

Phytochemical and Biological Validation of the Underexplored Medicinal Plant *Tamarix canariensis*

Validation phytochimique et biologique de la plante médicinale peu explorée *Tamarix canariensis*

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ABSTRACT

Introduction-Aim: This work highlights the phytochemical characterization and biological activities of *Tamarix canariensis*, an underexplored plant already recognized for its traditional therapeutic uses.

Methods: The phytochemical profile was assessed using HPLC-UV-MS/MS, on extracts obtained via maceration and ultrasound-assisted extraction. Antioxidant activity was quantified through DPPH and hydrogen peroxide scavenging assays, and anti-inflammatory potential was assessed by measuring inhibition of protein denaturation.

Results: The extracts were found to contain bioactive compounds such as quercetin glucuronide, 2'-hydroxy-3,4,4',6'-tetramethoxychalcone, and ferulic acid sulfate, with tamarixetin-3-O-hexoside detected exclusively in the hexane extract. They exhibited strong antioxidant effects, with IC₅₀ values of 242.62 ± 1.09 µg/mL (DPPH) and 243.95 ± 0.95 µg/mL (H₂O₂), which correlated closely with total phenolic content. Additionally, at a concentration of 2 mg/mL, the extracts inhibited protein denaturation by 90% in bovine albumin and 97.73% in egg white albumin.

Conclusion: These findings indicate that *Tamarix canariensis* is a promising natural source of phenolic compounds with potent antioxidant and anti-inflammatory properties, supporting its traditional medicinal use and underscoring its potential for pharmaceutical and healthcare applications.

Keywords: *Tamarix canariensis* ; HPLC-UV-MS/MS ; phytochemical profiling ; antioxidant activity ; antiinflammatory activity

RÉSUMÉ

Introduction – Objectif : Cette étude vise à caractériser le profil phytochimique et à évaluer les activités biologiques de *Tamarix canariensis*, une plante sous-explorée reconnue pour ses usages thérapeutiques traditionnels.

Méthodes: Le profil phytochimique a été évalué à l'aide de HPLC-UV-MS/MS, sur des extraits obtenus par macération et extraction assistée par ultrasons. L'activité antioxydante a été quantifiée par les tests DPPH et de piégeage du peroxyde d'hydrogène, et le potentiel anti-inflammatoire a été évalué en mesurant l'inhibition de la dénaturation des protéines.

Résultats: Les extraits contiennent des composés bioactifs, principalement la quercétine glucuronide, la 2'-hydroxy-3,4,4',6'-tétraméthoxychalcone et le sulfate d'acide férulique, avec la tamarixétine-3-O-hexoside détectée exclusivement dans l'extrait d'hexane. Ils ont montré de fortes activités antioxydantes, avec des valeurs IC₅₀ de 242,62 ± 1,09 µg/mL (DPPH) et 243,95 ± 0,95 µg/mL (H₂O₂), fortement corrélées au contenu total en phénols. De plus, à une concentration de 2 mg/mL, les extraits ont inhibé la dénaturation des protéines de 90 % dans l'albumine bovine et de 97,73 % dans l'albumine d'œuf.

Conclusion: Ces résultats indiquent que *Tamarix canariensis* est une source naturelle prometteuse de composés phénoliques aux propriétés antioxydantes et anti-inflammatoires puissantes, soutenant son utilisation traditionnelle en médecine et soulignant son potentiel pour des applications pharmaceutiques et de santé.

Mots-clés: *Tamarix canariensis* ; HPLC-UV-MS/MS ; profilage phytochimique ; activité antioxydante ; activité anti-inflammatoire.

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INTRODUCTION

Medicinal and aromatic plants have been used for therapeutic purposes for thousands of years and continue to play a central role in traditional medicine. Historical records document their use dating back over 5,000 years, Particularly in the medical systems of India, China, Egypt, Greece, Rome and Persia (1). It is important to note that medicinal and aromatic plants are rich in secondary metabolites, which are produced in response to environmental stimuli. These compounds, such as terpenes, essential oils, phenols and alkaloids, have numerous therapeutic properties, including antioxidant, antimicrobial and anti-inflammatory. In addition, they contribute to improving digestion, memory and immune function, and help to lower blood pressure and cholesterol levels (1).

Tamarix canariensis, known in French as « Tamaris des îles Canaries » (Canary Islands Tamarisk), is commonly known as "Torfa" in the Moroccan dialect, although its name may vary from one region to another. For example, it is called "Âmmây" and "Tammâyt" in Draa Souss, "L-fersîg" and "Âfersîg" in Tekna and the Sahara, "Âkawar" in Tekna and Draa (2). It is one of the many halophytic plants. This spontaneous Mediterranean species extends its range into tropical regions. It belongs to the genus *Tamarix*, which includes approximately 65 species native to Asia, Africa, and Europe (3). These plants are distinguished by their needle-like leaves, which are coated with salt excreted by specialized salt glands (4).

The genus *Tamarix* thrives in the Saharan bioclimatic zone, which covers 50% of Morocco's territory, stretching from Figuig to the Draa Valley. *Tamarix canariensis* is a common species in Mediterranean coastal areas, northern Europe, and the tropical, arid, and semi-arid regions of Asia. It demonstrates a strong capacity to adapt to diverse environments and exhibits remarkable resilience to harsh environmental conditions. Its flowering period occurs between March and July. Due to its ability to withstand coastal winds, this species is commonly used as an effective windbreak in coastal gardens. Additionally, its deep root system enables it to prevent the erosion of sandy soils (5–7). In Morocco, all *Tamarix* species are regarded as female, with the exception of *Tamarix aphylla*. On the other hand, the bark and leaf powder of *T. canariensis* and other *Tamarix* species are used in Saharan regions as antidiarrheal, hemostatic, and anti-hemorrhoidal remedies. Leaf infusions are used to treat colds and are believed to possess antirheumatic properties in the Draa Oasis, while fresh leaves are utilized in steam baths to relieve pain. In Asia, *T. canariensis* is employed for liver conditions and is known for its hepatotonic and anti-inflammatory benefits (3).

The interest in highlighting the *Tamarix canariensis* plant, the subject of this study, stems from its still largely under-exploited status in the medicinal and pharmaceutical fields, despite its therapeutic potential and traditional use. Very few studies have focused on its phytochemical profile. This project aims to contribute to the literature by providing new data on its phytochemical characteristics, as well as its biological activities. It also seeks to promote

a better understanding of this plant, with a view to its potential development in the pharmaceutical sectors.

Specifically, this study aims to conduct a comprehensive analysis of the plant's phytochemical composition and to identify its principal bioactive constituents. Special attention was given to evaluating its biological potential, particularly through the assessment of its antioxidant and anti-inflammatory activities.

METHODS

Plant material

The plant was collected from Sidi Rahhal (Casablanca region) in March 2019. The analyzed part of *Tamarix canariensis* Willd in this study corresponds to the aerial parts. The collection site is geographically located at Lambert coordinates: 33°28'47"N, 7°56'49"W. The botanical identification was carried out at the Laboratory of Ecology and Environment, Faculty of Sciences Ben M'Sik, by professors of botany. The collected plant material was air-dried and ground into a fine powder to prepare it for extraction.



Figure 1. Photo of the species *Tamarix canariensis* in its natural habitat, taken in Ain Diab(8).

Extraction

Two methods were compared for extracting phenolic compounds: maceration, aligned with traditional practices of infusion and decoction, and ultrasound-assisted extraction (UAE), a modern, economical, and widely used method. The choice of extraction solvent, and the solvent-to-water ratio, is also a key factor in the efficiency of the extraction process. In this study, the following solvents were used: methanol, ethanol, acetone, water, hexane, and ethyl acetate. For methanol, ethanol, and acetone, different solvent-to-water ratios (v/v) were tested: 100:0, 80:20, and 50:50.

Maceration

The plant material was mixed with solvent for 24 hours under magnetic stirring, then filtered using a Buchner funnel under vacuum. The mixture was centrifuged at 10,000 rpm for 10 minutes to separate the liquid from the solid particles. The supernatant was evaporated under a

rotary evaporator to remove the solvent and concentrate the extract. The dry residue was collected, stored in labeled Eppendorf tubes, and kept protected from light.

Ultrasound-assisted extraction (UAE)

The plant powder was extracted using a probe sonicator. The probe, operating at the highest power level (400 W), was immersed in the beaker containing the plant material and solvent for 15 minutes. An ice bath was used to prevent a temperature rise during the extraction process. The resulting extracts were then filtered, centrifuged at 10,000 rpm, and concentrated using a rotary evaporator. The dry residues were stored in Eppendorf tubes, protected from light, until further use (9).

Phytochemical profil

Total Phenolic Content (TPC)

Total polyphenols (TPC) are determined using the Folin-Ciocalteu method (10). Briefly, 50 µL of Folin-Ciocalteu reagent was added to 10 µL of each reconstituted sample, followed by a dilution with 600 µL of distilled water. After 10 min of incubation, 150 µL of Na₂CO₃ was added and the volume was made up to 1 mL with distilled water. Absorbance was measured on a spectrophotometer at 760 nm after incubation for 2 h in the dark at room temperature. The obtained results were compared to a calibration curve of gallic acid prepared under the same conditions (5–100 µg/mL), with a correlation coefficient (R²) of 0.998 (Appendix 1, Fig1(on line)).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of plants extracts was determined using a colorimetric method as previously described (11). Briefly, 250 µL of each extract was mixed with 1 mL of distilled water and 75 µL of 5% NaNO₂ solution. After a 6 min of incubation, 75 µL of 10% AlCl₃ solution was added. The mixture was left to stand for another 6 min before the addition of 1 mL NaOH solution (4%). The final volume was then adjusted to 2.5 mL with distilled water and the tubes were vigorously vortexed and then left to stand for 15 minutes, protected from light. Absorbance was measured at 510 nm and compared to a calibration curve prepared from a catechin solution under the same conditions, at concentrations ranging from 5 to 400 µg/mL. The calibration curve exhibited a determination coefficient (R²) of 0.997 (Appendix 1, Figure 2(on line)).

Flavonol Content (FC)

Flavonol content was assessed according to previously described methods. For this purpose, 500 µL aliquot of each extract was successively mixed with 500 µL of aluminum chloride (20 mg/mL) and 1.5 mL of sodium acetate (50 mg/mL). The mixtures were vortexed and incubated in the dark for 150 minutes. A spectrophotometer was used to measure absorbance at 440 nm, and the quantification was carried out using a quercetin calibration curve (5 - 200 µg/mL), with R² = 0.995 (12) (Appendix 1, Figure 3(on line)).

Condensed Tannin Content (CTC)

The condensed tannin content (CTC) was determined using the vanillin assay (13). For each sample, 100 µL was mixed with 1.5 mL of a 4% vanillin solution and 750 µL of concentrated hydrochloric acid (HCl). After vigorous vortexing, the tubes were incubated in the dark for 20 minutes. Absorbance was recorded at 500 nm, and the condensed tannin content was quantified using a catechin calibration curve prepared in the same conditions at different concentrations (5 - 400 µg/mL), with R² = 0.985.

2,2-Diphenyl-1-Picrylhydrazyl Radical-Scavenging Assay (DPPH)

The DPPH test was conducted as described bellow (14). 500 µL of each extract (1 mg /mL; methanol) were mixed with 1 mL of freshly prepared DPPH solution (0.1 mM; methanol). The tubes were rigorously shaken and kept in the dark for 60 minutes. DPPH reduction was measured at 515 nm and the scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{ADPPH - As}{ADPPH} \times 100$$

Where: ADPPH is the absorbance of DPPH and AS is the absorbance of DPPH when the sample is added at different concentrations. The IC₅₀ value is calculated from the percentage scavenging activity graph and Ascorbic acid was prepared at different concentrations and used as a positive control (Appendix 1, Figure 5(on line)).

Hydrogen Peroxide Scavenging Assay

According to a previous study (15), the hydrogen peroxide scavenging assay was performed as follows. A 50 mM phosphate buffer (pH 7.4) was mixed with a 40 mM hydrogen peroxide solution (H₂O₂). To assess the scavenging activity, 0.6 mL of the H₂O₂ solution was added to each sample. The mixtures were incubated for 10 minutes, and absorbance was measured at 230 nm. Ascorbic acid was used as a positive control prepared under the same operating conditions (Appendix 1, Figure 4 (on line)), while phosphate buffer was used as a negative control. The hydrogen peroxide scavenging activity (%) was calculated using the following equation.

$$\text{H2O2 scavenging activity (\%)} = \frac{A0 - As}{A0} \times 100$$

Where: As is the absorbance in the presence of sample or standard (ascorbic acid) and A₀ is the absorbance of the blank.

Bovine Serum Albumin Assay (BSA)

The Bovine Serum Albumin (BSA) denaturation assay was performed as follows (15). In each assay, 0.45 ml of BSA (0.5%; w/v) was mixed with 0.05 mL of extracts dissolved in phosphate buffer at various concentrations ranging from 200 to 2000 µg/mL. After thorough mixing, the samples were incubated in a water bath at 40°C for

25 minutes. Subsequently, phosphate buffer saline (2.5 ml; pH 6.3) was added to each tube. The negative control received 0.05 ml phosphate buffer solution instead of extract. As for the positive control, the anti-inflammatory drug Diclofenac was used under the same experimental conditions. The results obtained served as a reference to compare and validate the anti-inflammatory potential of the tested extracts. The absorbance was measured using a spectrophotometer at 660 nm, and the percentage inhibition of BSA denaturation was calculated using the following formula:

$$\text{Inhibition of BSA denaturation (\%)} = \frac{A1 - A2}{A1} \times 100$$

Where A1 is the absorbance of the negative control and A2 is the absorbance of the test sample.

Chicken Egg Albumin Assay (CEA)

The ability of *T. canariensis* extracts to inhibit egg albumin denaturation was assessed using the following protocol (16). In each test tube, 2.8 mL of phosphate-buffered saline (PBS), 2 mL of plant extract, and 0.2 mL of hen's egg albumin were mixed. Each tube was shaken vigorously and left at room temperature for 15 minutes, followed by an incubation in a water bath at 70°C for 10 minutes. Diclofenac was used as a standard anti-inflammatory reference, and absorbance was measured at 660 nm using a spectrophotometer. The following equation was used to determine the inhibition of CEA denaturation:

$$\text{Inhibition of CEA denaturation (\%)} = \frac{A1 - A2}{A1} \times 100$$

Where: A1 is the absorbance of the control and A2 is the absorbance of the test sample.

HPLC-UV-MS/MS analysis

Qualitative analysis of phenolic compounds was carried out using a high-performance liquid chromatography system (HPLC) coupled with a triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

The analytical method was based on reversed-phase separation using a Kinetex C18 column (100 × 4.6 mm; 2.6 µm particle size), enabling efficient separation of compounds. Chromatographic separation was conducted in gradient mode with two mobile phases : solvent A (0.1% aqueous solution of formic acid) and solvent B (methanol), according to the protocol described by Bouaouda et al. (17). Compound identification was based on comparing retention times and mass spectra with nine reference standards and further confirmed using the NIST-MS/MS spectral library.

Statistical analysis

The results of this study were obtained from triplicate analyses and are expressed as mean ± standard deviation (SD). Difference between extraction methods, solvents and plant parts were evaluated using two-way ANOVA,

followed by Tukey's post-hoc test to identify the most effective extraction procedures and solvent ratios for subsequent analyses. To compare biological activity results with controls, Student's t-test was applied, considering results statistically significant at $p < 0.05$ and highly significant at $p < 0.01$. Furthermore, IC_{50} values and Pearson coefficient (r) correlation matrices were calculated and illustrated using GraphPad Prism v.10.1.2 for Windows.

RESULTS AND DISCUSSIONS

Phytochemical profil

TPC, TFC, FC, and CTC

The results of phytochemical tests carried out on *T. canariensis* extracts revealed that all extracts are particularly rich in bioactive compounds such as TPC, TFC, FC and CTC as shown in Table 1. The yields obtained from the different extraction methods varied significantly, with a p-value of less than 0.05. The yields ranged widely, from 0.45% to 24.44%, reflecting the influence of varying experimental conditions. These results are consistent with findings from another species in the Tamaricaceae family. The yield of *Tamarix aphylla* using methanolic extraction was 20.5%, whereas the yield with ethyl acetate was only 1.6% (18). Statistically significant differences were observed depending on the extraction methods and solvents used, with a significance threshold set at $p < 0.05$. This indicates that the choice of extraction method and solvent significantly influences the quantity of compounds extracted.

In particular, UAE showed better results in terms of extraction efficiency compared to traditional maceration. This indicates that the use of UAE enables faster and more complete extraction of bioactive compounds.

Ethanol extracts (80:20) yielded the highest concentrations of TPC (145.14 ± 0.25 mgGAE/gDW), while acetone extracts (80:20) were more effective at extracting total flavonoids (89.78 ± 1.71 mgCE/gDW), flavonols (31.93 ± 0.18 mgQE/gDW) and condensed tannins (166.10 ± 1.35 mgCE/100gDW).

In contrast, hexane extracts showed the lowest concentrations, with 57.92 ± 0.833 mgGAE/gDW for TPC and 46.00 ± 0.33 mgCE/gDW for TFC.

Extracts obtained using (acetone 80:20), (methanol 80:20), and (ethanol 80:20) revealed the highest concentrations. These solvent-water mixtures are considered the most effective for extracting phenolic compounds from *T. canariensis* due to their balanced polarity (19). Consequently, these extracts were selected for the evaluation of biological activities and for the characterization of bioactive compounds.

Numerous studies have highlighted the richness of the *Tamarix* genus in bioactive compounds, and several species are considered important sources of phenolic compounds (20,21). However, the lack of data specifying the TPC, TFC, FC and CTC of *T. canariensis* underscores the need for further research aimed at a more in-depth characterization.

Table 1. Determination of TPC, TFC, F and CTC in T.canariensis extracts.

Extraction method	Extraction solvents		Yield (%)	TPC* mgGAE/gDW	TFC* mgCE/gDW	FC* mgQE/gDW	CTC* mgCE/100gDW
Maceration	Methanol	100	7.24 ± 0.15 ^a	42.81 ± 0.91 ^{ab}	32.33 ± 1.00 ^a	3.01 ± 0.32 ^a	14.00 ± 0.75
		80:20	10.05 ± 0.25 ^b	72.94 ± 0.19 ^a	51.39 ± 0.96 ^b	3.61 ± 0.51 ^b	18.33 ± 0.66
		50:50	7.45 ± 0.45 ^c	55.89 ± 0.63 ^b	43.11 ± 0.83 ^c	3.11 ± 0.33 ^c	16.05 ± 0.59
	Ethanol	100	0.77 ± 0.22 ^d	53.83 ± 0.76 ^c	45.06 ± 0.69 ^d	0.88 ± 0.19 ^d	12.86 ± 1.31
		80:20	9.27 ± 0.24 ^e	83.89 ± 0.91 ^c	56.67 ± 0.57 ^e	1.10 ± 0.48 ^e	17.29 ± 1.03
		50:50	8.49 ± 0.47 ^f	69.72 ± 0.25 ^d	47.78 ± 0.38 ^f	0.89 ± 0.19 ^f	14.10 ± 0.33
	Acetone	100	1.90 ± 0.19 ^g	46.67 ± 0.66 ^e	40.22 ± 0.50 ^g	6.31 ± 0.18 ^g	14.48 ± 0.33
		80:20	6.77 ± 0.09 ^j	62.56 ± 0.25 ^f	49.61 ± 0.50 ^h	16.43 ± 0.93 ^h	16.19 ± 0.16
		50:50	12.30 ± 0.34 ^j	54.31 ± 0.09 ^g	43.17 ± 1.29 ^j	12.51 ± 0.30 ^j	15.14 ± 2.23
	Water		8.59 ± 0.16 ^k	83.44 ± 0.75 ^h	42.94 ± 1.57 ^j	2.79 ± 0.07 ^j	16.05 ± 0.87
	Hexan		0.45 ± 0.05 ⁱ	34.33 ± 0.72 ⁱ	23.83 ± 0.66 ^k	0.06 ± 0.01 ^k	5.19 ± 0.43
	Ethyl acetate		0.58 ± 0.05 ^m	84.89 ± 0.00 ^j	64.72 ± 0.5 ^j	0.63 ± 0.03 ^j	10.00 ± 0.28
Ultrasound-assisted extraction	Methanol	100	14.28 ± 0.52 ^a	72.86 ± 0.77 ^a	59.78 ± 0.69 ^a	9.69 ± 0.68 ^a	18.81 ± 0.59
		80:20	23.66 ± 0.19 ^b	115.89 ± 0.5 ^a	73.56 ± 0.19 ^b	13.04 ± 0.41 ^b	33.71 ± 0.57
		50:50	21.66 ± 0.17 ^c	83.64 ± 0.34 ^b	66.44 ± 1.07 ^c	10.98 ± 0.11 ^c	31.38 ± 1.15
	Ethanol	100	14.13 ± 0.10 ^d	83.58 ± 0.50 ^c	78.11 ± 0.51 ^d	9.67 ± 0.51 ^d	20.19 ± 0.16
		80:20	21.22 ± 0.67 ^e	145.14 ± 0.25 ^c	84.72 ± 0.50 ^e	19.64 ± 0.41 ^e	26.81 ± 1.15
		50:50	15.82 ± 0.11 ^f	105.19 ± 1.04 ^d	80.44 ± 0.69 ^f	10.84 ± 0.32 ^f	22.43 ± 0.85
	Acetone	100	3.32 ± 0.43 ^g	81.17 ± 0.72 ^e	76.56 ± 1.07 ^g	12.34 ± 0.15 ^g	22.95 ± 0.87
		80:20	15.16 ± 0.24 ⁱ	98.56 ± 0.41 ^f	89.78 ± 1.71 ^h	31.93 ± 0.18 ^h	166.10 ± 1.35
		50:50	24.44 ± 0.30 ^j	86.96 ± 0.53 ^g	77.67 ± 1.66 ⁱ	20.47 ± 0.14 ⁱ	148.62 ± 0.33
	Water		11.22 ± 0.19 ^k	130.78 ± 2.71 ^h	73.39 ± 0.69 ^j	12.28 ± 0.32 ^j	35.90 ± 1.35
	Hexan		0.75 ± 0.20 ^l	57.92 ± 0.83 ^j	46.00 ± 0.33 ^k	2.10 ± 0.08 ^k	9.90 ± 1.35
	Ethyl acetate		0.74 ± 0.06 ^m	134.67 ± 0.88 ^j	82.28 ± 1.50 ^j	3.33 ± 0.33 ^l	20.90 ± 0.16

*Values are means ± standard deviation; values in each column with the same alphabet are significantly different (p<0.05); TPC: total polyphenolic content; TFC: total flavonoids content; FC: flavonols content; CTC: condensed tannins content

In vitro antioxidant activity

The antioxidant activity of T.canariensis was evaluated using the DPPH assay and H₂O₂ scavenging test, as shown in Figure 2.

The most significant free radical scavenging effects were observed in ethyl acetate extract (TAE) and aqueous extract (TAQ). In the DPPH assay, which measures an extract's ability to neutralize free radicals, the ethyl acetate extract exhibited the lowest IC₅₀ value (242.62 ± 1.09 µg/mL). This result indicates that the extract is highly effective at neutralizing radicals at low concentrations, which may be attributed to ethyl acetate's capacity to extract a wide range of semi-polar bioactive compounds, favored by its moderate polarity. These findings suggest that the antioxidant activity observed may be due to this class of bioactive substances, which are either not extracted or only poorly extracted by more polar solvents (22). In the same regard, a study on T. ramosissima showed that the tannin and ethyl acetate fractions significantly reduced DPPH radicals, with low IC₅₀ values (7.4 and 8.6 µg/mL), indicating high antioxidant activity (23).

The results of the H₂O₂ test revealed the effectiveness of T. canariensis aqueous extract (TAQ) to neutralize free radicals, with an IC₅₀ value of 243.95 ± 1.26 µg/mL. This result indicates that the extract exhibits a moderate capacity to eliminate hydrogen peroxide, suggesting that

TAQ possesses notable free radical scavenging potential at this concentration. This can be attributed to water's ability to dissolve water-soluble antioxidant compounds, such as polyphenols, flavonoids and phenolic acids (24). In a similar context, a study showed that the crude extract of T. aphylla leaves demonstrated a strong capacity to scavenge H₂O₂ radicals, achieving 57% activity at a concentration of 1000 µg/mL. With an IC₅₀ value of 123.4 µg/mL, the extract significantly reduced H₂O₂ radical formation in a concentration-dependent manner, suggesting notable antioxidant potential (25). In comparison, T.gallica and T.articulata exhibit stronger antioxidant activity than T.canariensis, as evidenced by their IC₅₀ values of 15.70 µg/mL and 7.69 µg/mL, respectively (20). Another study conducted on T. aphylla revealed that its extracts also possess moderate antioxidant activity, with an IC₅₀ values of 14.0 mg/mL (26).

In vitro anti-inflammatory activity

With the exception of the extract obtained with hexane, all Tamarix canariensis extracts exhibited strong inhibitory activity against protein denaturation, with inhibition rates exceeding 90% at a concentration of 2 mg/mL, as shown in Figure 3. The differences observed between the active extracts were minimal and statistically insignificant

($p > 0.05$), indicating comparable efficacy among them. For comparison, diclofenac sodium, used as a positive control due to its well-known anti-inflammatory properties, showed an inhibition rate of 97.73% at the same concentration.

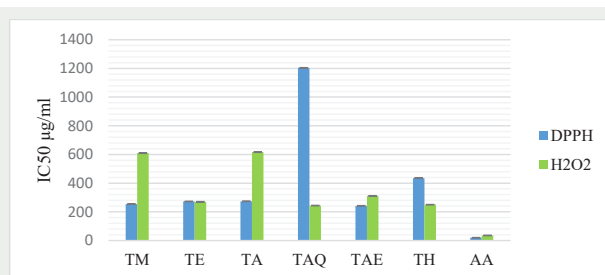


Figure 2. Results of DPPH and H2O2 scavenging tests for *T.canariensis* extracts

AA: ascorbic acid; TM: methanol extract; TE: ethanol extract; TA: acetone extract; TAQ: aqueous extract; TAE: ethyl acetate extract; TH: hexane extract;

The close inhibition values between *T. canariensis* extracts and diclofenac suggests that this plant has a strong

potential to inhibit protein denaturation, supporting the hypothesis of significant anti-inflammatory activity. The similar effectiveness of the various extracts in preventing protein denaturation highlights the robustness of the inhibitory effect exhibited by *T. canariensis*. This suggests the potential future use of *T.canariensis* as an anti-inflammatory agent, considering that protein denaturation is one of the main mechanisms involved in inflammatory disorders. Furthermore, its ability to inhibit protein denaturation also suggests its potential application as a food additive to protect proteins against thermal degradation (27). Species from the same family, namely *T.aphylla*, *T.dioica* and *T.africana*, have also been identified for their anti-inflammatory properties (28,29). One study demonstrated that essential oil from *Tamarix aphylla* possesses significant anti-inflammatory activity ; when administered at a dose of 316 mg/kg, it effectively reduced edema in an animal model of induced inflammation in rats (30).

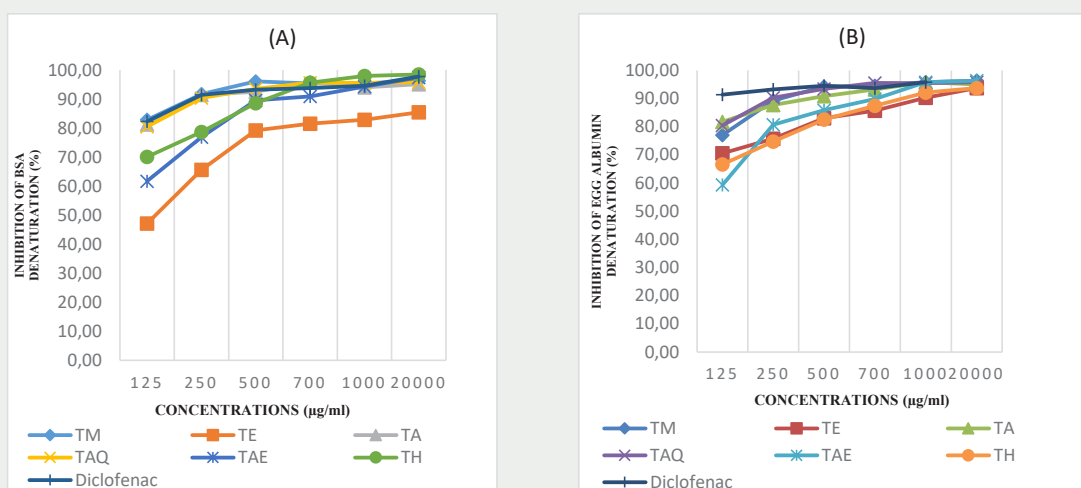


Figure 3. Results of denaturation inhibition of BSA (A) and CEA (B) from *T.canariensis* extracts.

TM: methanolic extract; TE: ethanolic extract; TA: acetonic extract; TAQ: aqueous extract; TAE: ethyl acetate extract; TH: hexane extract.

Correlation

The results of the Pearson correlation analysis for *Tamarix canariensis* extracts indicate significant positive correlations between extract yield, total phenolic compounds, flavonoids, flavonols, condensed tannins, and DPPH radical scavenging activity as shown in Figure 4. These findings suggest that higher concentrations of these phenolic compounds are associated with increased free radical scavenging capacity, highlighting the close relationship between their presence and the observed antioxidant activity. In contrast, for H_2O_2 scavenging assay and the protein denaturation inhibition tests, negative correlations were observed. This suggests that the phenolic content and extract yield are not the primary factors influencing these activities, and other bioactive compounds or mechanisms may contribute to the antioxidant and anti-inflammatory effects detected in

these assays.

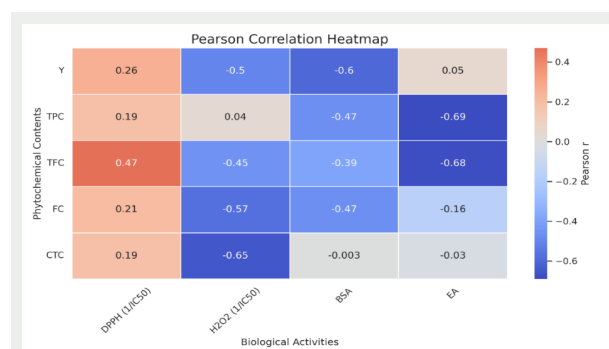


Figure 4. Pearson correlation heatmap between phytochemical content and biological activities of *Tamarix canariensis* extracts

Y: yield; TPC: total phenolic content; TFC: total flavonoid content; FC: flavonols; CTC: condensed tannins content; BSA: BSA denaturation test; EA: egg albumin denaturation inhibition test .

Phenolic profile analysis by HPLC-UV-MS/MS

The data presented in Table 4 provide a comprehensive overview of the HPLC-UV-MS/MS analysis results of *Tamarix canariensis*. This analysis revealed a profile of bioactive compounds, with flavonoids and phenolic acids representing the most abundant classes across all the extracts examined (Appendix 2 (on line)). Figure 5 presents the chemical structures of the major compounds identified in the extracts. Notably, quercetin—a flavonoid widely recognized for its potent antioxidant and anti-inflammatory properties—was detected in all analyzed extracts. Its conjugated derivative, quercetin glucuronide was particularly prominent and appeared as the major compound in the methanolic, ethanolic, and acetonic extracts, indicating its preferential extraction by solvents of intermediate polarity. On the other hand, the compound 2'-hydroxy-3,4,4',6'-tetramethoxychalcone, a member of the chalcone family, was found in higher concentrations in hexane and ethyl acetate extracts, this distribution suggests greater solubility of the molecule in low to moderately polar solvents. Additionally, ferulic

acid, a well-documented antioxidant phenolic acid, was identified as the predominant compound in the aqueous extract, highlighting its strong affinity for polar extraction media. Notably, tamarixetin-3-O-hexoside, a methoxylated derivative of quercetin, was exclusively detected in the hexane extract, emphasizing the solvent-specific extraction of certain compounds depending on their polarity and chemical structure.

In addition to quercetin metabolites, the presence of ferulic acid contributes to the antioxidant potential of the extracts and plays a significant role in food preservation due to its radical-scavenging properties. Tamarixetin, a methoxylated flavonoid, has been reported to exhibit renoprotective, antioxidant, and antibacterial activities (23,31). Moreover, the detection of myricitrin in the extracts is noteworthy, as this compound has demonstrated efficacy against periodontitis, an infectious inflammatory condition triggered by bacterial plaque (32). These findings corroborate the traditional medicinal uses of *Tamarix canariensis* in the treatment of rheumatic disorders, diarrhea, and its application as a gingival tonic (33).

Table 4. Results of HPLC-UV-MS/MS analysis of *T. canariensis* extracts.

Tentative identification	RT (min)	REF	Chemical Formula	Molecular Mass (g/mol)	[M-H] ⁻ (m/z)	Fragments (m/z)	Extracts					
							M	E	A	AQ	H	AE
2'-hydroxy-3,4,4',6'-tetramethoxychalcone	1.97	N	C ₁₉ H ₂₀ O ₆	344	343	313, 315	P	P	P	P	P*	P*
5-O-Caffeoylquinic acid	3.20	N	C ₁₆ H ₁₈ O ₉	354	353	191, 173	P	P	P	P	P	P
Quercetin 3'-methyl ether	4.77	N	C ₁₆ H ₁₂ O ₇	316.26	315	271, 255, 300	P	P	P	P	P	P
Gallic acid	6.50	S	C ₇ H ₆ O ₅	170	169	125	P	P	P	P	P	A
Chlorogenic acid	12.45	S	C ₁₆ H ₁₈ O ₉	354	353	191	P	P	P	A	P	P
Catechin	17.66	N	C ₁₅ H ₁₄ O ₆	290	289	245, 203	P	P	P	P	A	A
Ferulic acid	18.96	S	C ₁₀ H ₁₀ O ₄	194	193	134, 178	P	P	P	P	A	A
Myricitrin	20.22	N	C ₂₁ H ₂₀ O ₁₂	464	463	316, 317	P	P	P	P	A	P
Quercetin	20.95	S	C ₁₅ H ₁₀ O ₇	302	301	301, 271	P	P	P	P	P	P
Quercetin glucuronide	22.11	N	C ₂₁ H ₁₈ O ₁₃	478	477	301	P*	P*	P*	P	P	P
Ferulic acid sulfate	23.19	N	C ₁₀ H ₁₀ O ₇ S	274	273	178, 193	P	P	P	P*	A	A
Kaempferol-3-O-hexose-deoxyhexose	24.42	N	C ₂₇ H ₃₀ O ₁₅	594	593	284, 285	P	P	P	P	P	A
Tamarixetin-3-O-hexoside	24.70	N	C ₂₂ H ₂₂ O ₁₂	478	477	300, 315	A	A	A	A	P	A

M: methanol; E: ethanol; A: acetone; AQ: aqueous extract; H: hexane; AE: ethyl acetate; AS: spectral analysis; RT: retention time; P: presence; A: absence; *: majority peak.

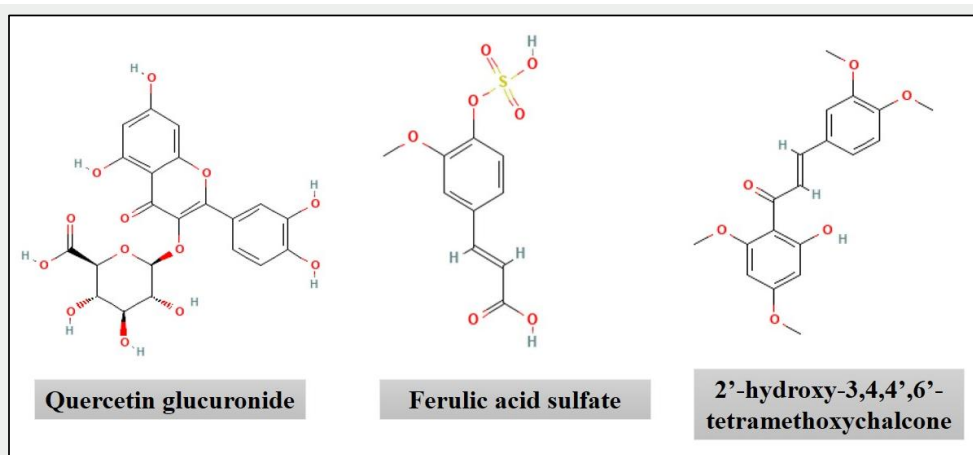


Figure 5. Chemical structures of the major compounds identified in the extracts

CONCLUSION

The results revealed the promising antioxidant capacity of *T. canariensis*, particularly in its aqueous extracts, which were notably rich in ferulic acid. These extracts exhibited strong DPPH radical scavenging activity and effectively inhibited protein denaturation, suggesting a potential anti-inflammatory effect. Overall, the findings validate the traditional medicinal uses of *T. canariensis* and contribute valuable new data on a previously underexplored Mediterranean species. This work opens promising avenues for the therapeutic and biotechnological valorization of *T. canariensis*, especially in the development of natural antioxidant and anti-inflammatory agents.

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