

Phenolic profile and biological activities of ultrasound-assisted aqueous-ethanolic extracts of *Tamarix hispida* Willd

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Abstract: *Tamarix hispida* Willd. (Tamarisk; locally known as “zhyngyl” in Kazakh) is a halophyte traditionally used for dysentery, rheumatism, and ulcers, with reported antioxidant and prolyl endopeptidase-inhibitory activities. This study investigated the phenolic profiles and biological activities of 10%, 50%, and 70% aqueous-ethanolic extracts (THU-10, THU-50, and THU-70) obtained by ultrasound-assisted extraction (UAE). Phenolic constituents were analyzed by HPLC-DAD, and antioxidant, enzyme inhibitory, anti-inflammatory, and cytotoxic activities were evaluated. Eleven phenolic compounds, including rutin, gallic acid, epicatechin, fisetin, and *p*-hydroxybenzaldehyde were identified, where rutin was the predominant quantified phenolic compound, reaching 2.78, 3.88, and 4.42 mg/g in THU-10, THU-50, and THU-70, respectively. THU-10 showed the strongest antioxidant activity in the ABTS and DPPH assays ($IC_{50} = 2.13 \pm 0.75$ and 6.01 ± 0.30 μ g/mL, respectively) and the strongest AChE and BChE inhibition ($IC_{50} = 21.90 \pm 0.70$ and 38.71 ± 0.19 μ g/mL, respectively). The extracts also inhibited α -amylase, α -glucosidase, and pancreatic lipase. THU-70 exhibited the strongest anti-inflammatory activity ($IC_{50} = 18.4 \pm 0.2$ μ g/mL). In the BSLA, the tested extracts showed no activity and were considered non-cytotoxic. UAE reduced extraction time from 48 h to 25 min and increased extract yield 3–4-fold. Overall, solvent composition significantly influenced the phenolic profiles and biological activities of *T. hispida* extracts.

Keywords: *Tamarix hispida*, ultrasound-assisted extraction, phenolic compounds, antioxidant activity, anti-inflammatory activity, HPLC-DAD

1 Introduction

The growing interest in naturally derived bioactive compounds as alternatives to synthetic pharmaceuticals has spurred extensive research on medicinal plants over the past two decades (Newman & Cragg, 2020; Atanasov et al., 2021). This trend is especially relevant to wound healing, antimicrobial resistance, and chronic inflammation, where plant-derived polyphenols, flavonoids, tannins, and terpenoids have demonstrated significant therapeutic potential (Gonfa et al., 2023; Vuolo et al., 2019). Among the most

pharmacologically promising families of Central Asian flora, Tamaricaceae stands out for the remarkable phytochemical diversity of its genera (Bahramsoltani et al., 2020).

Tamarix hispida Willd. is a halophytic shrub, widely distributed across the arid and semi-arid regions of Kazakhstan and Central Asia. These halophytic shrubs and small trees have long been used in traditional medicine to treat inflammatory conditions, skin disorders, gastrointestinal ailments, and liver diseases (Bahramsoltani et al., 2020). The phytochemical profile of *Tamarix* species is exceptionally rich, encompassing polyphenols, hydrolysable tannins, flavonoids, phenolic acids, triterpenoids, and alkaloids (Rasouli et al., 2017). Previous phytochemical investigations of *Tamarix* species have revealed a variety of biological active constituents associated with antioxidant, anti-inflammatory, antimicrobial, and enzyme-inhibitory activities. In particular, earlier investigations by our research group led to the

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isolation of biologically active terpenoids and triterpenoids from *T. hispida*. Isotamerixen, a triterpenoid isolated from *T. hispida*, was reported to possess antioxidant and prolyl endopeptidase-inhibitory activities (Sultanova et al., 2004). Other studies from our group also demonstrated antioxidant and antimicrobial activities in extracts of *T. ramosissima* (Sultanova et al., 2001; Sultanova et al., 2013). Although several phytochemical constituents of *T. hispida* have been reported, information on the biological activities of its aqueous-ethanolic extracts remains limited. In particular, systematic comparisons of extracts obtained using different ethanol-to-water ratios and evaluated using the same set of biological assays are lacking. These limits understanding of the relationship between extraction solvent composition, phenolic profile, and biological activity.

Extraction efficiency is a critical determinant of the bioactive content and pharmacological performance of plant extracts. Conventional maceration, while simple, is time-consuming (48–72 h) and often yields incomplete extraction (Zhumagaliyeva et al., 2021). Ultrasound-assisted extraction (UAE) has emerged as a powerful, energy-efficient alternative that exploits acoustic cavitation to disrupt cell walls and dramatically enhance mass transfer of bioactive constituents (Tiwari, 2015). Our previous work demonstrated that UAE of *T. hispida* under optimized conditions (35 kHz, 20–25°C, 25 min) increased extractable substance yield by 3–4-fold compared with maceration and elevated tannin content by approximately 25% (Zhumagaliyeva et al., 2021). However, the influence of different aqueous-ethanolic solvent compositions on the phenolic composition and multiple biological activities of UAE-derived *T. hispida* extracts has not been comprehensively evaluated.

Antioxidant and anti-inflammatory activities are particularly relevant because oxidative stress and inflammatory processes are closely associated with tissue damage and impaired wound healing (Irshad et al., 2025). Phenolic compounds, including flavonols such as rutin, fisetin, and quercetin; phenolic acids such as gallic, ferulic, and *p*-coumaric acids; and catechins are known to contribute to these biological effects (Chemat et al., 2017; Kumar & Pandey, 2013; Aslan et al., 2025; Labeled et al., 2026). In addition to antioxidant and anti-inflammatory effects, evaluating enzyme-inhibitory activities provides further insight into the broader pharmacological potential of phenolic-rich plant extracts (Gören & Topçu, 2025). Pancreatic lipase (PL), however, is a key enzyme involved in dietary fat digestion and intestinal lipid absorption. Therefore, inhibition of PL is considered a promising strategy for reducing fat absorption and managing obesity. Natural products are regarded as valuable sources for discovering novel PL inhibitors with potential anti-obesity effects (Ng & Tan, 2025). Therefore, assessing inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α -amylase, α -glucosidase, and pancreatic lipase can provide complementary information on their potential anticholinesterase, antidiabetic, and metabolic enzyme-inhibitory properties.

Phenolic-rich plant extracts, such as those described above, are of particular interest as candidate active ingredients for future topical and wound-care formulations, including polymer-based hydrogel delivery systems. Hydroxypropyl methylcellulose (HPMC), for instance, has been reviewed by our group as a biocompatible carrier matrix for bioactive substances (Amanzholkzy et al., 2025). However, characterization of the phytochemical composition and biological activities of the extracts is an essential prerequisite before their further development into pharmaceutical formulations. Accordingly, the present study aimed to: (i) prepare three aqueous-ethanolic extracts (THU-10, THU-50, THU-70) of *T. hispida* using UAE; (ii) characterize their phenolic profiles by HPLC-DAD; and (iii) comparatively evaluate their antioxidant, anticholinesterase, α -amylase, α -glucosidase, pancreatic lipase inhibitory, anti-inflammatory, and cytotoxic activities.

2 Materials and Methods

2.1 Chemicals, Reagents and Instruments

All reagents used were of analytical grade. Methanol (HPLC and analytical grade) and glacial acetic acid were supplied by Merck (Steinheim, Germany). Gallic acid ($\geq 99.0\%$), protocatechuic acid ($\geq 99.9\%$), (+)-catechin hydrate ($\geq 98.0\%$), rutin hydrate ($\geq 94.0\%$), ferulic acid ($\geq 99.0\%$), *p*-coumaric acid ($\geq 98.0\%$), and vanillic acid ($\geq 97.0\%$) were purchased from Sigma-Aldrich GmbH (Steinheim, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH, $\geq 99\%$, Sigma-Aldrich (USA)). All aqueous solutions were prepared with ultrapure water.

The principal instruments used in this study included a Sapphire 1.3 ultrasonic bath for ultrasound-assisted extraction; a Shimadzu high-performance liquid chromatography system equipped with a diode-array detector for phenolic analysis; a BioTek Synergy H1 96-well multimode reader (BioTek Instruments, Inc., USA) for absorbance-based assays; and a luminometer (Labsystems, Finland) for chemiluminescence measurements.

2.2 Plant Material

Aerial parts (leaves, stems) of *Tamarix hispida* Willd. were collected by Amanzholkzy Arailym at the flowering stage (September 2024) in the Almaty Region, Republic of Kazakhstan (GPS coordinates: N = 43.588006°, E = 77.884779°). The plant was identified by Dr. Lapshina and voucher specimens (No. 5239) are deposited at the Laboratory of Plant Resources, Institute of Botany and Phytointroduction, Almaty, Republic of Kazakhstan. The collected material was air-dried at room temperature in the absence of direct sunlight, then ground to a particle size of ≤ 1 mm and stored in a cool, dry place before extraction.

2.3 Ultrasound-Assisted Extraction (UAE)

Three distinct extracts (THU-10, THU-50, and THU-70) were prepared by treating the powdered dry plant material with 10%, 50%, and 70% (v/v) ethanol, maintaining a solid-to-liquid ratio of 1:4 (w/v). Extraction was performed in a

Sapphire 1.3 ultrasonic bath (35 kHz, 20–25°C) for 25 min. The resulting suspensions were then filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure to obtain dry extracts.

2.4 HPLC-DAD Analysis, Method Validation, and Measurement Uncertainty

The qualitative and quantitative profiling of phenolic constituents in the extracts was carried out using a Shimadzu high-performance liquid chromatography system equipped with a diode-array detector, following the analytical strategy described by (Tokul-Ölmez et al. 2020; Sicak et al., 2026). Data acquisition and processing were performed using LC-Solution software. Separation was carried out using an Inertsil ODS-3 reversed-phase analytical column (5 µm, 4.6 × 250 mm; GL Sciences, Tokyo, Japan), which was operated at 40°C during the procedure. The extract was prepared at a final concentration of 8 mg/mL in 80% methanol, filtered through a 0.45 µm membrane, and 20 µL of the solution was injected into the system. The mobile phase consisted of water containing 0.1% acetic acid (solvent A) and methanol with 0.1% acetic acid (solvent B). Chromatographic separation was performed using a multi-step gradient program with a total run time of 81 min at a flow rate of 1.0 mL/min. The gradient elution of mobile phase B was set as follows: 0–3 min, 2% B; 3–6 min, 5% B; 7–8 min, 6% B; 9–13 min, 10% B; 14–18 min, 25% B; 19–25 min, 30% B; 26–30 min, 40% B; 31–36 min, 42% B; 37–41 min, 54% B; 42 min, 55% B; 43–52 min, 56% B; 53–56 min, 65% B; 57–59 min, 75% B; 60–61 min, 85% B; 62–68 min, 95% B; 69–74 min, 100% B; 75–76 min, 80% B; 77–78 min, 50% B; and 79–81 min, returning to the initial condition of 2% B for column re-equilibration. The flow rate was maintained at 1.0 mL/min, and the total chromatographic run time was 81 min. Detection of phenolic compounds was performed over the wavelength range 200–400 nm, using UV spectra and retention times from authenticated standards to identify compounds. All measurements were conducted in triplicate, and results were expressed as milligrams of compound per gram of dry extract.

The chromatographic method was comprehensively validated in accordance with the ICH Q2(R1) guidelines, evaluating linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability (precision), and accuracy (recovery). Linearity was established across multi-point calibration curves, with determination coefficients (R^2) ≥ 0.9880 for all reference standards. Signal-to-noise criteria of 3:1 and 10:1 were adopted to evaluate the limit of detection (LOD) and the limit of quantification (LOQ), respectively. Repeatability was determined by seven replicate injections ($n = 7$) of standard solutions and expressed as percentage relative standard deviation ($RSD\% \leq 5.06\%$). Method accuracy was verified by recovery assays with spiked samples, yielding mean recovery percentages ranging from 96.14% to 108.00%.

The evaluation of measurement uncertainty was carried out in accordance with the EURACHEM/CITAC Guide CG 4 (*Quantifying Uncertainty in Analytical Measurement*) using a top-down validation approach (Ellison & Williams,

2012; AlMutairi et al., 2025; Al et al., 2025). The relative combined standard uncertainty ($u_{c,rel}$) for each quantified phenolic compound was calculated by combining the major independent uncertainty contributors, including method repeatability ($u_{rep} = RSD/100$), recovery/bias (u_{rec}), calibration curve regression (u_{cal}), and volumetric/weighing operations (u_{prep}):

$$u_{c,rel} = \sqrt{u_{rep}^2 + u_{rec}^2 + u_{cal}^2 + u_{prep}^2}$$

The expanded measurement uncertainty ($U_{rel}, \%$) was then established using a coverage factor of $k = 2$, corresponding to an approximate 95% confidence level:

$$U_{rel} (\%) = k \times u_{c,rel} \times 100 \quad (k = 2)$$

Quantitative phenolic contents of the extracts are reported as Mean $\pm U$ (mg/g dry extract), where U represents the expanded uncertainty calculated as $U = C \times (U_{rel}/100)$. All validation parameters, uncertainty budgets, and quantitative data are summarized in Tables 1 and S1.

Representative chromatograms of the reference standard mixture and *Tamarix hispida* extracts (THU-10, THU-50, and THU-70) are shown in Figure 1, confirming good resolution and peak symmetry for all compounds analyzed.

2.5 Biological Activity

2.5.1 In vitro Antioxidant Activity

The antioxidant capacities of the extracts (THU-10, THU-50, and THU-70) were assessed using three distinct assays: DPPH free radical scavenging, ABTS radical cation scavenging, and CUPRAC, with butylated hydroxytoluene (BHT) as a positive control.

2.5.1.1 Determination of 2,2-Diphenyl-1-picrylhydrazyl Free Radical Scavenging Activity of the THU-10, THU-50, and THU-70

The DPPH radical scavenging potential was assessed based on the protocol described by (Blois 1958) with slight modifications (Tel et al., 2016; Abdigali et al., 2025). Briefly, ethanolic preparations of THU-10, THU-50, and THU-70 at varying concentrations (40 µL each) were mixed with 160 µL of a 0.004% DPPH solution in ethanol. After incubating the reaction mixture in the dark for 30 min, optical density was measured at 517 nm utilizing a BioTek Synergy H1 96-well multimode reader (Vermont 05404, USA).

2.5.1.2 Determination of ABTS Cation Radical Scavenging Activity of the THU-10, THU-50, THU-70

The ABTS cation radical scavenging activity was performed according to the method reported by (Re et al., 1999), with slight modifications (Labeled et al., 2016; Abdigali et al., 2025). The ABTS cation radical reagent was produced through the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, kept in darkness for 12 h at ambient temperature. Before the assay, ethanol was used to dilute the working stock at a 1:88 ratio, targeting an optical density of

Table 1. Method validation parameters, expanded measurement uncertainty, and phenolic composition of THU-10, THU-50, and THU-70 extracts of *Tamarix hispida* Willd. determined by HPLC-DAD at 254 nM (mg/g dry extract)

Compound	RT ^a (min)	THU-10	THU-50	THU-70	Calibration Equation	R ² ^b	Linear Range (µg/mL)	λ _{max} , nM	LOD ^c (µg/mL)	LOQ ^c (µg/mL)	Recovery (%)	RSD ^d within Day (n = 7)	Expanded Uncertainty (u, %, k = 2)
Gallic acid	15.225	1.38 ± 0.15	<LOD	<LOD	y = 45540x - 84708	0.9995	5.00–62.5	254	4.64	14.06	108.00	2.35	10.96
Protocatechuic acid	24.625	<LOD	<LOD	0.08 ± 0.003	y = 65753x - 6932.1	0.9993	0.57–290	254	0.26	0.79	100.22	1.22	4.08
Catechin	30.274	<LOD	<LOD	<LOD	y = 2611.2x + 74392	0.9972	3.80–1950	254	1.20	3.63	96.14	3.78	9.76
p-Hydroxybenzaldehyde	33.367	1.21 ± 0.13	2.35 ± 0.25	<LOD	y = 34376x + 4239.6	0.9880	1.25–50	254	1.33	4.44	99.01	4.76	10.70
Vanillic acid	34.758	<LOD	<LOD	0.20 ± 0.02	y = 66764x + 46508	0.9998	0.39–200	254	0.29	0.88	100.16	5.06	11.26
Epicatechin	35.278	0.57 ± 0.05	0.94 ± 0.09	1.66 ± 0.15	y = 20976x + 7998.2	0.9999	3.9–500	254	0.95	2.86	101.25	4.00	9.20
p-Coumaric acid	40.874	<LOD	<LOD	<LOD	y = 18300x + 6153.3	0.9998	6.25–400	254	5.46	16.56	101.60	3.14	7.64
Ferulic acid	42.564	<LOD	<LOD	0.70 ± 0.05	y = 42245x + 110701	0.9992	2.34–300	254	3.96	11.99	100.99	3.20	7.58
Hesperidin	47.381	<LOD	<LOD	<LOD	y = 2949.5x + 9083.5	0.9995	3.00–180	254	2.99	9.73	100.39	2.68	6.54
Rutin	47.527	2.78 ± 0.20	3.88 ± 0.28	4.42 ± 0.32	y = 47899x + 56096	0.9995	0.46–235	254	0.43	1.29	99.28	3.01	7.20
Fisetin	51.243	0.59 ± 0.07	0.52 ± 0.06	0.93 ± 0.11	y = 100784x + 16688	0.9984	0.28–72.5	254	0.34	1.04	96.84	5.02	11.72

Note: ^aRT: Retention time (min); ^bR²: Coefficient of determination; ^cLOD: Limit of Detection (S/N = 3); LOQ: Limit of Quantification (S/N = 10); ^dRSD: Relative standard deviation of peak areas (intra-day repeatability, n = 7). Quantitative values for extracts are presented as mean ± expanded measurement uncertainty (U, mg/g, k = 2, 95% confidence level).

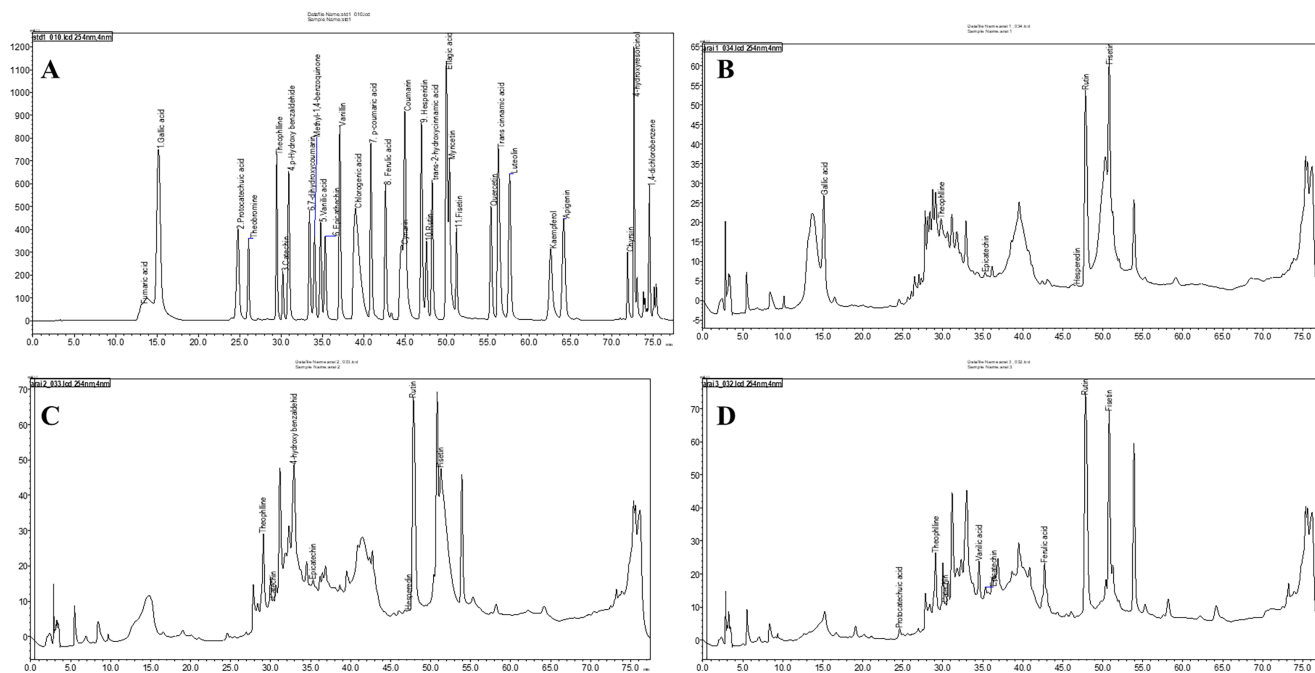


Figure 1. Representative HPLC-DAD chromatograms at 254 nm: (A) Mixture of authentic phenolic standards: 1. Gallic acid, 2. Protocatechuic acid, 3. Catechin, 4. *p*-Hydroxybenzaldehyde, 5. Vanillic acid, 6. Epicatechin, 7. *p*-Coumaric acid, 8. Ferulic acid, 9. Hesperidin, 10. Rutin, 11. Fisetin; (B) *Tamarix hispida* 10% ethanol extract (THU-10); (C) 50% ethanol extract (THU-50); and (D) 70% ethanol extract (THU-70)

0.700 ± 0.025 at 734 nm. An aliquot of 160 μ L of this reagent was then briefly mixed with 40 μ L of DMSO-solubilized THU-10, THU-50, and THU-70 at varying concentrations. The absorbance was measured at 734 nm after 10 min incubation using a 96-well multimode reader (BioTek Synergy H1, Vermont 05404, USA).

2.5.1.3 Determination of CUPRAC Reducing Antioxidant Capacity of the THU-10, THU-50, THU-70

The cupric reducing antioxidant capacity (CUPRAC) assay was performed according to (Apak et al., 2004). Briefly, 50 μ L of 10 mM Cu (II), 50 μ L of 7.5 mM neocuproine, and 60 μ L of NH_4Ac buffer (1 M, pH 7.0) were added to each well in a 96-well plate. Subsequently, 40 μ L portions of THU-10, THU-50, and THU-70, solubilized in DMSO across various concentrations, were introduced into the mixture. Following a 1 h incubation, absorbance readings were taken at 450 nm. All trials were executed in triplicate, incorporating DMSO as a negative control to track the reaction baseline. Time-dependent alterations in optical density were used to evaluate the bleaching rate. For the antioxidant assays, the effective concentration yielding 50% inhibition (IC_{50}) and, for CUPRAC, the level corresponding to 0.500 absorbance ($A_{0.5}$) were determined from plots of bleaching percentage against extract concentration. Every bioassay read-out was collected on a 96-well multimode microplate reader (BioTek Synergy H1, BioTek Instruments, Inc., Vermont 05404, USA).

2.5.2 In vitro Enzyme Inhibitory Activities

The enzyme inhibitory activities of THU-10, THU-50, and THU-70 were evaluated using acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α -amylase, α -glucosidase, and pancreatic lipase inhibition assays.

2.5.2.1 Determination of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibitory Activity of the THU-10, THU-50 and THU-70

The electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg) and horse reddish butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg) were used to determine the anticholinesterase activity in DMSO for THU-10, THU-50, and THU-70, with acetylthiocholine iodide and butyrylthiocholine chloride as substrates, using spectroscopic method (Ellman et al., 1961; Kızıltaş et al., 2021; Karta et al., 2025). Briefly, 10 μ L aliquots of the extracts (THU-10, THU-50, or THU-70) at different concentrations were pre-mixed with 130 μ L of 100 mM sodium phosphate buffer (pH 8.0) and 20 μ L of buffered AChE or BChE. Following a 15 min incubation period at 25°C, 20 μ L of 0.5 mM DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] and 20 μ L of either acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM) were introduced. The absorbance was then measured at 412 nm using a 96-well multimode reader (BioTek Synergy H1, Vermont 05404, USA). Galantamine was used as the positive control.

2.5.2.2 Determination of α -amylase Inhibitory Activity of the THU-10, THU-50 and THU-70

α -Amylase inhibitory activity of THU-10, THU-50, and THU-70 was tested using a spectroscopic method with slight modifications to (Quan et al., 2019). Briefly, the assay was performed in a 96-well plate by pre-incubating 25 μ L of test extracts at various concentrations with 50 μ L of α -amylase solution (0.1 U/mL in 20 mM phosphate buffer, 6 mM NaCl, pH 6.9) at 37°C for 10 min. The reaction was initiated by incorporating 50 μ L of a 0.05% starch substrate solution and maintained for an additional 10 min at 37°C. To halt the reaction, 25 μ L of 0.1 M HCl was added to each well, after which 100 μ L of Lugol's iodine reagent was introduced to detect remaining starch. The absorbance was then measured at 565 nm using a 96-well multimode reader (BioTek Synergy H1, Vermont 05404, USA). Acarbose was used as the positive control.

2.5.2.3 Determination of α -glucosidase Inhibitory Activity of the THU-10, THU-50 and THU-70

α -Glucosidase inhibitory activity of THU-10, THU-50, and THU-70 was determined using a spectroscopic method with slight modifications (Kim et al., 2000). Briefly, 10 μ L of the sample solution was mixed in a 96-well plate with 50 μ L of 10 mM phosphate buffer (pH 6.9), 25 μ L of PNPG (*p*-nitrophenyl- α -D-glucopyranoside) dissolved in 10 mM phosphate buffer (pH 6.9), and 25 μ L of α -glucosidase solution (0.1 U/mL, 10 mM phosphate buffer, pH 6.0). After maintaining the mixture at 37°C for 20 min, 90 μ L of 100 mM sodium carbonate was introduced into each well to quench the enzymatic reaction. The absorbance was then measured at 400 nm using 96-well multimode reader (BioTek Synergy H1, Vermont 05404, USA). Acarbose was used as the positive control.

2.5.2.4 Pancreatic Lipase Inhibitory Activity

Pig pancreatic lipase type II was prepared in a suitable buffer (10 mM MOPS (morpholinepropane sulfonic acid), and 1 mM EDTA, pH 6.8, at 2.5 mg/mL). 169 μ L of Tris buffer (100 mM Tris-HCl and 5 mM CaCl₂, pH 7.0) was added to prepare an enzyme buffer. Extracts across a 5–200 μ g/mL concentration range were treated with 1 mg/mL enzyme in buffer and kept at 37°C for 15 min. Following this initial step, the enzymatic reaction was initiated by adding 10 mM *p*-nitrophenyl butyrate substrate to the tubes and maintaining the mixture at 37°C for 30 min. The enzyme was then added, and the resulting color was measured at 405 nm. Using the absorbance values, the % inhibition values for each concentration were calculated. The IC₅₀ values were calculated from the graph of pancreatic lipase % inhibition activity versus sample amount. Orlistat was used as the positive standard (Mokhtar et al., 2023). Orlistat was used as the positive control.

2.5.3 Anti-inflammatory Activity—Oxidative Burst Assay Using Chemiluminescence Technique

The luminol-dependent chemiluminescence method detailed by (Helfand et al. 1982) was employed for the evaluation. Briefly, 25 μ L of diluted whole blood prepared in HBSS⁺⁺ (Hanks' Balanced Salt Solution supplemented with CaCl₂ and MgCl₂; Sigma, USA) was pre-incubated in triplicate with 25 μ L of each compound at 1, 10, and 100 μ g/mL in white half-area 96-well microplates (Costar, USA). Control wells were loaded with cells in HBSS⁺⁺ devoid of test agents. After a 15 min incubation at 37°C inside the luminometer chamber (Labsystems, Finland), 25 μ L portions of both serum-opsonized zymosan (SOZ; Fluka, Switzerland) and luminol (Research Organics, Cleveland, OH, USA) were added to all wells except the solvent blanks (HBSS⁺⁺ only). Cellular ROS generation was quantified as relative light units (RLU). The standard inhibitor ibuprofen yielded an IC₅₀ value of 11.2 μ g/mL.

2.5.4 Cytotoxicity—Brine Shrimp Lethality Assay (BSLA)

Cytotoxicity was evaluated by the brine shrimp lethality assay (BSLA) described by (Meyer et al., 1982). Eggs of *Artemia salina* were hatched in artificial seawater (38 g/L marine salt, pH 7.4) in a divided tray under illumination at 37°C for 48 h. Nauplii (larvae) were collected for use within 48–72 h of hatching.

The sample extracts were solubilized at 20 mg/mL using an appropriate vehicle (water or methanol). To achieve target concentrations of 10, 100, and 1000 μ g/mL subsequent to overnight solvent removal, volumes of 5, 50, and 500 μ L were transferred into distinct vials, with three replicates evaluated per concentration level. A total of 10 nauplii were transferred to each test vial, after which the volume was made up to 5 mL with seawater. Following this, incubation was carried out for 24 h under light conditions at 25–27°C. Surviving larvae were counted; etoposide served as the positive cytotoxic control. Lethal dose values (LD₅₀ with 95% confidence intervals) were determined using Finney's probit analysis software (Finney, 1971).

2.6 Statistical Analysis

The biological activity results are expressed as mean $\pm$ SEM of three parallel measurements. Statistical comparisons were performed using one-way ANOVA, with $p < 0.05$ considered statistically significant. HPLC-DAD quantitative data are reported as mean $\pm$ expanded measurement uncertainty (U, $k = 2$, 95% confidence level). Statistical analyses were performed using GraphPad Prism 9.0.

3 Results and Discussion

In the present study, three aqueous-ethanolic extracts of *Tamarix hispida* Willd. (THU-10, THU-50, and THU-70) were prepared by ultrasound-assisted extraction and comparatively evaluated to determine the influence of solvent composition on their phenolic profiles and biological activities. The extracts were characterized by HPLC-DAD and

evaluated for antioxidant, anticholinesterase, α -amylase, α -glucosidase, pancreatic lipase inhibitory, anti-inflammatory, and cytotoxic activities. The marked improvement in yield and tannin content achieved by UAE corroborates earlier data from our laboratory (Zhumagaliyeva et al., 2021) and aligns with mechanistic literature on acoustic cavitation-driven cell-wall disruption (Tiwari, 2015). The 3–4-fold yield enhancement is consistent with reports on other polyphenol-rich plants processed by UAE (Chemat et al., 2017), underscoring the industrial and pharmaceutical scalability of the approach.

3.1 Extraction Yield and Process Comparison

Ultrasound-assisted extraction at 35 kHz, 20–25°C for 25 min yielded a dry extract of 37.34% (THU-10), significantly exceeding the yield obtained by conventional maceration (48 h) under equivalent conditions. Compared with maceration, UAE reduced extraction time by approximately 115-fold (from 48 h to 25 min) and increased the yield of extractable substances by 3–4-fold across the tested frequency range of 20–35 kHz. Tannin content in UAE extracts reached approximately 10%, compared with 8% for maceration, representing an increase of about 25%. These results corroborate and extend our earlier observations (Zhumagaliyeva et al., 2021).

3.2 Phenolic Composition by HPLC-DAD

Phenolic compounds were identified in three extracts using HPLC-DAD at 254 nm and quantitative data are summarized in Table 1. Rutin, a flavonol glycoside, was the dominant compound across all three extracts, with concentrations increasing with ethanol content: 2.78 (THU-10) < 3.88 (THU-50) < 4.42 mg/g (THU-70). The epicatechin amount also increased with increasing the ethanol percentage of the extraction solvent (0.57, 0.94, and 1.66 mg/g extract, respectively). In contrast, gallic acid (1.38 mg/g) was detected only in THU-10, likely reflecting its preferential solubility in aqueous-rich solvents. *p*-Hydroxybenzaldehyde was present in THU-10 (1.21 mg/g), and THU-50 (2.35 mg/g) but absent in THU-70. Protocatechuic acid, vanillic acid, and ferulic acid were unique to THU-70, suggesting that more hydrophobic phenolic esters require higher ethanol concentrations for efficient extraction.

The HPLC-DAD analysis revealed that rutin was the principal polyphenol in all three extracts, and that its content increased monotonically with ethanol concentration (2.78 → 3.88 → 4.42 mg/g). Rutin is a well-established anti-inflammatory and antioxidant compound whose biological effects are mediated through inhibition of the xanthine oxidase enzyme, suppression of NF- κ B signaling, and free-radical scavenging (Ganeshpurkar & Saluja, 2017). The exclusive presence of gallic acid in THU-10 (1.38 mg/g) is noteworthy: gallic acid is one of the most potent natural antioxidants and demonstrates wound-healing, antimicrobial, and anti-inflammatory properties. Its preferential extraction in water-rich media reflects its high polarity and hydrophilicity. Epicatechin, fisetin, and *p*-hydroxybenzaldehyde, possessing documented antioxidant and anti-inflammatory activities (Anitha et al.,

2020; Fernandes & Salgado, 2016; Pal et al., 2016), contributed to the overall bioactivity profiles of the extracts.

3.3 Antioxidant Activities

The antioxidant activity of the *Tamarix hispida* extracts was evaluated using ABTS, DPPH, and CUPRAC assays (Table 2).

Among the three extracts, THU-10 exhibited the strongest antioxidant activity overall. It showed the lowest IC₅₀ values in the ABTS (2.13 ± 0.75 µg/mL) and DPPH (6.01 ± 0.30 µg/mL) assays. THU-50 and THU-70 showed weaker radical-scavenging activity, with ABTS IC₅₀ values of 4.68 ± 0.33 and 9.19 ± 0.54 µg/mL and DPPH IC₅₀ values of 15.16 ± 0.27 and 14.20 ± 0.66 µg/mL, respectively. In the CUPRAC assay, THU-10 also exhibited the highest reducing capacity among the extracts, as indicated by the lowest A_{0.5} value (10.51 ± 0.01 µg/mL), compared with THU-50 (24.28 ± 0.03 µg/mL) and THU-70 (22.10 ± 0.03 µg/mL). Since lower IC₅₀ and A_{0.5} values indicate greater antioxidant activity, these results consistently demonstrate the comparatively strong antioxidant potential of THU-10. The comparatively strong antioxidant activity of THU-10 may be associated with its specific phenolic composition, including gallic acid, epicatechin, rutin, fisetin, and *p*-hydroxybenzaldehyde. In particular, gallic acid was quantified only in THU-10, whereas differences in the concentrations and combinations of the other phenolic constituents may also contribute to the observed antioxidant effects.

An analysis of studies on different *Tamarix* species reveals a common trend, indicating that extracts obtained from various plant parts, including aerial parts, leaves, flowers, bark, and fruits, are rich in phenolic compounds and exhibit considerable antioxidant activity (Said et al., 2018; Chaturvedi et al., 2012; Dawood et al., 2021; Ksouri et al., 2009). A similar solvent-dependent variation in antioxidant activity has also been reported for other *Tamarix* species (Mahadi et al., 2024). In *Tamarix aphylla*, the ethyl acetate extract of the aerial parts exhibited pronounced antioxidant activity, with DPPH, ABTS, and CUPRAC values of 43.24 ± 0.53, 71.74 ± 0.30, and 113.79 ± 4.22 mg TE/g, respectively. Even more pronounced antioxidant activity was observed for the ethyl acetate extracts of *T. senegalensis*. The extract of its aerial parts showed DPPH, ABTS, and CUPRAC values of 475.95 ± 8.31, 596.13 ± 2.25, and 644 ± 28.36 mg TE/g, respectively. In contrast, the hexane extracts of both species generally exhibited considerably lower antioxidant activity. This pronounced dependence on the extraction solvent suggests that the compounds responsible for the antioxidant activity of *Tamarix* species are preferentially extracted within specific ranges of solvent polarity. Thus, the antioxidant behavior observed for *T. hispida* may be associated with differences in the qualitative and quantitative composition of phenolic compounds extracted at different solvent concentrations.

Previous studies on *Tamarix* species have also demonstrated substantial free-radical-scavenging activity. For example, *T. gallica* exhibited antioxidant activity in the DPPH assay, with an IC₅₀ value of 0.5 mg/mL (Chaturvedi

Table 2. The antioxidant activity results of THU-10, THU-50 and THU-70^a

Compounds	Antioxidant Activity		
	ABTS ⁺ Assay (IC ₅₀ µg/mL)	DPPH [•] Assay (IC ₅₀ µg/mL)	CUPRAC Assay (A _{0.5} µg/mL)
THU-10	2.13 ± 0.75	6.01 ± 0.30	10.51 ± 0.01
THU-50	4.68 ± 0.33	15.16 ± 0.27	24.28 ± 0.03
THU-70	9.19 ± 0.54	14.20 ± 0.66	22.10 ± 0.03
BHT ^b	5.61 ± 0.72	38.21 ± 0.28	4.91 ± 0.50

Note: ^aValues expressed herein are mean ± SEM of three parallel measurements. *p* < 0.05. ^b Reference compounds. Abbreviation: BHT, 2-tert-butyl-4-hydroxytoluene. IC₅₀: half-maximal inhibitory concentration, the concentration of an inhibitor required to reduce the biological activity by 50%. A_{0.5}: Half-Maximal Absorbance Effective Concentration, the concentration of an inhibitor required for 0.500 absorbance.

Table 3. IC₅₀ µg/mL[†] values of various enzyme inhibitory activities and anti-inflammatory activity (ROS assay) of THU-10, THU-50, THU-70^a

Samples	Anticholinesterase activity		Antidiabetic activity		Pancreatic lipase inhibitory activity	Anti-inflammatory activity
	AChE inhibitory activity	BChE inhibitory activity	α-Amylase inhibitory activity	α-Glucosidase inhibitory activity		
	IC ₅₀ µg/mL	IC ₅₀ µg/mL	IC ₅₀ µg/mL	IC ₅₀ µg/mL	IC ₅₀ µg/mL	IC ₅₀ µg/mL
THU-10	21.90 ± 0.70	38.71 ± 0.19	45.77 ± 0.12	91.31 ± 0.71	154.7 ± 0.80	65.4 ± 0.6
THU-50	29.08 ± 0.67	44.28 ± 0.97	88.55 ± 1.20	105.2 ± 0.55	180.7 ± 0.73	98.4 ± 1.0
THU-70	35.62 ± 0.70	43.05 ± 0.55	93.42 ± 1.66	128.6 ± 0.27	149.1 ± 0.32	18.4 ± 0.2
Galantamine ^b	4.75 ± 0.51	46.76 ± 0.18	NT	NT	NT	NT
Acarbose ^b	NT	NT	62.92 ± 1.84	15.71 ± 0.97	NT	NT
Orlistat ^b	NT	NT	NT	NT	17.66 ± 0.61	NT
Ibuprofen ^b	NT	NT	NT	NT	NT	11.2 ± 1.9

Note: ^aValues expressed herein are mean ± SEM of three parallel measurements, *p* < 0.05. ^b Reference compounds. NT: not tested.

et al., 2012). Phenolic compounds frequently reported in *Tamarix* species include gallic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ellagic acid, catechin, quercetin, rutin, isoquercetin, kaempferol, and related phenolic constituents (Aytar et al., 2025; Mahadi et al., 2024). Collectively, these findings support the importance of both solvent polarity and phenolic composition in determining the antioxidant properties of *Tamarix* extracts.

3.4 Anticholinesterase, Antidiabetic, Anti-Inflammatory and Pancreatic Lipase Inhibitory Activities

The results of the oxidative burst assay, based on the luminol-enhanced chemiluminescence technique, are presented in Table 3. THU-70 exhibited the highest anti-inflammatory activity (IC₅₀ = 18.4 ± 0.2 µg/mL), followed by THU-10 (IC₅₀ = 65.4 ± 0.6 µg/mL) and THU-50 (IC₅₀ = 98.4 ± 1.0 µg/mL). Among the extracts, THU-70's IC₅₀ is comparable to that of ibuprofen (11.2 ± 1.9 mg/mL), indicating a significant anti-inflammatory effect consistent with the high polyphenol content of the higher-ethanol extracts.

The anticholinesterase activity, the antidiabetic activity, and pancreatic lipase inhibitory of the *Tamarix Hispida* extracts were evaluated (Table 3). The anticholinesterase activity was assessed based on their inhibitory effects against

acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and expressed as IC₅₀ values. All extracts exhibited inhibitory activity against both AChE and BChE, although the IC₅₀ values varied among the extracts. For AChE inhibition, THU-10, THU-50, and THU-70 showed IC₅₀ values of 21.90 ± 0.70, 29.08 ± 0.67, and 35.62 ± 0.70 µg/mL, respectively. Based on these values, the AChE inhibitory activity decreased in the following order: THU-10>THU-50>THU-70, with THU-10 exhibiting the strongest inhibitory activity. A similar trend was observed for BChE inhibition, with THU-10 showing the lowest IC₅₀ (38.71 ± 0.19 µg/mL), followed by THU-70 (43.05 ± 0.55 µg/mL) and THU-50 (44.28 ± 0.97 µg/mL). Overall, THU-10 exhibited the strongest inhibitory activity against both enzymes among the investigated *Tamarix Hispida* extracts. The antidiabetic activity was assessed by determining the inhibitory effects of the extracts against α-Amylase and α-Glucosidase, while pancreatic lipase inhibitory activity was evaluated based on the inhibition of pancreatic lipase. The inhibitory activities were expressed as IC₅₀ values.

In contrast to the more extensively studied *Tamarix* species, *Tamarix hispida* is considered to be chemically rich in antioxidant constituents, including a potent

Table 4. Cytotoxicity of *Tamarix hispida* extracts against brine shrimp nauplii (n = 30 per concentration). Etoposide: positive control (70% mortality)

Extract	Dose (µg/mL)	Total shrimps	Survivors	Mortality (%)	Etoposide mortality (%)
THU-10	10	30	28	6.67	70
	100	30	26	13.34	
	1000	30	25	16.67	
THU-50	10	30	24	20.00	
	100	30	23	23.34	
	1000	30	21	30.00	

antioxidant triterpenoid as well as tannin- and flavonoid-containing compounds. However, direct evidence of its anti-inflammatory activity remains limited in the available literature (Sultanova et al., 2004; Zhumagaliyeva et al., 2021). Therefore, for the first time, the anti-inflammatory activity of *T. hispida* extracts obtained by ultrasound-assisted extraction (UAE) was evaluated using an oxidative burst assay based on the luminol-enhanced chemiluminescence technique, and the results are presented in Table 2. THU-70 exhibited the highest anti-inflammatory activity ($IC_{50} = 18.4 \pm 0.2$ mg/mL), and contained the highest amount of rutin and epicatechin. The lower activity of THU-50 ($IC_{50} = 98.4 \pm 1.0$ mg/mL) compared with THU-10 ($IC_{50} = 65.4 \pm 0.6$ mg/mL) may reflect an antagonistic interaction among its phenolic constituents at the tested concentration of 50 µg/mL or differences in bioavailability in the assay medium. Among the extracts, the IC_{50} of THU-70 is comparable to that of ibuprofen (11.2 ± 1.9 mg/mL); this observation indicates a significant anti-inflammatory effect, consistent with the high polyphenol content of the higher-ethanol extracts (Dabeek & Marra, 2019).

3.5 Cytotoxicity (Brine Shrimp Lethality Assay)

Cytotoxicity data for THU-10 and THU-50 against brine shrimp nauplii are presented in Table 4. All tested extracts showed mortality rates well below the cytotoxicity threshold ($LD_{50} < 1000$ µg/mL = cytotoxic per the Meyer criterion (Meyer et al., 1982)), with maximum mortalities of 16.67% (THU-10) and 30.00% (THU-50) at 1000 µg/mL, compared to 70% for the etoposide-positive control. These results confirm the safety of both extracts within the tested concentration range (10–1000 µg/mL) and support their suitability for further pharmacological studies.

The BSLA data classified all extracts as non-cytotoxic, with mortality rates of 6.67–16.67% for THU-10 and 20.00–30.00% for THU-50 at the highest tested dose (1000 µg/mL), compared with 70% for etoposide. These findings align with the generally recognized safety profile of polyphenol-rich *Tamarix* extracts (Irshad et al., 2025), and with earlier brine shrimp lethality data for related Tamaricaceae species. The non-cytotoxic profile strongly supports the prospective use of these extracts in topical wound-care formulations.

From a translational perspective, THU-10 emerges as the most promising extract for further pharmaceutical research (Amanzholkzy et al., 2025). This combination,

rich in gallic acid, rutin, and epicatechin, could in principle provide sustained polyphenol release and synergistic antioxidant/anti-inflammatory action; however, this remains a direction for future research. Certain limitations should be acknowledged. Complete cytotoxicity data for THU-70 are pending. Anti-inflammatory and antioxidant data for THU-50 and THU-70 obtained by maceration were not available for direct comparison within this study and will be addressed in future studies. *In vivo* models will be required to validate the *ex vivo* and *in vitro* findings reported here.

4 Conclusions

This study demonstrated that ultrasound-assisted extraction (UAE) provided an efficient approach for obtaining phenolic-rich extracts from *Tamarix hispida* Willd., reducing the extraction time from 48 h to 25 min and increasing the extraction yield by 3–4-fold compared with conventional maceration. HPLC-DAD analysis identified eleven phenolic compounds, with distinct compositional profiles observed among THU-10, THU-50, and THU-70. Rutin was the predominant quantified phenolic compound, reaching 2.78, 3.88, and 4.42 mg/g in THU-10, THU-50, and THU-70, respectively, whereas gallic acid was detected only in THU-10 (1.38 mg/g).

Among the investigated extracts, THU-10 exhibited the strongest overall antioxidant activity, with IC_{50} values of 2.13 ± 0.75 and 6.01 ± 0.30 µg/mL in the ABTS and DPPH assays, respectively, and an $A_{0.5}$ value of 10.51 ± 0.01 µg/mL in the CUPRAC assay. THU-10 also showed the strongest AChE ($IC_{50} = 21.90 \pm 0.70$ µg/mL), BChE ($IC_{50} = 38.71 \pm 0.19$ µg/mL), α -amylase ($IC_{50} = 45.77 \pm 0.12$ µg/mL), and α -glucosidase ($IC_{50} = 91.31 \pm 0.71$ µg/mL) inhibitory activities among the three extracts. In contrast, THU-70 exhibited the strongest pancreatic lipase inhibitory activity ($IC_{50} = 149.1 \pm 0.32$ µg/mL) and anti-inflammatory activity ($IC_{50} = 18.4 \pm 0.2$ µg/mL).

In the brine shrimp lethality assay, THU-10 and THU-50 showed low toxicity within the tested concentration range, with mortality rates of 16.67% and 30.00%, respectively, at 1000 µg/mL. THU-10 was characterized by stronger antioxidant, anticholinesterase, α -amylase, and α -glucosidase inhibitory activities, whereas THU-70 showed particularly strong anti-inflammatory activity.

Overall, the aqueous-ethanolic extract THU-10 was identified as a promising candidate for further pharmaceutical development. Future research will address the formulation of THU-10 into delivery systems, such as polymer-based hydrogels, as well as *in vivo* anti-inflammatory efficacy, wound-closure kinetics, and the identification of individual bioactive compounds responsible for the observed activities using LC-MS/MS.

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Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Artificial Intelligence (AI) Statement

The authors utilized ChatGPT (OpenAI) for enhancing language and correcting grammatical errors, while ChatGPT (OpenAI) was employed to aid in the creation of the graphical abstract and figures. All content generated with the assistance of AI was thoroughly reviewed, verified, and sanctioned by the authors, who assume complete responsibility for the precision and integrity of the final manuscript.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

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