Trop J Nat Prod Res, August 2025; 9(8): 3597 - 3606

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

HPLC/MS Characterization and Evaluation of the Antioxidant and Anti-Inflammatory Properties of Algerian Medicinal Plant: *Carthamus caeruleus* L. Extracts

Karima Djafri[1,2], Wiam Saadi[3], Abdelkader Saadi[2], Abdallah Noui [4,5]*, Fatima Z Bouarsa[1], Ana Borges[6], Mohammed Kouidri[2]

[1]*Laboratory of Natural Bio-Ressources, Hassiba Benbouali University of Chlef, 02000 Chlef, Algeria*
[2]*Faculty of Life and Natural Sciences, Hassiba Benbouali University of Chlef, Algeria*
[3]*Department of Biology, Faculty of Nature, Life and Earth Sciences, University of Djillali Bounaama, Khemis Miliana 44225, Algeria*
[4]*Laboratory of Management and Valorization of Agricultural and Aquatic Ecosystems, University Center of Tipaza, 42000, Algeria*.
[5]*National School of Nanoscience and Nanotechnology, Sidi Abdellah, Algiers, Algeria.*
[6]*CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.*

ARTICLE INFO

Article history:
Received 17 May 2025
Revised 22 June 2025
Accepted 25 June 2025
Published online 01 September 2025

ABSTRACT

Carthamus caeruleus L. (*Asteraceae*) has been traditionally recognized for its wound-healing, anti-inflammatory, and burn-relieving properties. The present study investigates the phytochemical composition, antioxidant potential, and anti-inflammatory activity of hydromethanolic and chloroformic extracts derived from its rhizomes. The hydromethanolic extract exhibited higher total phenolic content (138.07 ± 0.15 µg GAE/mg) and flavonoid concentration (35.87 ± 0.02 µg QE/mg). Phytochemical analysis using liquid chromatography coupled with mass spectrometry identified key compounds including feruloylquinic acid, dihydro-*p*-coumaric acid glucoside, and a ferulic acid derivative (isomer I). Antioxidant activity was assessed via lipid peroxidation inhibition, cellular antioxidant activity, hydrogen peroxide scavenging, and total antioxidant capacity. The hydromethanolic extract demonstrated strong lipid peroxidation inhibition (IC_{50} = 1.47 ± 0.17 mg/mL), high total antioxidant capacity (100.01 ± 0.09 µg GAE/mg), moderate cellular antioxidant activity (12.49 ± 1.13%), and hydrogen peroxide scavenging at 450 µg/mL.Anti-inflammatory activity was evaluated *in vitro* using LPS-stimulated RAW 264.7 macrophages and *in vivo* through carrageenan-induced paw edema in mice. The extract inhibited nitric oxide production (IC_{50} = 161 ± 0.36 µg/mL), reduced erythrocyte hemolysis (83.09 ±0.45 %), and protein denaturation (65.04 ± 1.86%) at 500 µg/mL. *In vivo*, a 53.57 ± 4.71% reduction in paw inflammation was observed at 1000 mg/kg. These findings support the traditional use of *Carthamus caeruleus* L. and highlight its potential as a source of antioxidant and anti-inflammatory agents. Further studies are required to isolate active constituents and evaluate their therapeutic relevance.

Keywords: *Carthamus caeruleus*, burn-relieving properties, Phytochemical analysis, High-performance liquid chromatography coupled with tandem mass spectrometry, Antioxidant potential, Anti-inflammatory activity.

Introduction

Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defenses, plays a critical role in various pathological conditions. While ROS play essential roles in cellular signaling and metabolic processes, their excessive accumulation-often triggered by environmental or physiological stressors—can inflict substantial damage on cellular components.[1] Such damage affects essential biomolecules, including proteins, lipids, and DNA, and has been implicated in the development of neurodegenerative disorders such as Alzheimer's, amyotrophic lateral sclerosis, and Down syndrome, as well as metabolic disorders like diabetes, obesity, and their associated microvascular complications.[2]

*Corresponding author; Email: a.noui@ensnn.dz
Tel: +213 655142134

Citation: Djafri K, Saadi W, Saadi A, Noui A, Bouarsa Z F, Borges A, Kouidri M. HPLC/MS Characterization and Evaluation of the Antioxidant and Anti-Inflammatory Properties of Algerian Medicinal Plant : *Carthamus caeruleus* L. Extracts. Trop J Nat Prod Res. 2025; 9(8): 3597 – 3606 https://doi.org/10.26538/tjnpr/v9i8.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Algeria, the largest country in the Mediterranean basin and in the Arab and African worlds, features a wide range of ecological zones, spanning nearly 2.4 million km² and 1.600 km coastline. This environmental diversity supports a rich flora comprising over 4.000 plant taxa. Combined with its deep-rooted cultural heritage, Algeria has long embraced phytotherapy as an integral part of traditional medicine, sustained by generations of ethnobotanical knowledge.[3] In this context, the present study focuses on *Carthamus caeruleus*, a member of the *Asteraceae* family and the *Carthamus* genus.[4]

These rare perennial herbaceous plants are adapted to sun-exposed Mediterranean habitats and are believed to have originated in Southwest Asia, later spreading to North Africa, Europe, and other regions.[5] Traditionally, *Carthamus caeruleus* L. has been used for wound healing, burn treatment, and inflammation relief.[6] These uses are supported by its richness in bioactive compounds such as rutin, quercetin, and caffeic acid, which have demonstrated promising antioxidant and anti-inflammatory properties in preliminary *in vitro* and *in vivo* studies.[7] However, despite these encouraging findings, the precise chemical composition of *Carthamus caeruleus* L. rhizome has not yet been thoroughly characterized using advanced analytical techniques. In this context, the present study aims to address this gap by conducting the first detailed profiling of *Carthamus caeruleus* L.

rhizome using liquid chromatography coupled with tandem mass spectrometry. This approach will enable the accurate identification of chemical compounds that may be responsible for its biological effects. Furthermore, the antioxidant and anti-inflammatory activities of the extract are evaluated to deepen the understanding of the pharmacological potential of this medicinal plant.

Materials and Methods

Plant Material and Extract Preparation

Rhizomes of *Carthamus caeruleus* L. were collected in the summer of 2023 from the Medjadja region, located in Chlef Province, northwestern Algeria (36°15'47.9"N, 1°23'50.9"E), following botanical identification by Professor Abdelkader Saadi, a botanist at the Faculty of Natural and Life Sciences, University of Chlef, Algeria. A voucher specimen was deposited in the herbarium under the reference number UCT-Cc-113-2023. The plant material was air-dried, ground, and extracted by maceration in methanol and chloroform, as described by Chaouche et *al*.[8] The obtained extracts were filtered, concentrated under reduced pressure, and stored at 4 °C until further analysis.

Assessment of extraction yield

The extraction yield of *Carthamus caeruleus* L. rhizomes was calculated to evaluate the efficiency of the extraction process using both hydro-methanolic and chloroformic solvents. The yield percentage was determined according to the following equation 1:

$$Yield(\%) = \frac{\text{Mass of dry extract \_mg}}{\text{Initial mass of sample \_ mg}} \times 100 \quad \text{(Eq. 1)}$$

Determination of total phenolic content (TPC)

The total phenolic content of the hydro-methanolic and chloroformic extracts of *Carthamus caeruleus* L. was determined using the Folin–Ciocalteu reagent method.[9] A calibration curve was constructed by mixing 1 mL aliquots of gallic acid standard solutions (50, 100, 150, 200, and 250 µg/mL) with 5.0 mL of diluted Folin–Ciocalteu reagent (1:10 dilution) and 4.0 mL of sodium carbonate solution (75 g/L). The reaction mixtures were incubated at room temperature for 30 minutes, after which the absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Optizen 2120, Mecasys Co. Ltd., Korea). TPC was expressed as micrograms of gallic acid equivalent/mg of dry weight µg.GAE mg/mL

Determination of total flavonoids (TF)

The total flavonoid content was evaluated using a colorimetric method. Briefly, 100 µL of the extract was mixed with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite solution. After a 5-minute, 0.3 mL of 10% aluminum chloride solution was added. Six minutes later, 2 mL of 1 M sodium hydroxide was incorporated into the mixture. The reaction mixture was immediately diluted with 3.3 mL of distilled water and thoroughly mixed. The absorbance was measured at 510 nm against a blank using a UV-Vis spectrophotometer (Optizen 2120, Mecasys Co. Ltd., Korea). A calibration curve was established using quercetin as the standard, and the total flavonoid content was expressed as micrograms of quercetin equivalents per milligram of sample (µg QE/mg).[10]

Metabolite analysis of Carthamus caeruleus L.

Phenolic profiles of *Carthamus caeruleus* L. extracts were characterized using liquid chromatography techniques. The analysis was carried in an Ultimate 3000 (Dionex Co., San Jose, CA, USA) apparatus equipped with an autosampler/injector, a binary pump, a column compartment, and an ultimate 3000 Diode Array Detector (Dionex Co., San Jose, CA, USA), coupled to a Thermo LTQ XL ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with and ESI source.[11] Chromatographic separation was achieved using a Hypersil Gold C18 column (100 mm x 2.1 mm i.d., 1.9 µm particle size, end-capped; ThermoScientific) held at 30°C. A gradient elution system was employed, consisting of (A) acetonitrile and (B) 0.1% formic acid (v/v). The gradient program began with a linear increase from 5% to 40% solvent A over 14.72 minutes, followed by a ramp to 100% A over 1.91 minutes. Solvent A was then held at 100% for 2.19 minutes before returning to the initial conditions.

The flow rate was maintained at 0.2 mL/min. UV-Vis spectra (200-700 nm) were acquired for all eluted peaks, with chromatograms specifically extracted at 280, 320, and 340 nm. Mass spectrometry data acquisition and instrument control were performed using Thermo Xcalibur Qual Browser software. The mass spectrometer operated in negative ionization mode with the ESI needle voltage set to 5.00 kV and the capillary temperature at 275°C. The scanned mass range was *m/z* 100-2000. Nitrogen gas (purity >99%) was used at a pressure of 520 kPa. Collision-induced dissociation tandem mass spectrometry (CID-MS/MS) and MSn experiments were conducted on precursor ions using helium as the collision gas (25-35 arbitrary units). The chromatographic characterization was based on the interpretation of UV spectral characteristics, mass spectral fragmentation patterns, and comparisons with previously reported data.

Determination of In Vitro Antioxidant Properties

Lipid peroxidation assay

Lipid peroxidation inhibition was evaluated in porcine brain homogenates using thiobarbituric acid reactive substances (TBARS) assay. The absorbance of the MDA-TBA complex was measured at 532 nm using ELX800 microplate Reader (Bio-Tek Instruments, Inc.; Winooski, USA) across extract concentrations ranging between 0.0195 and 0.625 mg/mL. The half-maximal effective concentration (EC_{50}), representing the concentration required for 50% inhibition, was determined with butylated hydroxytoluene (BHT) used as a standard reference compound .[11] The percentage inhibition was calculated using the following equation 2:

$$(\%)\text{inhibition} = \frac{\text{Control absorbance \_Sample absorbance}}{\text{Control absorbance}} \times 100 \quad \text{(Eq. 2)}$$

Cellular antioxidant activity (CAA) assay

The cellular antioxidant activity (CAA) was evaluated using a modified method based on Zhou et *al*. [12] employing murine macrophage RAW 264.7 cells (DSMZ). Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, *L*-glutamine, and antibiotics, and seeded at 2×10^4 cells/well in 96-well plates for 48 hours. Following HBSS washes, cells were treated with non-cytotoxic plant extracts (500-2000 µg/mL) and 50 µM DCFH-DA for 60 minutes at 37°C. After further washes, 600 µM AAPH was added, and fluorescence (excitation 485 nm, emission 535 nm) was monitored every 5 minutes for 60 minutes using a Biotek FLX800 plate reader. To quantify the inhibition of the oxidation reaction, results were presented as percentages, and the Cellular Antioxidant Activity (CAA) values were derived via the following equation 3:

$$CAA = \frac{\int AUCs}{\int AUCc} \times 100 \quad \text{(Eq. 3)}$$

∫AUCs signifies the area under the fluorescence-time curve for the sample, and ∫AUCc represents the area under the fluorescence-time curve for the control.

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity was evaluated based on the phosphomolybdenum assay, as outlined by Jimoh et *al.*[13] Five concentrations of the sample were prepared from the stock solution (1 mg/mL**):** 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL.

A reagent solution was freshly prepared by mixing equal volumes of 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. An aliquot of 0.2 mL of the sample (1 mg/mL) or standard solution was combined with 2 mL of the reagent. The reaction mixtures were incubated at 95 °C in a water bath for 90 minutes. After cooling to ambient temperature, the absorbance was recorded at 695 nm using a UV–Vis spectrophotometer.

A standard calibration curve was established with different concentrations of gallic acid (20-100 µg/mL), and the total antioxidant capacity was expressed as micrograms of gallic acid equivalents (µg GAE)/mg.

Determination of Hydrogen Peroxide (H_2O_2) reduction activity

The ability of *Carthamus caeruleus* L. rhizome extract to decompose hydrogen peroxide (H_2O_2) was assessed following the procedure described by Serteser et *al*., [14] Extract solutions were prepared at five concentrations (10, 50, 100, 500, and 1000 µg/mL) using 0.1 M phosphate buffer (pH 7.4). Briefly, 3.4 mL of each extract solution or of vitamin C (used as a positive control), both prepared in 0.1 M phosphate buffer (pH 7.4), were mixed with 0.6 mL of a 40 mM hydrogen peroxide solution prepared in the same buffer. For the negative control, the extract was replaced by phosphate buffer. The mixtures were incubated for 10 minutes in the dark at room temperature. Subsequently, the absorbance was measured at 230 nm using a UV-Visible spectrophotometer. The percentage of hydrogen peroxide decomposition was calculated using the following formula (equation 4):

$$H_2O_2 \text{ decomposition } (\%) = \frac{Ac - As}{Ac} \times 100 \quad \text{(Eq. 4)}$$

Anti-inflammatory potency – NO inhibition assay
Extracts were initially dissolved in sterile distilled water at 8 mg/mL, followed by serial dilutions to achieve test concentrations ranging from 0.125 to 8 mg/mL. RAW 264.7 murine macrophage cells, cultured in DMEM supplemented with 10% heat-inactivated FBS, glutamine, and antibiotics, were seeded at 5 x 10^5 cells/mL in 96-well plates and incubated for 24 hours.
Cells were then treated with 15 µL of extract dilutions (final concentrations 6.25-400 µg/mL) for one hour, subsequently stimulated with 30 µL of LPS (1 µg/mL) for an additional 24 hours, with dexamethasone (50 µM) as a positive control and unstimulated cells as a negative control. The amount of nitric oxide released was calculated by comparing the absorbance at 540 nm in an ELX800 Biotek microplate reader to the calibration curve. Finally, the extract concentration needed to inhibit NO output by 50% (EC_{50} µg/ml) was calculated.[15]

Erythrocyte Membrane Stabilization Assay
The anti-inflammatory potential of various *Carthamus caeruleus* L. extracts was evaluated *in vitro* using the human red blood cell (HRBC) membrane stabilization method, following the procedure of Fujiati et *al*.[16]. Gallic acid (200 µg/mL) was used as the standard, and the anti-inflammatory activity was assessed by determining the percentage inhibition of erythrocyte hemolysis. Blood samples were collected from healthy human volunteers after obtaining informed consent. The study was conducted in accordance with ethical guidelines and approved by the Institutional Ethics Committee (Approval No. 123/IEC/UCT/2023). The samples were stored at 4 °C for 24 hours prior to the experiment. A stock erythrocyte suspension was prepared by isolating erythrocytes from whole blood through centrifugation at 2500 rpm for 5 minutes, followed by removal of the supernatant. The erythrocyte pellet was washed three times with sterile saline solution (0.9% w/v NaCl), with each wash followed by centrifugation under the same conditions. After determining the packed cell volume, the erythrocytes were resuspended in phosphate-buffered saline (PBS; 10 mM, pH 7.4) to obtain a 40% (v/v) suspension. An aliquot of 50 µL from this erythrocyte stock suspension was added to a hypotonic buffer containing varying concentrations (500, 250, 125, 62.5, and 31.2 µg/mL) of *Carthamus caeruleus* L. extracts. A control sample, containing no plant extract, was also prepared. After a 10-minute incubation at room temperature, all samples were centrifuged at 5000 rpm for 5 minutes. The absorbance of the supernatant was measured at 540 nm using a UV spectrophotometer (equation 5).

$$\% \text{ inhibition of hemolysis } = \frac{As - Ac}{Ac} \times 100 \quad \text{(Eq. 5)}$$

Bovine Serum Albumin Denaturation-Based Assay.
The anti-inflammatory potential was evaluated through a modified version of the bovine serum albumin (BSA) denaturation method, as described by Elkolli et *al*.[17] Briefly, 100 µL of the plant extracts at different concentrations (500, 250, 125, 62.5, and 31.2 µg/mL µg/mL) was added to 500 µL of a 1% BSA solution. This preparation served as the experimental reaction mixture. The samples were first incubated at ambient temperature for 10 minutes, followed by heating at 51 °C for 20 minutes to induce protein denaturation. After cooling to room temperature, the absorbance was recorded at 660 nm using a spectrophotometer. Aspirin was employed as the standard anti-inflammatory agent. All experiments were performed in triplicate. The percentage inhibition of protein denaturation was calculated according to the following formula (equation 6):

$$\% \text{ Inhibition } = 100 - \frac{A1 - A2}{A0} \times 100 \quad \text{(Eq. 6)}$$

In the above formula, A_1 corresponds to the absorbance value of the test sample, A_2 refers to the absorbance of the control without BSA, and A_0 indicates the absorbance measured for the positive control, represented by the aspirin solution.

In vivo test
Animal models
Male Swiss albino mice (weighing approximately 30 g and aged 3–4 weeks) were obtained from the Pasteur Institute of Algiers, Algeria. The animals were housed in groups of three per standard plastic cage under controlled environmental conditions: a 12-hour light/dark cycle, temperature maintained between 25 and 35 °C, and relative humidity of 50–60%**.** All experimental procedures were conducted at the animal facility of Hassiba Ben Bouali Chlef, adhering to Algerian Law No. 12-235/2012 and the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA). A seven-day acclimation period was provided for the animals prior to experimentation. Animals undergoing oral administration of extracts or drugs were fasted for 18 hours preceding the experiment, with access to water.

Procedure
The carrageenan-induced paw edema model was employed to assess the anti-inflammatory potential of *Carthamus caeruleus* L. Prior to the experiment, animals were subjected to an 18-hour fast and received 5 mL of distilled water via gavage to reduce inter-individual variability in paw swelling. The right hind paw was designated as the untreated control. Experimental groups received *Carthamus caeruleus* L. extracts at doses of 250, 500 and 1000 mg/kg, Diclofenac at 10 mg/kg as a positive control, or distilled water at 5 mL/kg as a negative control. Sixty minutes following treatment administration, 0.05 mL of 1% carrageenan prepared in 0.9% NaCl solution was injected subcutaneously into the subplantar area of the left hind paw.[16]
Paw edema volumes were measured at 1, 2, 3, 4, and 5 hours post-carrageenan injection. The percentage inhibition of edema was then calculated using the following formula (equation 7):

$$\% = \frac{(\text{mean V left} - \text{V right control}) - (\text{mean V left} - \text{V right treated})}{(\text{V left} - \text{V right control})} \times 100$$

(Eq.7)

V left represents the mean edema volume of the left rat paw.
V right represents the mean edema volume of the right rat paw.

Statistical Analysis
Data are presented as means ± standard deviations, based on three independent replicates. Statistical analysis was carried out using one-way ANOVA under a completely randomized design, followed by the Newman–Keuls post hoc test for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$. Analyses were performed using StatBox software (version 6.5 Pro; Grimmer Logiciels, Paris, France), released between 1997 and 2002.

Results and Discussion

Extraction efficiency and bioactive compounds
The data summarized in Table 1 indicate that the hydromethanolic extract (HMEOH) of *Carthamus caeruleus* L. rhizomes yielded a significantly higher extraction efficiency (22.5 ± 0.26%) compared to the chloroformic extract (CHL, 19.2 ± 0.16%), corresponding to final dry extract masses of 1.7 g and 0.5 g, respectively. Regarding the quantification of bioactive compounds, the HMEOH extract showed a significantly higher total phenolic content (138.07 ± 0.15 µg GAE/mg DW) than the CHL extract (129.53 ± 0.20 µg GAE/mg DW) ($p < 0.05$). A similar pattern was observed for total flavonoids, with the

HMEOH extract containing 35.87 ± 0.02 µg QE/mg DW, significantly exceeding the amount detected in the chloroformic extract (16.75 ± 0.00 µg QE/mg DW).

Table 1: Extraction yield, total polyphenol content, and flavonoid content of *Carthamus caeruleus* rhizome extracts

Extract	Yield (% w/w)	Total Polyphenols (µg GAE/mg DW)	Flavonoids (µg QE/mg DW)
HMEOH	22.5± 0.26	138.07 ± 0.15[a]	35.87 ± 0.02[b]
CHL	19.2± 0.16	129.53 ±0.20[a]	16.75 ± 0.00[b]

Note: Values are expressed as mean ± standard deviation (n = 3). HMEOH: hydro-methanolic extract; CHL.: chloroformic extract; GAE: gallic acid equivalents; QE: quercetin equivalents; DW: dry weight. a–b: indicates that values with different letters in the same column are significantly different ($p<0.05$).

Table 2: Characterization of Compounds in *Carthamus caeruleus* L. Rhizome Extracts (Hydromethanolic and Chloroform) Using LC-MS/MS.

Peak	RT (min)	(*m/z*)	MS/MS Ions	HMEOH	CHL	Proposed compound
1	1.43	367	193	D	N.D	Feruloylquinic acid
2	8.27	191	191, 179	D	N.D	Caffeoylquinic acid isomer I
3	9.30	138	93	D	N.D	2-hydroxybenzoic acid
4	11.04	479	315, 301	D	N.D	Isorhamnetin *3-O*-glucoside
5	13.7	183	123	N.D	D	Syringaldehyde
6	13.13	179	135	D	N.D	Caffeic acid
		627	303, 257	D	N.D	Quercetin *O*-dihexoside
7	14.70	325	651,163	N.D	D	*P*-Coumaric acid glucoside
8	14.74	489	284,285, 489	D	N.D	Kaempferol *3- O*-acetyl hexoside
9	15.10	269	225, 151, 117	D	N.D	Apigenin
10	16.5	625	301, 284	N.D	D	Quercetin 7-*β-O*-diglucoside
11	18.25	585	301, 284	D	N.D	Quercetin *7-O*-galloyl-glucoside
12	18.47	286	201	D	N.D	Piperine
13	20.48	327	165, 121	D	D	Dihydro-*p*-coumaric acid glucoside
14	21.51	311	193, 149	D	N.D	Ferulic acid derivative isomer I
15	22.14	431	269	N.D	D	Apigenin-*7-O*- glucoside
16	22.45	497	254	N.D	D	Epicatechin glucoside
17	23.66	279	261	D	N.D	Linoleic acid

Note: HMEOH: hydromethanolic extract; CHL: chloroformic extract; RT: retention time; D-detected: N.D-not detected

Polyphenols, a structurally diverse group of secondary metabolites that includes flavonoids and tannins, have garnered substantial scientific attention for their wide range of biological properties, including antioxidant, anti-inflammatory, and antimicrobial activities.[18] In this study, the elevated levels of both total polyphenols and flavonoids in the HMEOH extract underscore its potential as a rich source of biologically active compounds.

The findings of our study are considerably higher than those previously reported in the literature. For instance, Baghiani et *al*.[4] and Ouda et *al*.[6] reported total polyphenol contents of 12.966 ± 0.727 and 13.08 ± 0.22 mg GAE/g dry extract, respectively. Similarly, the flavonoid contents in their extracts were notably lower, measured at 2.231 ± 0.146 and 5.02 ± 0.55 mg QE/g extract, respectively. These notable discrepancies may be primarily attributed to the nature of the extraction solvents used, particularly differences in polarity, which significantly affect the solubility and recovery of phenolic and flavonoid compounds. Moreover, additional variables such as the specific plant part analyzed, geographic and environmental origin, harvesting period, and methodological differences in extraction procedures are known to exert considerable influence on the phytochemical composition of plant-derived extracts.[19]

LC-MS identification of compounds in Carthamus caeruleus

The UHPLC chromatographic profiles of the hydromethanolic and chloroformic extracts from *Carthamus caeruleus* L. rhizomes are

presented in Figure 1 and Figure 2. The hydromethanolic extract exhibited a notably richer composition in phenolic constituents, consistent with its elevated total phenolic content (TPC) and total

Table 3: Antioxidant, anti-inflammatory properties of *Carthamus caeruleus* L. HMEOH (hydromethanolic extract) CHL (Chloroformic extract).

Experiments	HMEOH	CHL	Standard
	Antioxydant Activity		
TBARS inhibition IC_{50} (mg/mL)	1.47 ±0.17[b]	1.88 ±0.15[c]	20 ±0.78[a]
H_2O_2 IC_{50} (µg/mL)	450±2.87[b]	550 ± 1.56[c]	250±1.89[a]
CAA (%)	12.49 ±1.13[b]	4.09±0.36[c]	95±5[a]
TAC (µg/ GAE/mg)	100.01±0.09[b]	92.18±0.18[c]	127.63±0.48[a]
	Antiinflammatory Activity		
	(EC_{50} µg/mL)		
NO production inhibition	161.92±0.36[b]	199.80 ±0.21[c]	6.30±0.4[a]

Note: CAA– Cellular Antioxidant Activity (expressed as %) ; (µg/ GAE/mg) – µg Gallic acid equivalents/mg of extract;; (IC_{50}, EC_{50}) – the half-inhibitory concentration.Values are expressed as mean ± standard deviation (n = 3). Within each column, means followed by differentsuperscript letters (a, b, c) are significantly different at $p < 0.05$, as determined by one-way ANOVA.

Table 4: Anti-hemolytic activity of *Carthamus caeruleus* L. rhizome extracts (hydromethanolic and chloroformic) at various concentrations**.**

N/Standard	Concentration(µg/mL)	Extracts	
		HMEOH	CHL
1	31.25	21.52±0.01[a]	20.95± 2.12[b]
2	62.5	34.36±0.02[a]	21.58 ±0.41[b]
3	125	40.47 ±0.03[a]	30.39 ±1.08[b]
4	250	64.68±0.01[a]	66.88 ±0.34[b]
5	500	83.09 ±0.45[a]	79.76±0.78 [b]
Gallic Acid	200	86.56 ± 0.07	/

Note.
N: concentration number. Data are mean ± SD (n = 3) (from three replication). Different letters (a, b) in the same row indicate significant differences between extracts at the same concentration ($p < 0.05$).

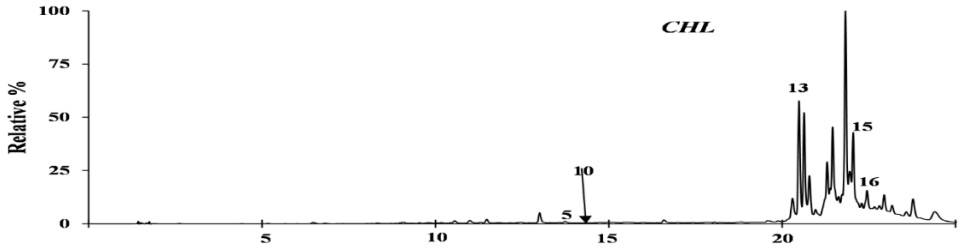

Figure 1: Chromatographic profiling of compounds in chloroformic extract of rhizomes of *Carthamus caeruleus* L.

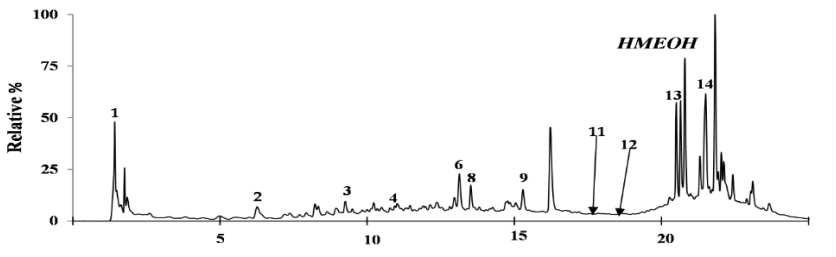

Figure 2: Chromatographic profiling of compounds in hydromethanolic extract of rhizomes of *Carthamus. caeruleus* L.

flavonoid content (TFC). This extract enabled the identification of 12 distinct compounds, compared to only 6 detected in the chloroformic extract. The detailed chemical composition of *Carthamus caeruleus* L. as elucidated by LC-MS/MS analysis, is provided in Table 2. The major constituents of the hydromethanolic extract correspond to peaks 1, 13, and 14 (Figure 1). Peaks 1 and 14 exhibited deprotonated molecular ions $[M–H]^-$ at *m/z* 367 and 311, respectively, both generating a characteristic fragment ion at *m/z* 193, which is indicative of ferulic acid.[20] These two compounds belong to the class of hydroxycinnamic acids and were specifically detected in the hydromethanolic extract. Furthermore, peak 13, observed in both extracts, exhibited a deprotonated molecular ion $[M–H]^-$ at *m/z* 327 and was identified as dihydro-*p*-coumaric acid glucoside. Another coumaric acid derivative was detected exclusively in the chloroformic extract as a minor peak (peak 7), corresponding to *p*-coumaric acid glucoside. These compounds showed precursor ions at *m/z* 327 and 325, respectively, and both generated a fragment ion at *m/z* 163, indicative of the coumaric acid moiety. The neutral loss of 162 amu confirmed the presence of a glucose unit.[21] MS profiling of chloroformic extract revealed the presence of flavonoids in three peaks 10, 15 and 16 at *m/z* 625, 431 and 497 respectively (Figure 2). Several quercetin derivatives were detected as minor peaks throughout the chromatographic profiles of both extracts. Among these, peak 10, detected exclusively in the chloroformic extract, was identified as quercetin 7-*β*-*O*-diglucoside based on its molecular ion at *m/z* 625 and its characteristic fragmentation pattern, including the successive losses of hexose and galloyl moieties, ultimately generating the quercetin aglycone at *m/z* 301. [22] Similarly, peak 11, found in the hydromethanolic extract, was assigned to quercetin 7-*O*-galloyl-glucoside.[23] Apigenin-*7*-*O*- glucoside (peak 15) $[M-H]^-$ at *m/z* 431 →269 was present only in the chloroformic extract. Its identification was supported by the presence of the apigenin aglycone (peak 9 detected only in the hydromethanolic extract) fragment at *m/z* 269.[24] Furthermore, the compound eluated in peak 16, characterized by an $[M-H]^-$ 497, was identified as epicatechin glucoside based on their UV spectra and MS fragmentation patterns consistent with previous studies, this compound was detected only in the chloroformic extract.[25] Caffeic acid and another of its derivative were also detected in the hydromethanolic extract in peaks 6 and 2 respectively. They were characterized by an $[M-H]^-$ ion at *m/z* 179 and 191 respectively identified as caffeic acid and caffeoyl quinic acid isomer I.[26] Simirgiotis et al. Quercetin *O*-dihexoside detected in hydromethanolic extract was co-eluted with caffeic acid in peak 6 matching that previously reported for quercetin *O* dihexoside found in *Carthamus* species.[23]

Peak 3 displayed a diagnostic deprotonated molecular ion at *m/z* 138 a benzoic acid derivative diagnostic fragment ion at *m/z* 93, resulting from the loss of CO_2. this compound was identified as 2-hydroxybenzoic acid and detected in hydromethanolic extract.[27]

Peak 4, also detected in hydromethanolic extract, exhibited was characterized by a $[M-H]^-$ molecular ion at *m/z* 479 . Upon MS/MS analysis, it revealed a fragmentation pattern typical of isoharmetin aglycone at *m/z* 315. This compound was identified as isorhamnetin *3*-*O*-glucoside.[28]

Beyond phenolics and flavonoids, syringaldehyde (peak 5 detected in chloroformic extract) [29] piperine (peak 12, in hydromethanolic extract) [24] and linoleic acid identified (peak 17, also in hydromethanolic extract.[25] Further diversifying the phytochemical profile of *Carthamus caeruleus* L. These identifications were based on comparisons of retention times and MS^2 spectra with established literature data.

The observed diferences in bioactive compound profiles between the the two extracts highlights a significant variation in their polarity. This disparity can be attributed to the nature of the extraction solvent, which critically affects the concentration and diversity of recovered phenolic compounds.[30]

Methanol, a highly polar solvent, is particularly effective for extracting a wide range of polar compounds, especially medium-polarity and polar phenolic compounds such as flavonoid glycosides and phenolic acids.[31] Our findings are consistent with previous studies indicating that the *Carthamus* genus is a rich source of flavonoids, which constitute the predominant class of secondary metabolites in these species.[32] For instance, *Carthamus tinctorius* L. has been reported to contain major flavonoids such as apigenin, quercetin, rutin, and myricetin, as well as phenolic acids including ferulic acid, *p*-coumaric acid, gallic acid, chlorogenic acid, and syringic acid.[33] Additionally, derivatives of ferulic and *p*-coumaric acids, along with alkaloids, have also been identified in the flowers of *Carthamus tinctorius*.[33]LC-MS analysis of *Carthamus oxycantha* L. roots also revealed the presence of syringic acid, piperine, *4*-hydroxybenzoic acid class compounds, and syringaldehyde.[34] Additionally, extracts from *Carthamus tinctorius* L. seeds have been shown to contain epigallocatechin, a 4-hydroxybenzoic acid derivative, and gallocatechin .[35]

Bioactive properties

Assessment of Antioxidant Capacity via CAA, TBARS , H_2O_2 Scavenging and TAC Assays

The hydromethanolic extract (HMEOH) consistently demonstrated superior antioxidant activity compared to the chloroformic extract (CHL), as evidenced by multiple in vitro assays ($p < 0.05$). Notably, HMEOH showed a significantly higher cellular antioxidant activity (CAA) value (12.49 ± 1.13%) compared to CHL (4.09 ± 0.36%). Similarly, in the TBARS assay, HMEOH exhibited a lower EC_{50} value (1.47 ± 0.17 mg/mL) than the chloroformic extract (1.88 ± 0.15 mg/mL), indicating stronger lipid peroxidation inhibition. This pattern was also observed in the hydrogen peroxide (H_2O_2) scavenging assay, where HMEOH achieved greater efficiency with an EC_{50} of 450 ± 2.87 µg/mL, outperforming CHL (EC_{50} = 550 ± 1.56 µg/mL) ($p < 0.05$). Moreover, the total antioxidant capacity (TAC), expressed in gallic acid equivalents (GAE), was significantly higher in the hydromethanolic extract (100.01 ± 0.09 µg GAE/mg dry extract) than in the chloroformic extract (92.00 ± 0.18 µg GAE/mg dry extract). Variations in antioxidant profiles are primarily attributed to differences in phytochemical composition, which can be influenced by the plant part used and the extraction solvent. Each bioactive compound contributes uniquely to the overall antioxidant effect of the extract.[36]

The pronounced antioxidant activity observed in the hydromethanolic rhizome extract may be attributed to its high flavonoid content particularly apigenin. Previous studies have demonstrated that apigenin exhibits potent antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.[37] Supporting this, Allemailem et al.[38] reported that apigenin effectively mitigates oxidative stress and mitochondrial damage induced by multi-walled carbon nanotubes in rat kidney mitochondria, highlighting its strong protective potential.

These results align with a recent study reporting that *Carthamus caeruleus* L. rhizome extracts exhibited superior antioxidant activity in the ABTS assay, with IC_{50} values of 0.14 and 0.19 µg/mL for the hydromethanolic and chloroformic extracts, respectively. The same study also demonstrated significantly higher nitric oxide (NO•) and superoxide anion ($O_2^{\bullet -}$) scavenging activities, with IC_{50} values of 1.39 and 1.85 mg/mL for the hydromethanolic and chloroformic extracts, respectively, and 0.89 and 2.98 mg/mL for superoxide radicals, respectively.

Anti-inflammatory activity

Evaluation of NO Inhibitory Activity

Nitric oxide (NO) is a key signaling molecule involved in the regulation of inflammation.[39] Under normal physiological conditions, it exhibits anti-inflammatory properties; however, under pathological conditions, its overproduction contributes to pro-inflammatory responses.[40] In this study, the hydromethanolic extract demonstrated significantly greater anti-inflammatory activity than the chloroformic extract, as evidenced by its superior inhibition of NO production (Table 3). The hydromethanolic extract exhibited an EC_{50} of 161.92 ± 0.36 µg/mL, which was significantly more effective ($p < 0.05$) than the chloroformic extract (EC_{50} = 199.80 ± 0.21 µg/mL). These findings support the traditional use of *Carthamus caeruleus* L. for inflammation management.Research on the *in vitro* anti-inflammatory properties of the *Carthamus* genus remains limited. However, studies on *Carthamus tinctorius* L. have reported comparable activity, with its

methanolic extract inhibiting 80% of NO production at 160 µg/mL, a result closely aligned with our findings for *Carthamus caeruleus* L.[41] Additionally, research by Sun et al. [42] demonstrated that honey extracts from *Carthamus tinctorius* L. flowers significantly reduce NO production in LPS-activated macrophages at concentrations as low as 2.5 and 5 µg/mL . These

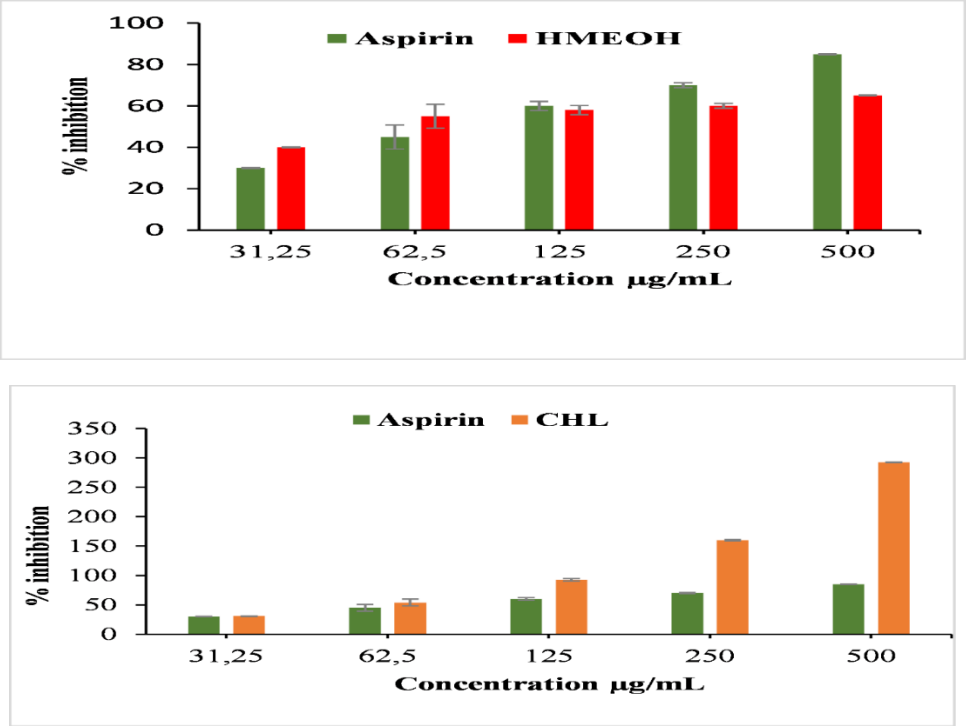

Figure 3: Comparative inhibition of protein denaturation by the hydromethanolic extract (HMEOH) (A), chloroformic extract (CHL) (B), and aspirin (ASA) at various concentrations ($p < 0.05$).

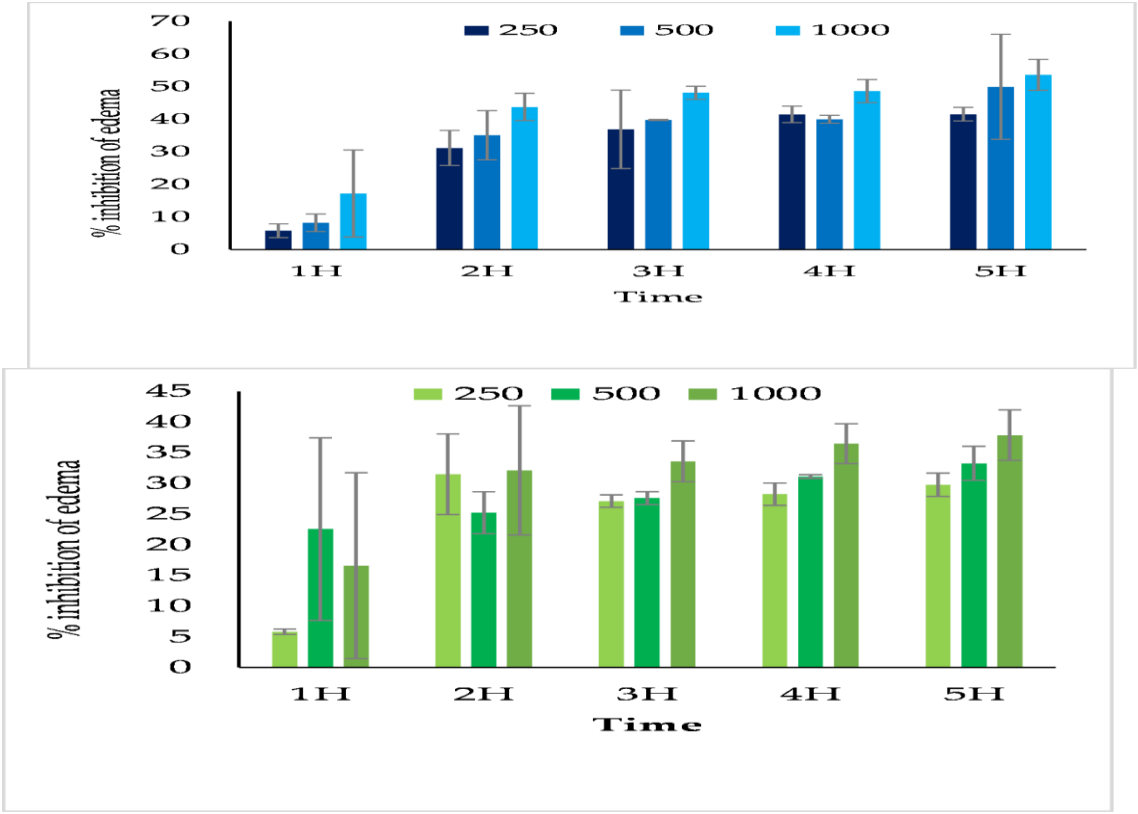

Figure 4: Inhibition of carrageenan-induced paw edema by the hydromethanolic extract (HMEOH) (A) and chloroformic extract (CHL) (B). Results are expressed as mean ± SD.

findings highlight the potential of *Carthamus* species as natural anti-inflammatory agents.

Inhibition of hemolysis

Table 4 presents the percentage inhibition of red blood cell (RBC) membrane hemolysis following treatment with *Carthamus caeruleus* L. rhizome extracts. The hydromethanolic and chloroformic extracts were evaluated in comparison with gallic acid. Both extracts exhibited statistically significant inhibition of RBC membrane lysis ($p < 0.05$) at various concentrations.

The results indicate that the hydromethanolic extract of *Carthamus caeruleus* L. rhizomes exhibits significantly higher anti-inflammatory activity than the chloroformic extract at all tested concentrations. A statistically significant difference was also observed between both extracts and gallic acid, which showed a hemolysis inhibition rate of $86.50 \pm 0.07\%$ at a concentration of 200 µg/mL.

Carthamus caeruleus L. extracts demonstrated a strong protective effect on human erythrocytes exposed to hypotonic stress. As reported by Belounis et al.[7], the aqueous extract of *Carthamus caeruleus* L. rhizomes effectively inhibited membrane lysis, with inhibition rates ranging from 35.8% to 98.13% across four different NaCl concentrations (0.7%, 0.5%, 0.3%, and 0.1%). This membrane-stabilizing activity is likely attributed to the presence of bioactive phytochemicals such as flavonoids and polyphenols, which are known to enhance membrane integrity and protect against oxidative and osmotic stress.[43]

Inhibition of Protein Denaturation

Both hydromethanolic and chloroformic extracts of *Carthamus caeruleus* L. rhizomes exhibited inhibitory effects on protein denaturation (Figure 3). The hydromethanolic extract showed notable activity, with inhibition rates ranging from $40.01 \pm 1.51\%$ to $65.31 \pm 1.86\%$ across concentrations from 31.25 to 500 µg/mL. In comparison, the chloroformic extract demonstrated inhibition percentages between $32.00 \pm 0.90\%$ and $60.00 \pm 1.09\%$. NO significant difference was observed between the two extracts at the highest tested concentration (500 µg/mL). These findings indicate that both extracts possess strong anti-denaturation activity, even at relatively low doses. To our knowledge, only one previous study has investigated the aqueous extract of *Carthamus caeruleus* L. rhizomes, reporting a potent inhibitory effect on ovalbumin denaturation, with a maximum inhibition rate of $80.86 \pm 2.23\%$ at 2000 µg/mL.[7] This supports the anti-inflammatory potential of the species, particularly through its ability to stabilize protein structures under stress conditions. Protein denaturation is widely acknowledged as a key initiator of inflammatory processes. It is intrinsically linked to structural changes in tissue proteins, which promote the formation of auto-antigens and trigger immune responses that contribute to the onset of inflammatory and arthritic disorders.[44] Plant-derived extracts appear to be promising and safer alternatives to conventional non-steroidal anti-inflammatory drugs (NSAIDs), which are often associated with undesirable side effects including gastrointestinal toxicity, hepatotoxicity, renal dysfunction, hypertension, and allergic reactions .[45] The anti-denaturation activity of *Carthamus caeruleus* L. extracts may be attributed to their high phenolic content. These polyphenolic compounds are capable of interacting with proteins, potentially forming stabilizing complexes, particularly with albumin, thereby preserving protein conformation and preventing denaturation under stress-induced conditions.

Effect of Carthamus caeruleus L. Extracts on Carrageenan-Induced Paw Edema in mice

The carrageenan-induced paw edema model was employed in mice to assess the *in vivo* anti-inflammatory efficacy of *Carthamus caeruleus* L. extracts. This well-established model, known for its ability to simulate acute inflammation, allows for the observation and quantification of the inflammatory response. These results are further illustrated in Figure 4. Throughout the experiment, no signs of toxicity were observed in mice at any tested doses, confirming the excellent safety profile of the extracts. As expected, carrageenan administration successfully induced a significant ($P < 0.005$) edema from the first hour, validating the reability of the inflammatory model. Diclofenac (10 mg/kg), used as the standard anti-inflammatory drug, effectively reduced this edema from the second to the fifth hour. Notably, both chloroformic and hydromethanolic extracts demonstrated significant anti-inflammatory activity in the carrageenan-induced edema model, particularly between the second and fifth hours. Interestingly, the anti-inflammatory effects of the extracts were dose-independent, with all tested doses (250, 500, and 1000 mg/kg) showing efficacy. Inflammation is a natural protective process in the body, which allows the immune system to target and neutralize foreign or dangerous agents.[46]Analysis of the results confirms that both the hydromethanolic and chloroform extracts possess significant anti-inflammatory potential. This finding aligns with previous studies have demonstrated the anti-inflammatory efficacy of *Carthamus caeruleus* L. rhizomes, which aligns with our current findings Ospelt et al.[47] and Benhamou et al.[48] Research conducted on samples collected from Tizi Ouzou, Tipaza, and Setif has specifically shown that *Carthamus caeruleus* L. rhizomes effectively attenuate carrageenan-induced inflammation in experimental animal models.

Conclusion

This study identifies *Carthamus caeruleus* L. as a promising candidate for the development of plant-based therapeutic agents, owing to its potent antioxidant and anti-inflammatory properties. The hydromethanolic extract, in particular, exhibited significant bioactivity in both *in vitro* and *in vivo* assays, likely due to its high content of phenolic compounds. Compounds such as feruloylquinic acid, caffeoylquinic acid, and kaempferol 3-*O*-acetylhexoside may play a key role in modulating oxidative and inflammatory pathways, contributing synergistically to the observed pharmacological effects. These findings support the traditional use of *Carthamus caeruleus* L. and provide a scientific basis for its further exploration as a source of bioactive molecules. Nonetheless, additional studies are needed to isolate and characterize the active constituents, investigate their mechanisms of action, assess their pharmacokinetic and toxicity profiles, and confirm their efficacy in relevant preclinical models.

Conflicts of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors extend their sincere thanks to the Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Portugal, for their valuable support.

References

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxid. Med. Cell Longev. 2017; 2017: 8416763. DOI: 10.1155/2017/8416763
2. Marwick TH, Ritchie R, Shaw JE, Kaye D. Implications of underlying mechanisms for the recognition and management of diabetic cardiomyopathy. J. Am. Coll. Cardiol. 2018; 71(3): 339–351. DOI: 10.1016/j.jacc.2017.11.019
3. Dahmane T, Kaci Z, Hadj Mohamed N, Abed A, Mebkhout F. Ethnobotanical study of spontaneous medicinal plants Gouraya's National Park (Bejaia-Algeria). Egypt J. Bot. 2023; 63(3): 1083–1100. DOI : 10.21608/ejbo.2023.202565.2294
4. Baghiani A, Boumerfeg S, Belkhiri F, Khennouf S, Charef N, Harzallah D, Lekhmici A, Attia Abdel-Wahhab M. Antioxidant and radical scavenging properties of *Carthamus caeruleus* L extracts growing wild in Algerian flora. Comun. Sci. 2010; 1(2): 128–136.

5. Dahmani MM, Laoufi R, Selama O, Arab K. Gas chromatography coupled to mass spectrometry characterization, antiinflammatory effect, wound-healing potential, and hair growth-promoting activity of Algerian *Carthamus caeruleus* L (*Asteraceae*). Indian J. Pharmacol. 2018; 50: 123–129. DOI : 10.4103/ijp.IJP_65_17
6. Ouda AN, Fatiha M, Sadia M, Zohra SF, Noureddine D. *In vivo* anti-inflammatory activity of aqueous extract of *Carthamus caeruleus* L rhizome against carrageenan-induced inflammation in mice. Jordan J. Biol. Sci. 2021; 14(3): 529–535. Doi:10.54319/jjbs/140319
7. Belounis Y, Moualek I, Sebbane H, Dekir A, Bendif H, Garzoli S, Houali K. Phytochemical characterization and antibacterial activity of *Carthamus caeruleus* L. aqueous extracts: *In vitro* and in silico molecular docking studies. Chem. Biodivers. 2025; 22(1): e202402662.
8. Chaouche TM, Haddouchi F, Ksouri R, Atik-Bekkara F. Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. J. Chin. Med. Assoc. 2014; 77(6): 302–307.
9. Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug *Gul-e-Zoofa* (*Nepeta bracteata* Benth). J. Taibah Univ. Med. Sci. 2017; 12(4): 360–363.
10. Abeywardhana KW, Abeysinghe DC, Dharmadasa RM, Aththanayake AML. Determination of optimum maturity stage for *Ocimum sanctum* L. grown under different growing systems in terms of therapeutically active secondary metabolites. World J. Agric. Res. 2014; 2(4): 159–162.
11. Afonso AF, Pereira OR, Fernandes Â, Calhelha RC, Silva AM, Ferreira IC, Cardoso SM. Phytochemical composition and bioactive effects of *Salvia africana*, *Salvia officinalis* 'Icterina' and *Salvia mexicana* aqueous extracts. Molecules**.** 2019; 24(23): 4327.
12. Zhou J, Gao G, Zhang S, Wang H, Ke L, Zhou J, Rao P, Wang Q, Li J. Influences of calcium and magnesium ions on cellular antioxidant activity (CAA) determination. Food Chem**.** 2020; 320: 126625.
13. Jimoh MO, Afolayan AJ, Lewu FB. Antioxidant and phytochemical activities of *Amaranthus caudatus* L. harvested from different soils at various growth stages. Sci. Rep. 2019; 9(1): 12965. Doi:10.1038/s41598-019-49276-w
14. Serteser A, Kargioğlu M, Gök V, Bağci Y, Özcan MM, Arslan D. Determination of antioxidant effects of some plant species wild growing in Turkey. Int. J. Food Sci. Nutr. 2008; 59(7–8): 643–651.
15. Medini F, Bourgou S, Lalancette K, Snoussi M, Mkadmini K, Coté I, Abdelly C, Legault J, Ksouri R. Phytochemical analysis, antioxidant, anti-inflammatory, and anticancer activities of the halophyte *Limonium densiflorum* extracts on human cell lines and murine macrophages. S. Afr. J. Bot**.** 2015; 99: 158–164. Doi:10.1016/j.sajb.2015.04.007.
16. Fujiati F, Haryati H, Joharman J, Utami SW. In vitro metabolite profiling and anti-inflammatory activities of *Rhodomyrtus tomentosa* with red blood cell membrane stabilization methods. Rep Biochem Mol Biol. 2022;11(3):502.
17. Elkolli H, Elkolli M, Ataya FS, Salem-Bekhit MM, Zahrani SA, Abdelmageed MWM, Ernst B, Benguerba Y. *In vitro* and in silico activities of *E. radiata* and *E. cinerea* as an enhancer of antibacterial, antioxidant, and anti-inflammatory agents. Molecules. 2023; 28(20): 7153. Doi:10.3390/molecules28207153.
18. Ouriagli T, Amnay A, Raoui SM, Errachidi F, Chahdi FO, Chabir R. Alkaloids from *Marrubium vulgare* L.: Antioxidant and anti-inflammatory activities as a function of extraction methods. Trop J Nat Prod Res. 2023; 7(7).
19. Rathod NB, Elabed N, Punia S, Ozogul F, Kim SK, Rocha JM. Recent developments in polyphenol applications on human health: A review with current knowledge. Plants. 2023; 12(6): 1217
20. Fernandes JC, Spindola H, De Sousa V, Santos-Silva A, Pintado ME, Malcata FX, Carvalho JE. Anti-inflammatory activity of chitooligosaccharides *in vivo*. Mar Drugs. 2010; 8(6): 1763–1768. Doi:10.3390/md8061763.
21. Kramberger K, Barlič-Maganja D, Bandelj D, Baruca Arbeiter A, Peeters K, Miklavčič Višnjevec A, Jenko Pražnikar Z. HPLC-DAD-ESI-QTOF-MS determination of bioactive compounds and antioxidant activity comparison of the hydroalcoholic and water extracts from two *Helichrysum italicum* species. Metabolites. 2020; 10(10): 403. doi:10.3390/metabo10100403.
22. Goufo P, Singh RK, Cortez I. A reference list of phenolic compounds (including stilbenes) in grapevine (*Vitis vinifera* L.) roots, woods, canes, stems, and leaves. Antioxidants. 2020; 9(5): 398. Doi:10.3390/antiox9050398
23. Kramberger K, Barlič-Maganja D, Bandelj D, Baruca Arbeiter A, Peeters K, Miklavčič Višnjevec A, Jenko Pražnikar Z. HPLC-DAD-ESI-QTOF-MS determination of bioactive compounds and antioxidant activity comparison of the hydroalcoholic and water extracts from two *Helichrysum italicum* species. Metabolites. 2020; 10(10): 403. Doi:10.3390/metabo10100403.
24. Sruthi D, Zachariah TJ. Phenolic profiling of *Piper* species by liquid chromatography-mass spectrometry. J Pharm Anal. 2016; 25(2): 123–132.
25. Liang J, Sun J, Chen P, Frazier J, Benefield V, Zhang M. Chemical analysis and classification of black pepper (*Piper nigrum* L.) based on their country of origin using mass spectrometric methods and chemometrics. Food Res Int. 2021; 140: 109877. Doi:10.1016/j.foodres.2020.109877.
26. Simirgiotis MJ, Benites J, Areche C, Sepúlveda B. Antioxidant capacities and analysis of phenolic compounds in three endemic *Nolana* species by HPLC-PDA-ESI-MS. Molecules. 2015; 20(6): 11490–11507. Doi:10.3390/molecules200611490.
27. Pérez-Magariño S, Bueno-Herrera M, Asensio-S-Manzanera MC. Characterization of bioactive phenolic compounds extracted from hydro-distillation by-products of Spanish *Lamiaceae* plants. Molecules. 2024; 29(22): 5285. Doi:10.3390/molecules29225285.
28. Zhu Z, Zhong B, Yang Z, Zhao W, Shi L, Aziz A, Rauf A, Aljohani ASM, Alhumaydhi FA, Suleria HAR. LC-ESI-QTOF-MS/MS characterization and estimation of the antioxidant potential of phenolic compounds from different parts of the lotus (*Nelumbo nucifera*) seed and rhizome. ACS Omega. 2022; 7(17): 14630–14642. Doi:10.1021/acsomega.1c07018
29. Zhu W, Sun S, Yang F, Zhou K. UHPLC/MS identifying potent α-glucosidase inhibitors of grape pomace via enzyme immobilized method. J Food Sci. 2018; 83(4): 1131–1139. Doi:10.1111/1750-3841.14087.
30. Lee JE, Jayakody JTM, Kim JI, Jeong JW, Choi KM, Kim TS, Seo C, Azimi I, Hyun J, Ryu B. The influence of solvent choice on the extraction of bioactive compounds from *Asteraceae*: A comparative review. Foods. 2024; 13(19): 3151. Doi:10.3390/foods13193151.
31. Babbar N, Oberoi HS, Sandhu SK, Bhargav VK. Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. J Food Sci Technol. 2014; 51: 2568–2575. Doi:10.1007/s13197-012-0754-4.
32. Ji Y, Guo S, Wang B, Yu M. Extraction and determination of flavonoids in *Carthamus tinctorius*. Open Chem. 2018; 16(1): 1129–1133. doi:10.1515/chem-2018-0119
33. Salem N, Msaada K, Hamdaoui G, Limam F, Marzouk B. Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). J Agric Food Chem. 2011; 59(9): 4455–4463. Doi:10.1021/jf1049936.
34. Baban MM, Ahmad SA, Abu-Odeh AM, Baban M, Talib WH. Anticancer, immunomodulatory, and phytochemical screening of *Carthamus oxyacantha* M. Bieb growing in the north of Iraq. Plants. 2023; 13(1): 42. Doi:10.3390/plants13010042.
35. Yu SY, Lee YJ, Kim JD, Kang SN, Lee SK, Jang JY, Lee HK, Lim JH, Lee OH. Phenolic composition, antioxidant activity and

anti-adipogenic effect of hot water extract from safflower (*Carthamus tinctorius* L.) seed. Nutrients. 2013; 5(12): 4894–4907. Doi:10.3390/nu5124894.

37. Madunić J, Madunić IV, Gajski G, Popić J, Garaj-Vrhovac V. Apigenin: A dietary flavonoid with diverse anticancer properties. Cancer Lett. 2018; 413: 11–22. Doi:10.1016/j.canlet.2017.10.041.
38. Allemailem KS, Almatroudi A, Alharbi HOA, AlSuhaymi N, Alsugoor MH, Aldakheel FM, Rahmani AH. Apigenin: A bioflavonoid with a promising role in disease prevention and treatment. Biomedicines. 2024; 12(6): 1353. Doi:10.3390/biomedicines12061353
39. Azerlyn DRN, Dwijayanti DR, Masruri M, Widodo N. Exploring the *In Vitro* Anti-Inflammatory Effect and In Silico Toxicity Profile of *Curcuma aeruginosa* Roxb. Extract in RAW 264.7 Macrophages. Trop J Nat Prod Res. 2025; 9(5): 1964 – 1972
40. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacol. 2007; 15: 252–259. Doi:10.1007/s10787-007-0013-x.
41. Jun MS, Ha YM, Kim HS, Jang HJ, Kim YM, Lee YS, Kim HJ, Seo HG, Lee JH, Lee SH, Chang KC**.** Anti-inflammatory action of methanol extract of *Carthamus tinctorius* involves in heme oxygenase-1 induction. J Ethnopharmacol. 2011; 133(2): 524-530.Doi :10.1016/j.jep.2010.10.029
42. Sun LP, Shi FF, Zhang WW, Zhang ZH, Wang K. Antioxidant and anti-inflammatory activities of safflower (*Carthamus tinctorius* L.) honey extract. Foods. 2020; 9(8): 1039. Doi:10.3390/foods9081039.
36. Giamperi L, Bucchini A, Bisio A, Giacomelli E, Romussi G, Ricci D. Total phenolic content and antioxidant activity of *Salvia* spp. exudates. Nat Prod Commun. 2012; 7(2). Doi:10.1177/1934578X1200700221
43. Martins CAF, Campos ML, Irioda AC, Stremel DP, Trindade ACLB, Pontarolo R. Anti-inflammatory effect of *Malva sylvestris*, *Sida cordifolia*, and *Pelargonium graveolens* is related to inhibition of prostanoid production. Molecules. 2017; 22: 1883. Doi:10.3390/molecules22111883
44. Reshma AK, Arun KP. *In vitro* anti-inflammatory, antioxidant and nephroprotective studies on leaves of *Aegle marmelos* and *Ocimum sanctum*. Asian J Pharm Clin Res. 2014; 7.
45. Modak D, Paul S, Sarkar S, Thakur S, Bhattacharjee S. Validating potent anti-inflammatory and anti-rheumatoid properties of *Drynaria quercifolia* rhizome methanolic extract through *in vitro*, *in vivo*, in silico and GC-MS-based profiling. BMC Complement Med Ther. 2021; 21: 89.
46. Musfiroh I, Muchtaridi M, Fristiohady A, Ikram NKK. Anti-inflammatory activity of Qutsh Al Hindi (*Saussurea lappa*) root fractions: *in vitro* assay and characterization of its active compound. Trop J Nat Prod Res. 2024; 8(11).
47. Ospelt C, Gay S. TLRs and chronic inflammation. Int J Biochem Cell Biol. 2010; 42(4): 495–505. Doi:10.1016/j.biocel.2009.10.010.
48. Benhamou A, Fazouane F. Ethnobotanical study, phytochemical characterization and healing effect of *Carthamus coeruleus* L. rhizomes. Int J Med Aromat Plants. 2013; 3(1): 61–68.