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Assessment of antioxidant, anti-inflammatory, antimicrobial, antidiarrheal and antipyretic activities of *Tamarix dioica* stem bark extracts by in vitro and in vivo methods

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ABSTRACT

Introduction: This study evaluated the traditional uses of *Tamarix dioica* stem bark extracts, with emphasis on antioxidant, anti-inflammatory, antimicrobial, antidiarrheal, and antipyretic activities, using both laboratory- and animal-based experimental approaches, to evaluate its traditional uses.

Methods: The methanolic and n-hexane extracts of *T. dioica* stem bark were analyzed for phytochemical constituents, total phenolic and flavonoid content, and antioxidant activity. Radical scavenging capacity was determined through DPPH and nitric oxide assays using ascorbic acid as a reference standard. Additional evaluations included antimicrobial screening, thrombolytic and membrane stabilization assays, and cytotoxic assessment. Biological activities, including analgesic, antidiarrheal, and antipyretic effects, were examined in Swiss albino male mice at oral doses of 200 and 400 mg/kg body weight.

Results: Among the tested extracts, the methanolic fraction demonstrated stronger antioxidant performance than the n-hexane fraction, producing lower IC_{50} values in both DPPH and nitric oxide scavenging assays (28.91 ± 0.64 µg/mL and 26.24 ± 0.58 µg/mL, respectively). Antimicrobial testing revealed mild inhibitory effects, with the n-hexane fraction producing inhibition zones up to 8 mm against selected bacterial strains and 7 mm against *Candida albicans*. Both extracts reduced diarrheal frequency, achieved 70.27% inhibition at the higher dose. Pronounced analgesic and antipyretic responses were observed in the n-hexane extract-treated groups, with maximal writhing suppression of 78.43% ($P < 0.001$) and temperature reduction of 4.4 % ($P < 0.001$).

Conclusion: Stem bark extracts of *T. dioica* exhibited multiple pharmacological activities, particularly antioxidant, antimicrobial, analgesic, and antidiarrheal effects, confirming its traditional uses. These findings support continued investigation into the isolation and characterization of the active phytochemicals responsible for the observed responses.

Implication for health policy/practice/research/medical education:
The stem bark extracts exhibit antioxidant, antimicrobial, antidiarrheal and analgesic activities, highlighting their potential for bioassay-guided isolation and identification of active compounds. Furthermore, the findings support the potential of this plant to be recognized and developed as a medicinal resource.

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Introduction

For millennia, medicinal plants have formed the foundation of healthcare with traditional knowledge of their therapeutic benefits preserved and transmitted across generations (1). The bioactive compounds they contain have shown significant potential in preventing diseases and promoting health (2), and their role in discovering novel protein-targeted drugs has gained

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increasing attention (3). The *Tamarix* genus, commonly known as tamarisk, consists of more than 60 salt-tolerant species that have been traditionally used in Asia and Africa to address injuries, inflammation and issues related to the spleen and liver (4). *Tamarix dioica*, commonly referred to as 'Jhau Gacchh' in Bangladesh, belongs to the *Tamaricaceae* family and has traditionally been used for various purposes, including carminative, diuretic and astringent properties in treating conditions such as liver inflammation, leucorrhea and postpartum recovery. Its leaves and barks display antibacterial and antifungal activities against pathogens, including *Aspergillus fumigatus, Trichophyton rubrum, Candida glabrata, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae,* due to the presence of biologically active elements, including flavonoids, saponins, tannins, alkaloids and phenolics (5,6). However, the pharmacological properties of the stem bark of *T. dioica,* particularly its anticoagulant, fever-reducing, anti-inflammatory and antioxidative effects have yet to be thoroughly investigated (7).

The antioxidant, antimicrobial, thrombolytic, antipyretic, and inflammation-reducing properties of the stem bark are evaluated in this study through in vivo and in vitro investigations using methanol and n-hexane extracts. The results would not only support the ethnomedicinal uses of *T. dioica* but also establish a scientific foundation for its prospective therapeutic applications, guiding future investigations into its bioactive compounds.

Methods

Plant material collection and extraction

Stem bark samples of *T. dioica* were collected and authenticated by a qualified taxonomist from Ramna Park, Dhaka, Bangladesh. A voucher specimen was preserved in the herbarium of the Department of Pharmacy, University of Asia Pacific (Accession No. UAP_Herb/1553_022). After collection, the bark was dried and ground into coarse powder. Approximately 600 g of powdered material was separately extracted using methanol and n-hexane as solvents. Each extraction was performed by immersing the plant powder in 2.5 L of solvent for fourteen days with periodic agitation to improve extraction efficiency. The mixtures were filtered and concentrated using a rotary evaporator. The concentrated extracts were preserved under suitable storage conditions until further use (8).

Preliminary phytochemical screening

Qualitative phytochemical analysis was performed for the major classes of secondary metabolites. Screening procedures were carried out to identify compounds, including flavonoids, alkaloids, glucosides, carbohydrates, saponins, steroids, and tannins, using established phytochemical protocols (9,10).

Determination of total phenolic contents

Total phenolic concentration was estimated using the Folin–Ciocalteu colorimetric technique. Extract solutions and gallic acid standards were prepared across a concentration range of 50–250 µg/mL. For analysis, one milliliter of each sample was mixed with diluted Folin–Ciocalteu reagent, followed by sodium carbonate solution. Standard preparations were incubated for thirty minutes, whereas extract mixtures remained at room temperature for one hour. Absorbance values were measured spectrophotometrically at 765 nm. Total phenolic concentration was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (11).

Determination of total flavonoid content

Flavonoid content was quantified using an aluminum chloride-based colorimetric assay. One milliliter of each sample solution was mixed with methanol, aluminum chloride solution, potassium acetate, and distilled water to achieve the required reaction volume. Samples were incubated at ambient temperature for thirty minutes before absorbance measurements were obtained at 415 nm. Flavonoid concentration was determined using quercetin as the reference standard and reported as milligrams of quercetin equivalents per gram of extract (12).

Evaluation of total antioxidant capacity

Antioxidant potential was measured using the phosphomolybdenum reduction method. Aliquots of extract and ascorbic acid standard solutions (25–400 µg/mL) were individually combined with the reagent containing sulfuric acid, sodium phosphate, and ammonium molybdate. Following incubation at 95 °C for ninety minutes, the mixtures were cooled and analyzed at 695 nm (13). Antioxidant capacity was calculated relative to the ascorbic acid calibration curve and expressed as milligrams of ascorbic acid equivalent per gram.

DPPH radical scavenging assay

Free radical scavenging activity was assessed using the DPPH method. Freshly prepared DPPH solution was combined with extract or standard solutions at multiple concentrations. The reaction mixtures were maintained at room temperature for thirty minutes, and the absorbance was subsequently recorded at 517 nm against a blank (14). The percentage of radical inhibition was calculated as:

$$\text{DPPH scavenging activity (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where:
A_0 = absorbance of control
A_1 = absorbance of extract or standard

Nitric oxide radical scavenging assay

Nitric oxide scavenging capacity was evaluated using sodium nitroprusside. Reaction mixtures containing sodium nitroprusside and sample solutions at varying

concentrations were incubated at 30 °C for two hours. Following incubation, the reagent solution was added, and the absorbance was recorded at 550 nm. The percentage of nitric oxide inhibition was subsequently calculated (15).

Thrombolytic activity assessment

Fresh human blood samples obtained from healthy volunteers were transferred into pre-weighed tubes and incubated at 37 °C to allow clot formation. After serum removal, the clot weight was recorded. Extract-treated groups received 100 µL of plant extract, negative controls received distilled water, and positive controls received streptokinase. Following ninety minutes of incubation at 37 °C, residual clot weight was measured and the percentage of clot dissolution was calculated (16).

Membrane stabilization assay

Anti-inflammatory potential was examined through membrane stabilization testing under hypotonic and heat-induced hemolytic conditions. For hypotonic hemolysis assessment, the erythrocyte suspension was mixed with phosphate buffer and hypotonic saline solution. Plant extracts and acetylsalicylic acid were added accordingly. Samples were centrifuged, and the absorbance of the supernatant was measured at 540 nm. Heat-induced membrane stabilization was evaluated using duplicate reaction systems, where treated erythrocyte suspensions were exposed either to elevated temperature or maintained under cooling conditions before absorbance analysis (17).

Brine shrimp cytotoxicity bioassay

Cytotoxic potential was determined through the brine shrimp lethality assay. Extract solutions at multiple concentrations were prepared using dimethyl sulfoxide. Nauplii were transferred into saline-containing tubes with test samples. Vincristine sulfate served as the positive control, and DMSO-containing tubes acted as negative controls. Following twenty-four hours of exposure, mortality rates were recorded, and LC_{50} values were calculated using linear regression (18).

Antimicrobial activity evaluation

Antimicrobial effectiveness of the extracts was determined using the disc diffusion technique. Sterile filter paper discs (6 mm diameter) containing designated concentrations of extract were placed onto Mueller–Hinton agar plates previously inoculated with test microorganisms. Diffusion of the extract into the culture medium allowed the formation of growth inhibition zones surrounding active samples. Antibacterial activity was compared with ciprofloxacin (5 µg/disc), while antifungal assessment used nystatin and ketoconazole (50 µg/disc) as reference agents. Solvent-treated discs served as negative controls. Antimicrobial efficacy was expressed by measuring inhibition zone diameters (19).

Experimental animals

Healthy male Swiss albino mice weighing approximately 25–30 g were selected for in vivo pharmacological evaluations. Animals were obtained from the Animal Resources Facility of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka. Experimental animals were maintained under controlled environmental conditions, including regulated temperature, relative humidity, and alternating light–dark cycles. Standard laboratory feed and water were supplied throughout the study period.

Evaluation of antidiarrheal activity

Antidiarrheal activity was investigated using the castor oil–induced diarrhea model (20). Animals were randomly allocated to several groups comprising control, standard treatment, and extract-treated groups. The control group received distilled water, whereas loperamide served as the reference drug. Test groups received methanol or n-hexane extracts orally at doses of 200 and 400 mg/kg body weight. One hour after treatment administration, castor oil was delivered orally to induce diarrhea. Animals were observed for four hours, and the number and consistency of fecal outputs were documented. Antidiarrheal effectiveness was determined by comparing reductions in stool frequency across groups.

Assessment of peripheral analgesic activity

Peripheral analgesic activity was examined using the acetic acid–induced writhing model. Animals were divided into six groups and treated with vehicle control, standard analgesic drug, or extract preparations at designated doses. The control group received oral Tween-80 solution, while diclofenac sodium was administered as the standard treatment. Experimental groups received methanolic or n-hexane extracts at oral doses of 200 and 400 mg/kg. After an adaptation interval, abdominal writhing was induced through intraperitoneal administration of acetic acid. Writhing responses were counted over a defined observation period and inhibition percentages were calculated relative to controls (21).

Evaluation of antipyretic activity

Antipyretic effects were assessed using Brewer's yeast–induced pyrexia. Fever was produced through subcutaneous administration of yeast suspension based on body weight. Rectal temperature measurements were recorded before and after induction. Animals were subsequently treated with vehicle control, paracetamol, or plant extracts at selected dose levels. Body temperature was monitored at predetermined intervals following treatment administration to determine fever-reducing effects (21).

Acute toxicity study

Acute toxicity was assessed following dose-escalation

procedures. Animals were assigned to multiple groups and orally administered increasing concentrations of methanol and n-hexane extracts. Control animals received physiological saline. Experimental groups received extract doses ranging from lower to higher concentrations (500, 1000, 1500, 2000, 4000 mg/kg body weight) to observe safety margins. Animals were continuously monitored for behavioral changes, signs of toxicity, and mortality during the observation period (22).

Statistical analysis

All experiments were carried out twice, and the results were expressed as mean ± standard error of mean (SEM). Data analysis was performed using Microsoft Excel software. Statistical comparisons between the experimental and control groups were carried out using a two-sample t-test assuming equal variance. Levels of statistical significance were categorized as follows:

- $P \leq 0.05$ → statistically significant
- $P \leq 0.01$ → highly significant
- $P \leq 0.001$ → extremely significant

Regression analysis was additionally employed to determine IC_{50} and LC_{50} values for antioxidant and cytotoxic evaluations.

Results

Phytochemical screening

Qualitative phytochemical evaluation confirmed the presence of multiple bioactive secondary metabolites within the stem bark extracts of *T. dioica*. Both methanol and n-hexane fractions contained glucosides, saponins, flavonoids, and alkaloids. Carbohydrates and tannins showed variable distribution between solvent fractions, whereas steroids and glycosides were not detected. These findings indicate that extraction solvent polarity influenced the phytochemical profile recovered from the stem bark. The results of phytochemical screening are summarized in Table 1.

Table 1. Phytochemical screening of *Tamarix dioica* stem bark extracts

Experiment	Methanol	n-Hexane
Carbohydrate	-	+
Glycoside	-	-
Glucoside	+	+
Saponin	+	+
Steroid	-	-
Tannin	+	-
Flavonoid	+	+
Alkaloids		
Wagner's reagent	+	+
Hager's reagent	+	+
Dragendroff's reagent	+	+

Note: "+" = Present; "-" = Absent.

Total phenolic content

The concentration of total phenolic compounds differed between the two extracts. The methanolic fraction demonstrated a substantially greater phenolic content, yielding 35.61 ± 1.33 mg GAE/g, while the n-hexane fraction contained 16.16 ± 0.23 mg GAE/g (Table 2). This result indicates that methanol provided more efficient extraction of phenolic constituents compared with the less polar solvent.

Total flavonoid content

Flavonoid quantification revealed lower overall concentrations than total phenolic content but showed a similar extraction pattern. The methanolic extract contained 30.29 ± 0.41 mg QE/g, considerably exceeding the value observed in the n-hexane fraction (3.91 ± 0.16 mg QE/g). These findings suggest enhanced extraction of flavonoid compounds in the polar solvent system (Table 2).

Total antioxidant capacity

Assessment of antioxidant potential demonstrated measurable antioxidant properties in both extracts. Using ascorbic acid equivalence calculations, the extracts displayed appreciable reducing ability, with the methanolic fraction showing stronger antioxidant-associated characteristics than the n-hexane extract, indicating a relationship between antioxidant performance and elevated phytochemical content (Table 2).

DPPH radical scavenging activity

The free radical neutralization assay showed concentration-dependent antioxidant effects (Figure 1). The reference compound ascorbic acid produced the strongest scavenging response with an IC_{50} value of 15.60 ± 0.29 µg/mL. Among plant extracts, the methanolic extract demonstrated greater DPPH inhibitory capacity (IC_{50} =

Table 2. Total phenol, flavonoid, antioxidant capacity, and free radical scavenging potential of *Tamarix dioica* stem bark extracts

Extracts/standards	Total phenol (mg gallic acid equivalent, (GAE)/g)	Total flavonoid (mg quercetin equivalent (QE)/g)	Total antioxidant (mg ascorbic acid equivalent, (AAE)/g)	DPPH free radical scavenging assay (IC_{50} µg/mL)	Nitric oxide-free radical scavenging (IC_{50} µg/ml)	Brine shrimp lethality bioassay (LC_{50} µg/mL)
Methanol extract	35.61±1.33	30.29±0.41	45.40±2.24	28.91 ± 0.64	26.24 ± 0.58	136.77 ± 2.95
n-Hexane extract	16.16±0.23	3.19±0.16	51.45±1.92	98.63 ± 2.15	47.32 ± 1.08	59.16 ± 1.31
Ascorbic acid	-	-	-	15.60 ± 0.29	16.03 ± 0.31	-
Vincristine	-	-	-	-	-	2.47 ± 0.06

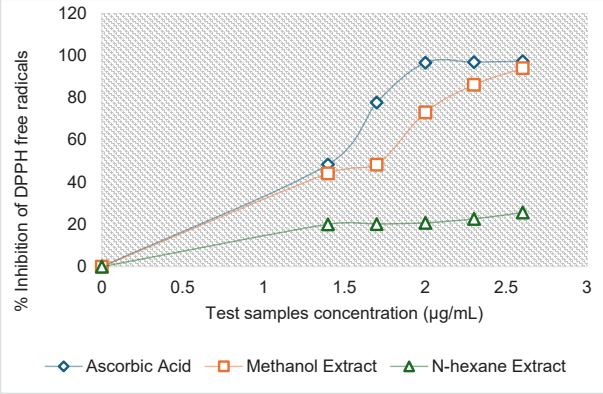

Figure 1. The percentage inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals by *Tamarix dioica* stem bark at various concentrations.

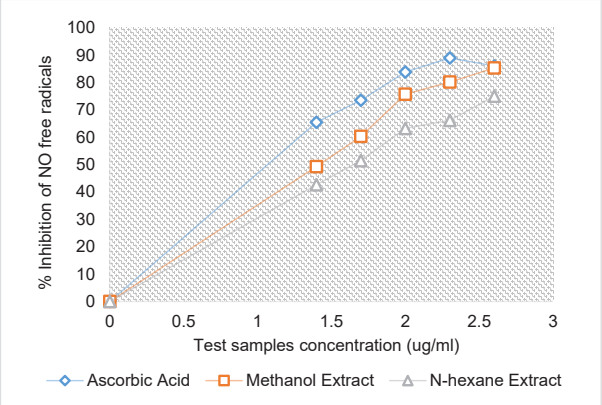

Figure 2. The percentage inhibition of NO (nitric oxide) free radicals by *Tamarix dioica stem* bark at different concentrations.

28.91 ± 0.64 µg/mL) compared with the n-hexane extract (IC_{50} = 98.63 ± 2.15 µg/mL). Lower IC_{50} values observed in the methanol fraction indicate greater efficiency in neutralizing DPPH free radicals (Table 2).

Nitric oxide scavenging activity

Both extracts exhibited the ability to suppress nitric oxide radicals; however, the extent of inhibition varied. The methanolic fraction generated a stronger scavenging response with an IC_{50} value of 26.24 ± 0.58 µg/mL, whereas the n-hexane extract demonstrated weaker activity (47.32 ± 1.08 µg/mL). Ascorbic acid remained more active than both extracts. Overall, these results support a comparatively stronger antioxidant potential for the methanol-derived extract (Figure 2 and Table 2).

Thrombolytic activity

Clot dissolution studies demonstrated measurable but limited thrombolytic activity in the tested extracts. The n-hexane fraction produced greater clot lysis (16.34 ± 0.83%) than the methanol fraction (9.56 ± 0.93%). Nevertheless, both activities remained substantially lower than the positive control streptokinase (66.38 ± 0.90%). These findings indicate poor thrombolytic effects under the present experimental conditions (Table 3).

Membrane stabilizing activity

Anti-inflammatory potential was estimated through membrane stabilization against hypotonic and thermal stress. The methanol extract produced stronger inhibition of erythrocyte membrane disruption compared with the n-hexane fraction under both test conditions. Observed inhibition percentages suggested moderate membrane-protective activity, although the standard acetylsalicylic acid remained more effective overall (Table 3).

Brine shrimp cytotoxicity assay

Cytotoxic evaluation using the brine shrimp lethality model demonstrated measurable biological activity in both extracts. The calculated LC_{50} values showed that the n-hexane fraction possessed relatively greater cytotoxic potential than the methanolic extract. However, neither extract approached the potency of the reference standard vincristine sulfate. These findings suggest the presence of biologically active constituents while indicating comparatively mild cytotoxic effects (Table 2).

Antimicrobial activity

Antimicrobial screening demonstrated low to moderate inhibitory activity against selected microbial strains (Tables 4 and 5). Both extracts produced measurable inhibition zones against gram-positive and gram-negative bacteria, as well as fungal organisms. At the highest concentration tested, inhibition zones generally ranged between 5 and 8 mm, whereas standard antimicrobial agents produced substantially larger inhibition diameters. The n-hexane fraction showed slightly broader inhibitory effects against tested organisms, including fungal activity against *Candida albicans*.

Table 3. Thrombolytic and membrane-stabilizing characteristics (% inhibition of hemolysis) of different extracts and the standard

Extracts	Thrombolytic activity	% Inhibition of hemolysis ± SEM	
	% of clot lysis	Hypotonic solution	Heat induced
Methanol extract	9.56±0.93	47.03±0.251	43.67±0.567
n-Hexane extract	16.34±0.83	26.48±0.840	15.63±0.675
Streptokinase	66.38± 0.90	-	-
Distilled water	1..07±0.21	-	-
Acetyl salicylic acid	-	55.71±1.210	69.59±0.965

Table 4. Antimicrobial properties of a methanol extract from *Tamarix dioica* stem bark

Test microorganism	Zone of inhibition in mm							
	50 µg/disc	100 µg/disc	150 µg/disc	200 µg/disc	250 µg/disc	Ciprofloxacin 5 µg/disc	Nystatin 50 µg/disc	Ketoconazole 50 µg/disc
Bacillus subtilis	Nil	7	7	7	8	40	-	-
Staphylococcus aureus	Nil	Nil	7	7	7	30	-	-
Escherichia coli	Nil	Nil	Nil	Nil	5	32	-	-
Pseudomonas spp.	Nil	7	7	7	8	38	-	-
Candida albicans	Nil	Nil	Nil	5	6	-	8	Nil

Table 5. Antimicrobial properties of n-hexane extract from *Tamarix dioica* stem bark

Test microorganism	Zone of inhibition in mm							
	50 µg/disc	100 µg/disc	150 µg/disc	200 µg/disc	250 µg/disc	Ciprofloxacin 5 µg/disc	Nystatin 50 µg/disc	Ketoconazole 50 µg/disc
Bacillus subtilis	Nil	6	7	7	8	40	-	-
Staphylococcus aureus	6	6	6	7	8	30	-	-
Escherichia coli	6	6	6	7	7	32	-	-
Pseudomonas spp.	6	6	7	7	8	38	-	-
Candida albicans	6	6	6	6	7	-	8	Nil

Antidiarrheal activity

Administration of *T. dioica* stem bark extracts resulted in a noticeable reduction in diarrheal episodes induced during the experimental period. Both methanolic and n-hexane fractions demonstrated dose-responsive antidiarrheal effects. At 200 mg/kg body weight, moderate suppression of fecal output was observed, whereas administration at 400 mg/kg produced stronger inhibition. The methanol extract reduced defecation frequency by 56.78% and 70.27% at the lower and higher doses, respectively. Likewise, the n-hexane extract achieved reductions of 67.57% and 70.27%. These outcomes indicate that both extracts possess considerable antidiarrheal potential, comparable to that of the reference treatment (Figure 3).

Peripheral analgesic activity

Evaluation of analgesic response using the writhing model demonstrated meaningful pain-relieving effects for both extract fractions. The methanolic extract reduced abdominal writhing by 56.86% at 200 mg/kg and 70.59% at 400 mg/kg. Greater inhibition was observed with the n-hexane fraction, which produced 60.78% and 78.43% suppression at equivalent doses. The findings indicate a clear dose-dependent analgesic response, with the n-hexane extract exhibiting superior efficacy under the study conditions (Figure 3).

Antipyretic activity

Both extracts exhibited measurable fever-reducing

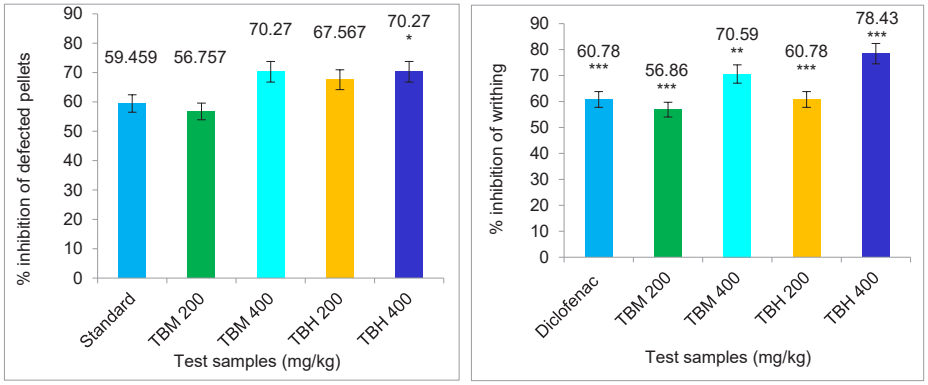

Figure 3. Percentage inhibition of defecated pellets by extracts (left) and % inhibition of writhing by extracts (right) against respective standards. TBM: methanolic stem bark extract of *Tamarix dioica*, TBH: n-hexane stem bark extract of *Tamarix dioica*. A t-test with equal variances was used to compare the data sets between the standard and treatment groups. *, **, and *** statistically significant compared to the respective standard group at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

effects in the yeast-induced pyrexia model. Temperature reduction became more apparent at the higher treatment dose and after longer observation periods. Following administration of the methanol extract, body temperature declined by 2.2% and 2.9% at 200 mg/kg and 400 mg/kg, respectively. In comparison, the n-hexane fraction produced reductions of 2.1% and 4.4% (Figure 4). These findings suggest that *T. dioica* stem bark may contain constituents capable of modulating fever-related physiological pathways.

Acute toxicity study

Acute toxicity assessment demonstrated a favorable safety profile for both extracts. Throughout the observation period, no mortality, severe adverse reactions, or abnormal behavioral responses were recorded in treated animals. The absence of toxic manifestations across tested concentrations suggests that both methanol and n-hexane stem bark extracts were well tolerated under the experimental conditions and may possess a relatively wide margin of safety up to 4000 mg/kg BW dose.

Discussion

The present investigation explored the pharmacological potential of methanolic and n-hexane stem bark extracts of *T. dioica* using multiple in vitro and in vivo experimental approaches. The findings collectively demonstrate that the plant contains bioactive constituents capable of producing diverse biological responses. Preliminary phytochemical analysis confirmed the occurrence of several secondary metabolites, including flavonoids, alkaloids, glucosides, and saponins. Differences between solvent fractions indicate that extraction efficiency is influenced by solvent polarity and may alter the concentration of active constituents obtained from plant material (23). Quantitative analysis revealed that the methanolic fraction contained greater concentrations of phenolic and flavonoid compounds. Because these phytochemicals are widely recognized for their electron-donating and radical-neutralizing capabilities, their increased abundance likely contributed to the stronger antioxidant responses observed in antioxidant assays (24). Results obtained from DPPH and nitric oxide scavenging experiments demonstrated that the methanol extract possessed superior free-radical inhibition compared with the n-hexane extract. Lower IC_{50} values indicate greater antioxidant effectiveness and support the role of phenolic-rich fractions in reducing oxidative stress–related damage (25).

Thrombolytic analysis demonstrated limited clot-dissolving capability for both fractions, although the n-hexane extract performed better than the methanolic fraction. Despite these observations, the activity remained substantially lower than the reference thrombolytic agent, suggesting that additional purification or compound isolation may be required to enhance efficacy (26).

Evaluation of membrane stabilization indicated moderate anti-inflammatory effects. The stronger protection demonstrated by the methanol fraction against hypotonic and thermal hemolysis suggests greater ability to preserve membrane integrity under inflammatory conditions (27).

Cytotoxicity assessment using brine shrimp lethality showed detectable biological activity in both extracts. The comparatively lower LC_{50} values observed for the n-hexane extract may indicate enrichment of biologically active constituents with greater cellular effects (5). Antimicrobial testing showed mild inhibitory effects against selected bacterial and fungal strains. Although inhibition zones remained smaller than those generated by standard antimicrobial agents, the findings support the possibility that stem bark extracts contain compounds with antimicrobial potential (28).

In animal experiments, both extracts produced favorable pharmacological responses. Reduction in diarrheal

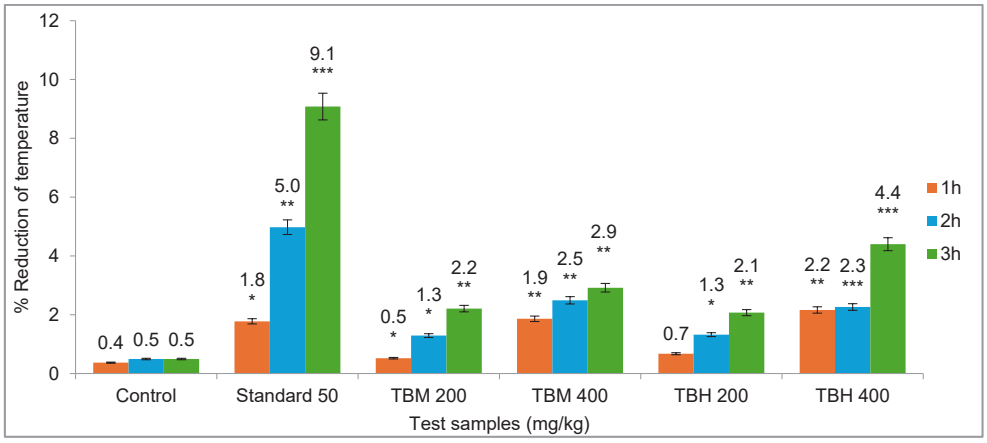

Figure 4. % Decrease in temperature from the initial temperature by plant extracts and standard. TBM: methanolic stem bark extract of *Tamarix dioica*, TBH: n-hexane stem bark extract of *Tamarix dioica*. A t-test with equal variances was used to compare the data sets between the control and treatment groups. *, **, and *** statistically significant compared to the control group at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

frequency may reflect interference with intestinal secretion, motility, or inflammatory pathways involved in gastrointestinal disturbances (29). Analgesic evaluation demonstrated stronger activity in the n-hexane fraction, suggesting that less polar constituents may contribute to peripheral pain modulation mechanisms. Similarly, both extracts reduced elevated body temperature in pyrexia models, indicating potential influence on pathways associated with inflammatory mediators (30). In the antipyretic assessment, both extracts produced significant temperature-reducing effects against yeast-induced pyrexia, particularly at a higher dose (400 mg/kg), which were comparable to standard paracetamol. A previous study reported that the methanolic extract of *T. dioica* showed significant antipyretic activity in animal models by effectively reducing Brewer's yeast-induced fever (31). Overall, the observed activities suggest that *T. dioica* stem bark contains chemically diverse constituents capable of exerting antioxidant and therapeutic effects across multiple biological systems.

Although the present investigation demonstrated multiple pharmacological effects of *T. dioica* stem bark extracts, several limitations should be acknowledged. First, the study evaluated crude extracts rather than purified phytochemical constituents. Because plant extracts contain complex mixtures of compounds, the precise molecules responsible for the observed activities could not be identified. Isolation and characterization of active constituents are necessary to establish compound-specific pharmacological profiles. Second, although in vitro and animal-based evaluations provide valuable preliminary evidence, these findings cannot directly predict clinical effectiveness in humans. Additional investigations involving mechanistic studies, pharmacokinetic evaluation, and long-term safety assessment are required before therapeutic translation. Third, the current work focused primarily on biological activity outcomes without detailed molecular investigations. Future studies incorporating biochemical pathway analysis, receptor-level evaluation, and omics-based approaches may provide deeper insight into mechanisms underlying antioxidant, anti-inflammatory, analgesic, and antimicrobial responses. Despite these limitations, the findings establish an experimental basis for continued investigation into *T. dioica* as a potential source of medicinal compounds.

Conclusion

The methanolic extract contained higher levels of phenolics and flavonoids corresponding to enhanced antioxidant activity in DPPH and nitric oxide scavenging assays, whereas the n-hexane fraction exhibited stronger thrombolytic and analgesic effects, indicating that solvent polarity impacts the extraction and potency of bioactive constituents. Both extracts demonstrated notable membrane-stabilizing activity, with the methanolic fraction showing superior protection against hypotonic and heat-induced hemolysis, suggesting significant anti-inflammatory potential. In vivo evaluations confirmed dose-dependent antidiarrheal, analgesic and antipyretic activities comparable to standard drugs. Overall, *T. dioica* stem bark represents a promising source of natural bioactive compounds, warranting further studies to isolate specific constituents and elucidate their precise mechanisms of action across different regions.

Authors' contribution

Conceptualization: Sanjeeda Ahmed, Samiha Mehnaz, A.H.M. Nazmul Hasan.
Formal analysis: Md. Khokon Miah Akanda.
Investigation: Sanjeeda Ahmed, Samiha Mehnaz, Al-Kifa Omi.
Methodology: A.H.M. Nazmul Hasan, Md. Khokon Miah Akanda.
Supervision: A.H.M. Nazmul Hasan.
Writing-original draft: Al Kifa Omi.
Writing-review & editing: A.H.M. Nazmul Hasan, Al Kifa omi.

Conflict of interests

The authors declare that they have no competing interests.

Data availability statement

All information and data are safely stored by the authors and is available upon request.

Declaration of AI-assisted tools in the writing procedure

No AI software has been used to prepare this manuscript.

Ethical considerations

Ethical approval for the study was obtained from the Research Ethics Committee, Department of Pharmacy, University of Asia Pacific (Ref: UAP/REC/2023/211), following a thorough review of the research protocol. All laboratory animal handling procedures adhered to the ARRIVE guideline, and ethical standards were strictly maintained throughout the study.

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