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OPEN ACCESS Original Article

Evaluation of Diuretic and Antibacterial Activities of *Tribulus terrestris* Ethanolic Extract

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ABSTRACT

Background: Renal disorders like urinary tract infections and imbalances in body fluids and electrolytes still create significant health and economic challenges, particularly in areas with fewer resources.
Objective: This study aimed to assess the *diuretic* and antibacterial effects of the ethanol extract of *Tribulus terrestris* (EETT) through both *in vivo* and *in vitro* experimental approaches.
Methods: The diuretic activities of ethanol extract of *Tribulus terrestris* (EETT) were evaluated in rodent models, while its antibacterial potential was investigated *in vitro*. Diuretic activity was assessed in rats by measuring urine output and electrolyte excretion (sodium, potassium, and chloride) following administration of EETT at the different doses; furosemide served as the reference diuretic. Antibacterial activity was tested in vitro using the disc diffusion technique on four bacterial strains: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae.*
Results: EETT produced a dose-dependent diuretic effect, with the highest dose (450 mg/kg) significantly increasing urine output ($p < 0.05$) and sodium excretion ($p < 0.01$) to levels comparable with furosemide. Potassium excretion also rose significantly, whereas chloride excretion decreased at higher doses, suggesting a possible chloride-sparing mechanism. Antibacterial testing revealed moderate inhibition of *Staphylococcus aureus* (15 mm) and *Pseudomonas aeruginosa* (23 mm), while *Escherichia coli and Klebsiella pneumoniae* exhibited no detectable susceptibility under current testing conditions.
Conclusion: The ethanol extract of *Tribulus terrestris* showed dose-dependent diuretic effects and moderate antibacterial activity. These findings support further research, including clinical studies, to explore its potential as a complementary treatment for kidney-related disorders.

Keywords: *Tribulus terrestris, diuretic activity, antibacterial activity.*

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INTRODUCTION

Kidney disorders constitute a major global health burden, contributing to rising morbidity, mortality, and healthcare costs [1,2]. These include chronic kidney disease (CKD) [1], acute kidney injury (AKI) [3], nephrolithiasis [4], and urinary tract infections (UTIs) [5], all of which can lead to renal dysfunction and adverse clinical outcomes [6,7]. Specifically, CKD affects about 9–10% of the global population and is projected to become the fifth leading cause of death by 2050 [1]. In comparison, AKI affects more than 13 million people annually, with 85% of the burden occurring in low- and middle-income countries [7].

In many low- and middle-income countries, including Yemen, kidney diseases pose a growing public health challenge. Regional studies have identified several key contributors: infections [8], obstructive uropathies (notably urolithiasis) [9], hypertension [10], and behavioral or environmental factors such as khat chewing [11], consumption of untreated water [12], and limited access to renal healthcare services [8, 13, 14]. These risk factors contribute to rising rates of renal failure locally and reflect global patterns where limited renal care access worsens disease progression.

Given the global burden of kidney diseases and the limitations of conventional therapies, interest has grown in exploring complementary approaches, particularly those with potential antiurolithiatic, diuretic, or antibacterial effects. Medicinal plants, rich in bioactive compounds, have been widely studied in this context. *Tribulus terrestris (T. terrestris*), traditionally used for urinary and renal ailments, is one such plant. However, despite its traditional applications, well-characterized experimental investigations evaluating its multi-targeted effects remain limited. During this century, many bacteria resistant to the antibiotics available in the health market have been enhanced by the indiscriminate use of these antibiotics. Most of these antibiotics have unpleasant side effects depending on the individual. Hence, researchers had to search for alternative agents for microbes [15]. This study aimed to assess the diuretic and antibacterial effects of the ethanol extract of *Tribulus terrestris* (EETT) through both *in vivo and in vitro* experimental approaches.

METHODOLOGY

Plant Material and Extraction

The aerial and subterranean parts of *Tribulus terrestris (T. terrestris),* including leaves, stems, roots, and fruits, were collected between May and June 2023 from wild vegetation zones in Taiz and Ibb governorates, located in Yemen's central highlands at altitudes of 1800–2000 meters above sea level (Figure 1). Taxonomic identification was confirmed by a botanist at the Department of Botany, Faculty of Science, Sana'a University, and a voucher specimen No. 721 was deposited at the Yemeni National Herbarium, Sana'a University (Figure 2).

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Figure 1. *Tribulus terrestris* photographed in its natural habitat in rural Ibb Governorate, Yemen.

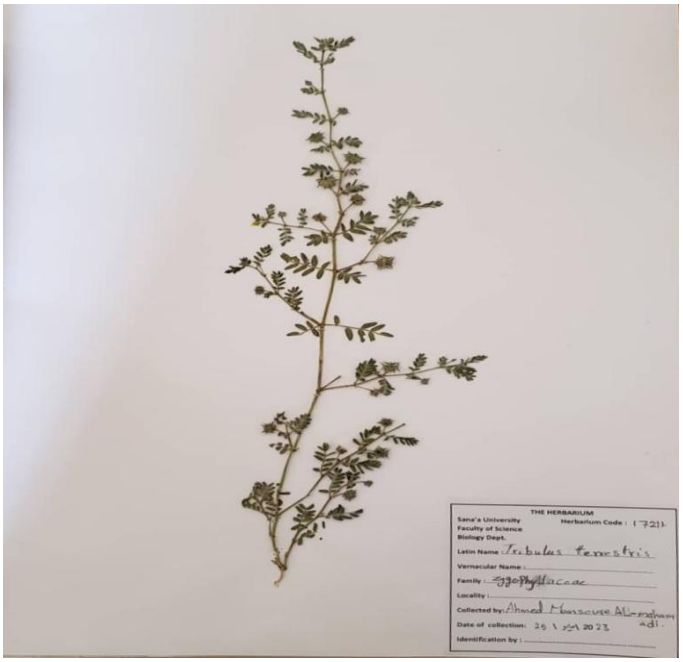

Figure 2. *Tribulus terrestris* herbarium specimen stored at the Faculty of Science, University of Sana'a, Yemen

Sequential extraction was performed using n-hexane, acetonitrile, and ethanol to maximize phytochemical diversity. The extraction method was adapted from Sasidharan et al. [16] and Altemimi et al. [17]. Based on a previous preliminary assessment conducted earlier in this project, the ethanol extract of *Tribulus terrestris* (EETT) demonstrated superior renal activity compared to the acetonitrile extract and was

therefore selected for further biological evaluation. The n-hexane extract was excluded due to insufficient yield.

Phytochemical Screening

Qualitative phytochemical screening of the ethanol extract was conducted using standard colorimetric and precipitation-based methods [18–20]. These protocols are commonly employed to identify major classes of secondary metabolites, such as saponins, alkaloids, flavonoids, anthraquinones, tannins, terpenoids, and resins.

Preparation of Extract Solution for Biological Testing

For biological assays, the dried ethanol extract was reconstituted in a mixture of DMSO and normal saline and sterilized using 0.45 µm Whatman® filter membranes under aseptic conditions. The final concentration of DMSO in the working solution was adjusted to 4%. A matching solvent mixture (4% DMSO in saline) was used as a negative control in all experiments.

Experimental Animals

Adult male Wistar rats weighing 180±20 g were obtained from the animal facility of the University of Science and Technology in Sana'a, Yemen. Animals were housed in groups of six per cage under controlled environmental conditions (23±2°C, 12-hour light/dark cycle), with free access to standard laboratory chow and water. The animals were acclimatized to the laboratory conditions for two weeks before the initiation of experimental procedures.

Ethics Approval

All animal experiments were conducted in accordance with institutional guidelines, and ethical approval (no. EAC/UST233) was obtained from the Ethical Committee of the University of Science and Technology prior to the commencement of the experiments.

Diuretic Effect Measurement

A total of 30 normal, adult male Wistar rats were randomly allocated into five groups, each consisting of six animals, to evaluate the diuretic activity. The study involved a single-dose administration followed by a 5-hour urine collection period. To standardize hydration status, all animals were deprived of water for 24 hours prior to the experiment. Bladders were gently emptied by applying light pressure to the pelvic area and tail traction. Immediately afterward, each rat received 15 mL of normal saline orally to establish uniform fluid loading conditions. The treatment groups were organized as follows:Group I (negative control) received an intraperitoneal (IP) injection of the vehicle (DMSO in normal saline); Group II (positive control) was administered furosemide at a dose of 20 mg/kg intraperitoneally. Groups III, IV, and V received IP injections of the EETT at doses of 150, 300, and 450 mg/kg body weight, respectively.

The selection of EETT doses was based on a previously published study by Chaudhary et al. [21] and further refined through a preliminary pilot experiment conducted in our laboratory to confirm safety and tolerability. All rats were housed in individual metabolic cages for the duration of the 5-hour collection period. Urine output was measured volumetrically using a graduated cylinder, and electrolyte concentrations (Na^+, K^+, and Cl^-) were analyzed using standard colorimetric techniques. Diuretic efficacy was evaluated using the following indices [22]:

- Diuretic Action = (Urine volume in test group) / (Urine volume in control group)
- Diuretic Activity = (Urine volume in test group) / (Urine volume in furosemide group)
- Electrolyte Index = (Urinary electrolyte excretion in test group) / (in control group).

All urine output and electrolyte measurements were performed by investigators blinded to the group allocation.

Antibacterial Assay

The EETT was prepared as previously described and was dissolved in sterile distilled water containing 4% DMSO to obtain a stock solution of 100 mg/mL. Filter paper discs (6 mm, Whatman® No. 1) were impregnated with 50 µL of this solution (equivalent to 5 mg/disc), dried at 37 °C for 24 hours, and stored at 4 °C until use [23].

Antibacterial activity was evaluated using the disc diffusion method on Mueller-Hinton agar (MH). Each MH plate was uniformly inoculated with the bacterial suspension using a sterile cotton swab. EETT-

impregnated discs were aseptically placed on the agar surface with adequate spacing. Discs containing standard antibiotics—amoxicillin-clavulanate (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), and ceftriaxone (5 µg)—served as positive controls. DMSO-only discs were used as negative controls to exclude solvent-related effects.

The plates were incubated at 37°C for 24 hours, after which the diameters of the inhibition zones were measured in millimeters using a transparent ruler. All experiments were conducted in triplicate, and the results are reported as the mean zone of inhibition (ZOI). A plant extract producing inhibition zones >10 mm was considered to exhibit notable antibacterial activity [24].

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism, version 8.0.2 (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± standard error of the mean (SEM). Differences between groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparisons test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Extract Yield and Phytochemical Screening

The ethanol extract of *Tribulus terrestris* (EETT) yielded approximately 5.7% (w/w), based on the weight of the dried powdered plant material. The qualitative phytochemical analysis revealed the presence of major phytoconstituents, including saponins, alkaloids, flavonoids, anthraquinones, and tannins.

Diuretic Activity: Experimental Assessment

EETT exhibited a dose-dependent diuretic effect in rats, as evidenced by increased urine output and alterations in urinary electrolyte concentrations. While the overall diuretic response was milder than that of furosemide, the extract significantly enhanced sodium and potassium excretion at higher doses. Notably, chloride excretion showed an inverse profile, suggesting a possible chloride-sparing effect.

Effect of EETT on Urinary Volume

Urinary volume collected over a 5-hour period showed a dose-dependent increase in EETT-treated rats. While the 150 mg/kg and 300 mg/kg doses did not produce statistically significant changes (6.00 and 7.00 mL, respectively; $p > 0.05$), the 450 mg/kg dose significantly increased urine output (7.92 mL; $p < 0.05$) compared to the control group (5.67 mL). The furosemide-treated group exhibited the highest diuretic response (9.17 mL; $p < 0.01$ vs. control).

Calculated diuretic action values for EETT were 1.06, 1.23, and 1.40 for 150, 300, and 450 mg/kg doses, respectively, compared to 1.62 for furosemide. Corresponding diuretic activity values, relative to furosemide, were 0.65, 0.76, and 0.86. These results are presented in Table 1.

Table 1: Urinary volume and diuretic indices in Wistar rats after single-dose administration of EETT

Groups	Urine volume (mL/5h)	Diuretic actiona	Diuretic activityb
Control	5.67 ± 0.42	1	--
Furosemide 20 mg/kg	9.17 ± 0.87 **	1.62	1
EETT 150 mg/kg	6.0 ± 0.26 ns	1.06	0.65
EETT 300 mg/kg	7.0 ± 0.52 ns	1.23	0.76
EETT 450 mg/kg	7.92 ± 0.01 *	1.4	0.86

Data are presented as mean ± SEM (n = 6). *p < 0.05, *p < 0.01, ns: not significant, all versus control group. EETT: ethanol extract of *Tribulus terrestris*. a. Diuretic action was calculated as the ratio of urine volume in the treated group to that in the control group. b. Diuretic activity was calculated as the ratio of urine volume in the treated group to that in the furosemide group.

Effect of EETT on Urinary Sodium Concentration

Urinary sodium concentration in the control group was 55.24 mmol/L. EETT at 150 mg/kg produced a non-significant increase (69.58 mmol/L; $p > 0.05$), whereas 300 and 450 mg/kg doses led to significant elevations—120 and 135.4 mmol/L, respectively ($p < 0.01$). The furosemide-treated group showed a

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urinary sodium concentration of 113.8 mmol/L ($p < 0.05$). Sodium excretion at 450 mg/kg approached or slightly exceeded that observed with furosemide, indicating a comparable diuretic effect at this dose. The corresponding sodium excretion indices were 1.3, 2.2, and 2.5 for 150, 300, and 450 mg/kg doses, respectively, compared to 2.1 for furosemide. These results are summarized in Table 2.

Effect of EETT on Urinary Potassium Concentration

Urinary potassium concentrations exhibited a dose-dependent increase in EETT-treated groups. No significant difference was observed at 150 mg/kg (33.64 mmol/L, $P > 0.05$) compared to the control group (20.54 mmol/L). However, significant increases were detected at 300 mg/kg (41.12 mmol/L; $p < 0.05$) and 450 mg/kg (43.62 mmol/L; $p < 0.05$). The furosemide-treated group recorded the highest potassium concentration (49.26 mmol/L; $p < 0.01$ vs. control). Corresponding potassium excretion indices were 1.6, 2.0, and 2.1 for the 150, 300, and 450 mg/kg EETT groups, respectively, while the furosemide group reached an index value of 2.4. These findings are summarized in Table 2.

Table 2: Urinary electrolyte concentrations and excretion indices in Wistar rats treated with EETT and furosemide

Group	Urine Na^+ (mmol/L)	Urine K^+ (mmol/L)	Urine Cl^- (mmol/L)	Na^+ Index	K^+ Index	Cl^- Index
Control	55.24 ± 4.03	20.54 ± 1.94	79.3 ± 4.14	1	1	1
Furosemide 20 mg/kg	113.8 ± 13.63 *	49.26 ± 3.57 **	141.2 ± 21.80 ns	2.1	2.4	1.8
EETT 150 mg/kg	69.58 ± 2.58 ns	33.64 ± 4.31 ns	79.38 ± 18.12 ns	1.3	1.6	1.0
EETT 300 mg/kg	120.0 ± 12.09 **	41.12 ± 4.34 *	51.62 ± 38.37 ns	2.2	2.0	0.7
EETT 450 mg/kg	135.4 ± 19.30 **	43.62 ± 6.67 *	20.04 ± 8.79 ns	2.5	2.1	0.3

Data are presented as mean ± SEM (n = 6). *$p < 0.05$, *$p < 0.01$, ns: not significant, all versus control group. EETT: ethanol extract of *Tribulus terrestris.*

Effect of EETT on Urinary Chloride Concentration

Urinary chloride concentration in the control group was 79.30 mmol/L. No significant change was observed with EETT at 150 mg/kg (79.38 mmol/L) or 300 mg/kg (51.62 mmol/L; $p > 0.05$). However, a significant reduction was noted at 450 mg/kg (20.04 mmol/L; $p < 0.01$). In contrast, the furosemide group exhibited a non-significant increase in chloride excretion (141.2 mmol/L). Chloride excretion indices for EETT decreased progressively with dose: 1.0, 0.7, and 0.3, respectively, compared to 1.8 in the furosemide group. The results are summarized in Table 2.

Antibacterial Activity of EETT and Comparison with Standard Antibiotics

The treatment with EETT exhibited inhibitory activity against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), producing mean inhibition zones of 15 mm and 23 mm, respectively. No zone of inhibition was observed against *Escherichia* coli (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), indicating that no detectable antibacterial effect was observed under the current conditions against these strains. Among the standard antibiotics tested, ciprofloxacin and levofloxacin showed strong activity against *S. aureus* and *P. aeruginosa*, with inhibition zones ranging from 19 to 24 mm. However, both agents exhibited small inhibition zones against *E. coli* and *K. pneumoniae* (≤10 mm), but the strains were classified as resistant based on interpretive criteria.

Amoxicillin and ceftriaxone displayed minimal or no activity, with all isolates classified as resistant to ceftriaxone and partially resistant to amoxicillin. These findings are illustrated in Figure 3.

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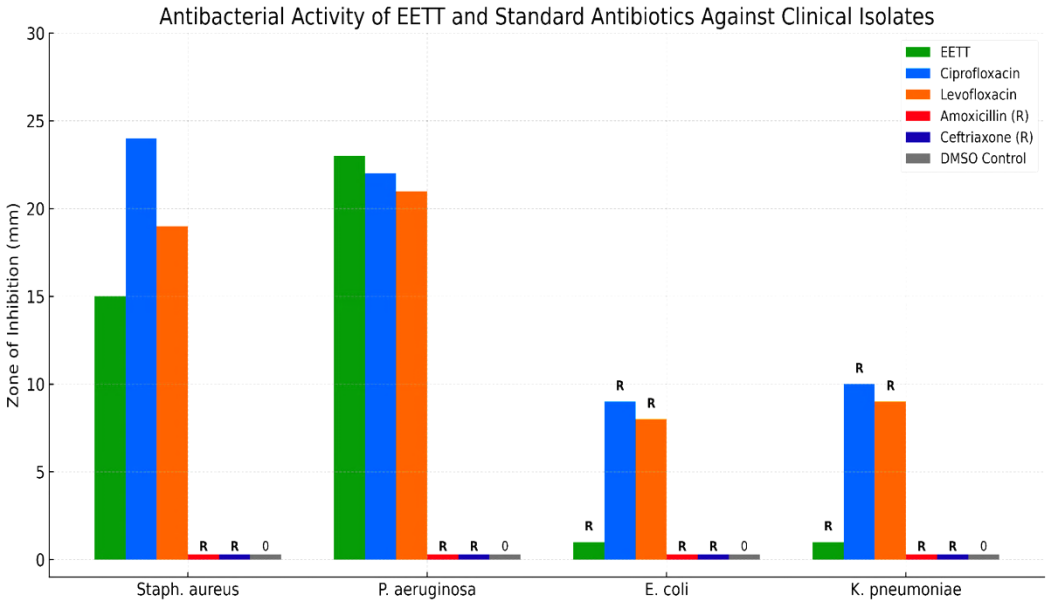

Figure 3. Antibacterial activity of EETT and selected antibiotics against clinical isolates. Results are expressed as mean inhibition zone diameter (mm) based on the disc diffusion assay.

EETT = ethanol extract of *Tribulus terrestris*; R = resistant; *S. aureus* = *Staphylococcus aureus; P. aeruginosa* = *Pseudomonas aeruginosa; E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae.*

DISCUSSION

This study provides experimental evidence supporting the diuretic and antibacterial potential of ethanol extract of *Tribulus terrestris* (EETT) in rodent models. These findings contribute to the expanding body of research on the therapeutic potential of phytomedicines for managing renal disorders and microbial infections. Phytochemical analysis of EETT revealed the presence of bioactive constituents such as saponins, flavonoids, tannins, and alkaloids. These compounds are widely recognized for their pharmacological activities. Saponins, flavonoids, alkaloids, and tannins are reported to exert diuretic actions through modulation of renal tubular transport mechanisms [25]. In addition, the antibacterial effect observed *in vitro* may be attributed to a synergistic action among multiple phytoconstituents present in the extract, including saponins, flavonoids, alkaloids, and tannins. These compounds are known to interfere with bacterial viability by disrupting cell membranes and inhibiting key enzymatic activities. Previous studies have reported that the antimicrobial properties of many medicinal plants often result from the combined effects of several classes of secondary metabolites [26]. The presence of these constituents in EETT may collectively explain its effects observed in this study.

EETT induced mild, dose-dependent increases in urine volume and electrolyte excretion, most evident at 300 and 450 mg/kg doses. Sodium and potassium excretion increased significantly at these doses. At 450 mg/kg, chloride excretion decreased markedly compared to control, suggesting a selective saluretic effect favoring sodium and potassium over chloride. Minimal changes were observed at 150 mg/kg. Although the overall diuretic effect was lower than furosemide, it remains pharmacologically relevant.

A previous study using an aqueous extract at 5 g/kg reported stronger diuretic responses, highlighting the influence of extraction solvent, dose, and experimental design on herbal pharmacological outcomes. The observed electrolyte modulation may involve renal tubular mechanisms, possibly influenced by flavonoids and alkaloids, though further mechanistic studies are required. Future research should explore renal enzyme activity, hormonal involvement, and molecular pathways, using larger sample sizes to validate these findings [27].

The changes in electrolyte excretion could involve renal tubular modulation, with possible

contributions from flavonoids and alkaloids, though this requires further investigation. Additionally, alkaloids present in the extract may contribute to the observed increase in urine output [28–30].

Overall, the urinary effects observed in this study align with previous reports describing the mild diuretic and saluretic activity of *T. terrestris*. These findings support its ethnopharmacological application in urinary disorders and highlight its potential as a complementary agent for promoting fluid balance and supporting renal function [31–33]. Future studies are recommended to investigate the underlying mechanisms of action, including renal enzyme activity, hormonal involvement, and molecular pathways, and to use larger sample sizes to further validate and generalize these findings.

EETT showed moderate activity against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), but was ineffective against *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*). This differential susceptibility may reflect structural differences in bacterial cell walls or varying sensitivity to the extract's phytochemicals. While Gram-positive bacteria are generally more sensitive to plant extracts, EETT also inhibited *P. aeruginosa,* suggesting additional specific interactions. The disc diffusion concentration used here (5 mg/disc) differs from standard antibiotics; thus, results are indicative rather than directly comparable. Future studies assessing MIC and MBC are recommended to further characterize the extract's antibacterial spectrum. These findings are consistent with previous studies reporting that an EETT at 15% w/v produced inhibition zones of 20 mm and 18 mm against S. *aureus* and *P. aeruginosa*, respectively [34]. Similarly, another study demonstrated that methanolic extracts of *T. terrestris* fruits exhibited inhibitory effects against these organisms with minimum inhibitory concentrations (MICs) ranging from 1.25 to 2.5 mg/mL, indicating concentration-dependent efficacy [35].

In contrast, EETT did not inhibit the growth of *E. coli* or *K. pneumoniae*, which also showed resistance to the panel of standard antibiotics tested. This lack of response may be attributed to inherent structural features of Gram-negative bacteria, such as the presence of an outer membrane, efflux pumps, and a high capacity for biofilm formation, all of which can impede antimicrobial penetration and activity. These findings are consistent with previous reports that found no antibacterial effect of Yemeni *T. terrestris* extracts against *E. coli* or *K. pneumoniae*, suggesting a geographical influence on phytochemical composition [36].

A recent Yemeni study conducted at the University of Aden offers important local insight into the antibacterial potential of *T. terrestris.* The researchers evaluated the effects of both methanolic and aqueous extracts against *S. aureus* and *E. coli*, using the disc diffusion method and measuring zones of inhibition at two time points: 24 and 72 hours. Their results indicated that the methanolic extract had superior efficacy, particularly against *S. aureus*, with a maximum inhibition zone of 16.6 mm at 800 mg/mL after 24 hours, while no significant activity was detected against E. coli under either condition [37]. In summary, EETT demonstrated mild, dose-dependent diuretic and saluretic effects. It also showed moderate antibacterial activity against *S. aureus* and *P. aeruginosa*, while *E. coli* and *K. pneumoniae* were unaffected."

CONCLUSION

The present study provides experimental evidence supporting the diuretic and antibacterial properties of ethanol extract of *T. terrestris* in rodent models. The extract increased urinary output and promoted dose-dependent excretion of sodium and potassium without significant change in chloride levels, indicating a chloride-sparing profile. It also demonstrated moderate inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa in vitro.* These findings highlight the multi-targeted pharmacological potential of *T. terrestris* and reinforce its value as a plant-based adjunct for managing renal and urinary tract disorders. Further mechanistic studies and clinical validation are warranted to confirm its efficacy and safety.

Limitations

Although the findings are promising, this study has some limitations. Detailed phytochemical profiling of EETT—such as by gas chromatography–mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC)—was not performed, which limits the ability to attribute the observed effects to specific constituents. The antibacterial evaluation

was limited to in vitro assays, without determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), or biofilm inhibition. In addition, a formal toxicity or safety assessment was not conducted. These aspects provide opportunities for future studies to further explore the extract's pharmacological profile, antibacterial activity, and safety, ideally using larger sample sizes and comprehensive toxicological evaluations.

Abbreviations

AKI=Acute Kidney Injury, CKD=Chronic Kidney Disease, EETT=Ethanol Extract of *Tribulus terrestris*, MBC= Minimum Bactericidal Concentration, MIC=Minimum Inhibitory Concentration, *T. terrestris*=*Tribulus terrestris*, ZOI=Zone of Inhibition.

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Author Contributions

Ahmed Al-mohamadi conducted the experimental procedures and formulated the study's theoretical framework. Imadeldin M. Taj Eldin conceptualized and designed the overall study and supervised the pharmacological investigations. Doa'a Ibrahim supervised the practical implementation of the laboratory work. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] WHO. "'The top 10 causes of death'" 2024. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed May 10, 2025).

[2] United States Renal Data System. USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States 2023. https://doi.org/https://usrds-adr.niddk.nih.gov/2024.

[3] Hoste EAJ, Kellum JA, Selby NM, Zarbock A, Palevsky PM, Bagshaw SM, et al. Global epidemiology and outcomes of acute kidney injury. Nat Rev Nephrol 2018;14:607–25.

[4] Awedew AF, Han H, Berice BN, Dodge M, Schneider RD, Abbasi-Kangevari M, et al. The global, regional, and national burden of urolithiasis in 204 countries and territories, 2000&2013;2021: a systematic analysis for the Global Burden of Disease Study 2021. E Clinical Medicine 2024;78. https://doi.org/10.1016/j.eclinm.2024.102924.

[5] He Y, Zhao J, Wang L, Han C, Yan R, Zhu P, et al. Epidemiological trends and predictions of urinary tract infections in the global burden of disease study 2021. Sci Rep 2025;15:4702. https://doi.org/https://doi.org/10.1038/s41598-025-89240-5.

[6] Webster AC, Nagler E V, Morton RL, Masson P. Chronic Kidney Disease. The Lancet 2017;389:1238–52.

[7] Francis A, Harhay MN, Ong ACM, Tummalapalli SL, Ortiz A, Fogo AB, et al. Chronic kidney disease and the global public health agenda: an international consensus. Nat Rev Nephrol 2024;20:473–85. doi: 10.1038/s41581-024-00820-6.

[8] Al-Rohani M. Renal failure in Yemen. Transplant Proc, vol. 36, Elsevier; 2004, p. 1777–9. https://doi.org/10.1016/j.transproceed.2004.06.024.

[9] Al Rohani M, Aljawshaei H, Aduolimi E. Acute renal failure in Yemeni patients. Saudi Journal of Kidney Diseases and Transplantation 2011;22:829–33.

[10] Nassar MY, Al-Shamahy HA, Al-Samawi AS, Asba NWA, El-Nono IH, Masood HA. Underlying Primary Causes of Chronic Renal Failure: A Three-Year Study in Al-Thawra General Hospital, Sana'a, Yemen. Yemeni Journal for Medical Sciences 2016;10:24–9.

[11] Al-Mohani SK, Al-Awadi RHA, Tajaldeen ME, Al-Mogammer AM, Al-Shaghdari MM, Alshopi OF, et al. Associated risk factors of renal failure among patients attending Hemodialysis center at Al-Thwara Authority Hospital in IBB city, Yemen: A cross sectional study. Journal of Advanced Research and Reviews 2023; 18:446–54. DOI: 10.30574/wjarr.2023.18.3.1038.

[12] Badheeb AM. Causes of chronic renal failure in hemodialysis unit: a single center experience in Yemen. Saudi Journal of Kidney Diseases and Transplantation 2006;17:66–9.

[13] Dahnan M, Assabri AM, Khader YS. Risk factors for end-stage renal failure among patients on hemodialysis in Aljomhory hospital, Sa'adah governorate, Yemen: hospital-based case-control study. JMIR Public Health Surveill 2019;5:e14215. doi:10.2196/14215.

[14] Al-Hegami MA, Al-Mamari A, Al-Kadasse AS, Al-Gasha'a FAS, Al-Hag S, Al-Hegami AAS. Prevalence and risk factors of hepatitis B and hepatitis C virus infections among patients with chronic renal failure in Zabeed city, Yemen Republic. Open J Med Microbiol 2015;5:136–42. DOI: 10.4236/ojmm.2015.53017.

[15] Maqlam T. Antibacterial activity of Commiphora Gileadensis bark extract against Staphylococcus aureus as cream, gel and ointment formulations. Yemeni Journal for Medical Sciences. 2024 Nov 29;18(1):80-5.

[16] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 2011;8(1).

[17] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 2017;6. https://doi.org/10.3390/plants6040042.

[18] Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan. Nigeria: Spectrum Books Ltd 1993:191–289.

[19] Trease GE, Evans WC. Pharmacognosy 15 ed. London: Bailer Tindal 2002.

[20] Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998.

[21] Chauhdary Z, Saleem U, Ahmad B, Shah S, Shah MA. Neuroprotective evaluation of Tribulus terrestris L. in aluminum chloride induced Alzheimer's disease. Pak J Pharm Sci 2019;32.

[22] Singla S, Kumari C, Soni A, Goyal S. The in-vivo diuretic activity of methanolic extract of Quercus leucotrichophora A. Camus seeds. Phytomedicine Plus 2022;2:100346. https://doi.org/https://doi.org/10.1016/j.phyplu.2022.100346.

[23] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71–9. doi.org/10.1016/j.jpha.2015.11.005.

[24] Leite SP, Vieira JRC, de Medeiros PL, Leite RMP, de Menezes Lima VL, Xavier HS, et al. Antimicrobial activity of Indigofera suffruticosa. Evidence-Based Complementary and Alternative Medicine 2006;3:261–5. doi: 10.1093/ecam/nel010.

[25] Hailu W, Engidawork E. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of Ajuga remota Benth (Lamiaceae) leaves in mice. BMC Complement Altern Med 2014;14:1–8. doi.org/10.1186/1472-6882-14-135.

[26] Ştefănescu R, Tero-Vescan A, Negroiu A, Aurică E, Vari C-E. A comprehensive review of the phytochemical, pharmacological, and toxicological properties of Tribulus terrestris L. Biomolecules 2020;10:752. doi.org/10.3390/biom10050752.

[27] Al-Ali M, Wahbi S, Twaij H, Al-Badr A. Tribulus terrestris: preliminary study of its diuretic and contractile effects and comparison with Zea

mays. J Ethnopharmacol 2003;85:257–60. doi.org/10.1016/S0378-8741(03)00014-X.

[28] de Souza AM, Silva Lara L da, Previato JO, Lopes AG, Caruso-Neves C, da Silva BP, et al. Modulation of sodium pumps by steroidal saponins. Zeitschrift Für Naturforschung C 2004;59:432–6. doi.org/10.1515/znc-2004-5-626.

[29] Paredes MD, Romecín P, Atucha NM, O'Valle F, Castillo J, Ortiz MC, et al. Beneficial effects of different flavonoids on vascular and renal function in L-NAME hypertensive rats. Nutrients 2018;10:484. doi: 10.3390/nu10040484.

[30] Meléndez-Camargo ME, Contreras-León I, Silva-Torres R. Diuretic effect of alkaloids fraction extracted from Selaginella lepidophylla (Hook. et Grev.) Spring. Bol Latinoam Caribe Plantas Med Aromat 2014;13:92–9.

[31] Jabbar A, Nazir A, Khalil J, Ansari NI, Janjua KM, Javed F. To compare the effects of Tribulus terristris with hydrochlorothiazide on urine volume and electrolytes. Ann King Edw Med Univ 2015;21:149.

[32] Jabbar A, Nazir A, Khalil J, Ansari NI, Qureshi KM, Javed F. To Compare the Effects of Tribulus Terristris with Furosemide on Urine Volume and Electrolytes. Ann King Edw Med Univ 2012;18:196. doi.org/10.21649/akemu.v18i2.400.

[33] Sivapalan SR. Biological and pharmacological studies of Tribulus terrestris Linn-A review. Int J Multidiscip Res Dev 2016; 3:257–65. doi.org/10.1177/1934578X1986839.

[34] Sharma R, Godatwar P. In Vitro Anti-microbial effect of various extracts of Ġokṣura (Tribulus terrestris) fruits on common pathogens causing Urinary Tract Infection. Journal of Ayurveda and Integrated Medical Sciences 2022;7:64–9.

[35] Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. J Zhejiang Univ Sci B 2008; 9:154–9. doi.org/10.1631/jzus.B0720251.

[36] Ali NAA, Jülich W-D, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol 2001; 74:173–9.

[37] Bin-Obedaan AKA, Farea ATS, Abd-rabbo BBO, Albayti FAA, Al-Hanaka NAA, Ba-abbad OAA, et al. Antibacterial activity of Tribulus terrestris L. against Escherichia coli and staphylococcus aureus. Electronic Journal of University of Aden for Basic and Applied Sciences 2024;5:74–85. doi.org/10.47372/ejua-ba.2024.1.330.