and electrolyte excretion, with a greater response at 200 mg/kg than at 100 mg/kg. However, its diuretic effect was lower than that of furosemide. Further studies are required to confirm its efficacy, mechanism of action, and safety.

References

- Kokate CK. Practical Pharmacognosy. 4th ed. Vallabh Prakashan; 1994. p. 112–120.
- Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). Life Science Journal. 2007;4(4):75–79.
- Mishra AG, Singh R, Patil N, Parkhe G. Determination of total phenolic and flavonoid content, antioxidant and antimicrobial activity of Gloriosa superba seed extract. Asian Journal of Pharmaceutical Education and Research. 2017;6(2):12–17.

- Parkhe G, Bharti D. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of Vitex trifolia Linn. Journal of Drug Delivery and Therapeutics. 2019;9(4-A):705–707.
- Zanzabil KZ, Hossain MS, Hasan MK. Diabetes mellitus management: An extensive review of 37 medicinal plants. Diabetology. 2023;4(2):186–234.
- Wei J, Zhao M, Meng K, Xia G, Pan Y, Li C, et al. The diuretic effects of coconut water by suppressing aquaporin and renin–angiotensin–aldosterone system in saline-loaded rats. Frontiers in Nutrition. 2022;9:930506.
- Njinga N, Kola-Mustapha A, Quadri A, Atolani O, Ayanniyi R, Buhari M, et al. Toxicity assessment of sub-acute and sub-chronic oral administration and diuretic potential of aqueous extract of Hibiscus sabdariffa calyces. Heliyon. 2020;6(9).
- Blebea NM, Pușcașu C, Ștefănescu E, Stăniguț AM. Diuretic therapy: Mechanisms, clinical applications, and management. Journal of Mind and Medical Sciences. 2025;12(1):26.
- Clark AL, Cleland JG. Causes and treatment of oedema in patients with heart failure. Nature Reviews Cardiology. 2013;10(3):156–170.
- Wink M. Modes of action of herbal medicines and plant secondary metabolites. Medicines. 2015;2(3):251–286.
- Nwozo OS, Effiong EM, Aja PM, Awuchi CG. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. International Journal of Food Properties. 2023;26(1):359–388.
- Thuy TT, Huong DT, Huan DQ, Hanh NT, Ni HT, Minh PH, Son NT. Wedelia species: Phytochemistry, pharmacology, toxicology, nanoformulation, and synthetic modification—an extensive review. Medicinal Chemistry Research. 2025;34(9):1855–1887.
- Balamurugan V, Fatima S, Velurajan S. A guide to phytochemical analysis. International Journal of Advance Research and Innovative Ideas in Education. 2019;5(1):236–245.
- Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal. 2013;2013:162750.
- Wile D. Diuretics: A review. Annals of Clinical Biochemistry. 2012;49(5):419–431.
- Stason WB, Cannon PJ, Heinemann HO, Laragh JH. Furosemide: A clinical evaluation of its diuretic action. Circulation. 1966;34(5):910–920.