JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF THE ANTIOXIDANT, HEMOLYTIC, AND ANTIBACTERIAL PROPERTIES OF LEAVES AND RHIZOMES OF *CARTHAMUS CAERULEUS* L.

*Karima Djafri**[1], *Abdelaziz Merouane*[2], *Abdelkader Saadi* [3], *Abdallah Noui* [4, 5], *Yavuz Selim Çakmak*[6]

Address(es): Karima Djafri,
[1] Laboratory of Natural Bio-Ressources, Departement of Biotechnology , Faculty of Life and Natural Sciences ,University of Chlef ,02000, Algéria.
[2] Higher School of Saharan Agriculture of Adrar, 01000, Algeria.
[3] Faculty of Life and Natural Sciences, Hassiba Benbouali University of Chlef, 02000, 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, 16000, Algeria.
[6] Faculty of Science and Arts, Aksaray University, 68100, Turkey.

*Corresponding author: k.djafri@univ-chlef.dz

https://doi.org/10.55251/jmbfs.11067

ARTICLE INFO

Received 24. 2. 2024
Revised 31. 3. 2026
Accepted 28. 4. 2026
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

Carthamus caeruleus (L.), a member of the Asteraceae family, is traditionally recognized for its therapeutic properties, particularly in the treatment of burns and inflammatory disorders. Hydro-methanolic extracts of leaves and rhizomes were prepared and analyzed for their qualitative and quantitative phytochemical composition. Antioxidant activity was evaluated using three methods: DPPH, FRAP, and β-carotene assays. Additionally, hemolytic activity was assessed on human erythrocytes as a measure of cytotoxicity, and antibacterial activity was determined using the microdilution method. The leaf extract exhibited higher contents of phenolic and flavonoid compounds compared to the rhizome extract ($p < 0.05$). Whereas the rhizome extracts showed a higher tannin content than the leaves. HPLC analysis identified seven components in the leaves and a mixture of ten phenolic acids and flavonoids in the rhizome extracts. Antioxidant and antibacterial assays revealed the superiority of the leaf extracts over the rhizome extracts ($p < 0.05$), with comparable potential to BHT according to DPPH assays (IC_{50} = 11.3 ± 0.5 µg/mL), β-carotene bleaching (74.94% ± 0.5), and FRAP activity (0.58 ± 0.076 mg/mL). The leaf extracts exhibited a minimum inhibitory concentration (MIC) ranging from 0.6 to 5 mg/mL. Hemolytic activity against human erythrocytes ranged from 0.86% to 14.45%. Overall, the species *Carthamus caeruleus* (L.) is characterized by its richness in bioactive compounds, particularly in the leaves, which could serve as a potential source of natural antioxidants and antibacterial agents with beneficial therapeutic properties.

Keywords: *Carthamus caeruleus* (L). natural antioxidants, antioxidant activity, phytochemical screening, hemolytic activity

INTRODUCTION

Carthamus caeruleus (L)., a taxonomically distinct species within the family Asteraceae, belongs to the genus *Carthamus*, which comprises 25 species distributed across various regions, including parts of Africa, Northern India, and Spain. Its original geographic origin is in the eastern Mediterranean region **(Toubane et *al.*, 2017)**. *C. caeruleus* L. is considered a rare species, typically found in damp forest clearings, plains, and along stream banks, thriving particularly in clay and siliceous-clay soils **(Saffidine, 2018)**.
The genus *Carthamus* is well known for its rich phytochemical diversity and broad spectrum of biological activities. Analyses of the aerial parts of *C. lanatus* and the seeds of *C. tinctorius* have led to the identification of several bioactive compounds, including fatty acids, serotonin derivatives, flavonoids, and hydroxy saffron yellow. These molecules exhibit pharmacological properties such as bone protection and antioxidant effects, which justify their traditional use in the prevention of cardiac and rheumatic diseases **(Taskova et *al.*, 2003).**
In Algeria, traditional practices involve the use of *C. caeruleus* L. rhizomes as a natural remedy for burns, inflammation, and skin regeneration. These medicinal effects are attributed to the presence of various bioactive compounds, including carotenoids, flavonoids, terpenoids, steroids, tannins, saponins, coumarins, quinones, and mucilage **(Ouda et *al.*, 2021).**
To the best of our knowledge, this is the first report on the identification and analysis of polyphenols in *C. caeruleus* L. using HPLC, highlighting its valuable therapeutic properties. The present study focuses on the valorization of this species through the screening and HPLC profiling of phenolic compounds extracted from its rhizomes and leaves, as well as the evaluation of their antioxidant and hemolytic activities.

MATERIAL AND METHODS

Plant collection and extract preparation

Leaves and rhizomes of *C. caeruleus* L., identified by Professor Saadi Abdelkader, were collected in June 2022 from the Labiod Medjadja region, Chlef, in northwestern Algeria. The plant materials were air-dried, powdered, and then extracted by maceration using a hydro-methanolic solution (80:20, v/v) according to the method described by **Chaouche et *al.* (2014)**. The resulting extracts were stored at 4 °C until further analysis.

Preliminary phytochemical screening

The hydro-methanolic extracts obtained from the leaves and rhizomes of *C. caeruleus* L. were subjected to preliminary phytochemical screening to assess the presence or absence of major classes of bioactive compounds, including flavonoids, tannins, alkaloids, saponins, terpenoids, and mucilage. The phytochemical tests were performed based on the appearance of coloration, turbidity, or precipitation using specific reagents for each phytochemical group **(Hosni et *al.*, 2020)**.

Assay for total phenolic, flavonoids and condensed tannins

The total phenolic content was quantified following the protocol described by **Djamilatou et *al.* (2021)** using the Folin-Ciocalteu method. The phenolic content was expressed as µg gallic acid equivalents per mg of extract (µg GAE/mg) using a calibration curve generated from a gallic acid reference solution. Flavonoid content was determined using the aluminum chloride method, with quercetin as the standard, and expressed as µg QE/mg **(Hayat et *al.*, 2020)**. Condensed tannins were measured using the vanillin/HCl method, and the results were compared to a standard curve established with catechin at concentrations ranging from 0 to 200 µg/mL **(El Cadi et *al.*, 2020)**.

Identification of Phenolic Compounds

The phenolic compound profiles of the different extracts were analyzed using an HP-Agilent 1292 Infinity HPLC system, equipped with a C18 reverse-phase column and a diode array detector (DAD), following the method described by **Fedoul et *al.* (2022)**. For each sample, 10 µL of a methanolic solution (20 mg/mL) was injected into the system.
The HPLC system was equipped with a 250 × 4.6 mm C18 Ultra Sphere-ODS column. Detection was performed at multiple wavelengths: 254, 278, 287, and 330 nm. The mobile phase consisted of solvent A (3% acetic acid in water) and solvent B (methanol). A gradient elution program was applied at a constant flow rate of 0.8 mL/min as follows: 93% A–7% B (0.1 min), 72% A–28% B (20 min), 75% A–25% B (8 min), 70% A–30% B (7 min), 67% A–33% B (15 min), 58% A–42% B (2 min), 50% A–50% B (8 min), 30% A–70% B (3 min), 20% A–80% B (2 min), and finally 100% B for the last 5 minutes.
For compound identification, the following phenolic standards were used: 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, benzoic acid, catechin hydrate, chlorogenic acid, caffeic acid, epicatechin, gallic acid, hesperidin, p-coumaric acid, quercetin, rosmarinic acid, sinapic acid, syringic acid, trans-cinnamic acid, and trans-ferulic acid.
Peaks in the chromatograms were identified by comparing both the retention times and UV spectra of the analytes with those of authentic standards under the same chromatographic conditions. Quantification of each phenolic compound was performed using external calibration curves at concentrations of 1, 5, 10, 20, 50, 70, and 100 ppm. All compounds exhibited strong linear correlations, with R^2 values ranging from 0.9970 to 0.9999 (see Table). All standard solutions were freshly prepared in methanol and filtered through 0.45 µm syringe filters prior to analysis. Each measurement was performed in triplicate, and the results were reported as ppm (mg/kg or mg/L) for each phenolic compound detected in the extracts.

Antioxidant activity

The antioxidant activity was evaluated at various concentrations using a 10% methanol solution as the solvent. Three complementary *in vitro* assays were employed: DPPH **(Mayouf et *al.*, 2019)**, FRAP **(Gheffour et *al.*, 2015)**, and the β-carotene bleaching assay **(Merouane et *al.*, 2020)**. BHT (butylated hydroxytoluene) was used as the reference standard.

Hemolytic assay

The *in vitro* hemolytic potential of extracts from both plant parts was assessed using a suspension of human erythrocytes in phosphate-buffered saline (PBS, pH 7.4) **(Haddouchi et *al.*, 2018)**. Blood was collected from a healthy volunteer into heparinized tubes and then centrifuged at 2,500 rpm for 10 minutes to separate the plasma. The resulting erythrocyte pellet was washed twice with PBS and resuspended in an equal volume of the previously removed plasma. The erythrocyte suspension was subsequently diluted 20-fold with PBS to obtain the working solution.
To assess hemolytic activity, 20 µL of each extract at various concentrations were added to 1,980 µL of the prepared erythrocyte suspension. The tubes were then incubated at 37 °C for 60 minutes. After incubation, 250 µL samples were collected from each tube, mixed with 750 µL of PBS, and immediately placed in an ice bath to stop the reaction. The samples were then centrifuged at 2,500 rpm for 10 minutes, and the absorbance was measured at 548 nm.

Antibacterial activity

The antibacterial activity was evaluated by dissolving the extracts in sterile distilled water to obtain a final concentration of 20 mg/mL **(Pires et *al.*, 2018)**.
Clinical bacterial isolates used in this study were obtained from patients admitted to various units of the Hospital Center of Trás-os-Montes and Alto Douro (Vila Real, Portugal). The study included five Gram-negative species: *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Morganella morganii*. Three Gram-positive bacteria were also tested: *Enterococcus faecalis, Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA).
The minimum inhibitory concentration (MIC) was determined using the microdilution method combined with the p-iodonitrotetrazolium chloride (INT) colorimetric assay.

Statistical study

All results are presented as mean ± standard deviation of three replicates. The data were statistically analyzed using a one-way analysis of variance (ANOVA) with complete randomization, followed by pairwise comparisons of means using the Newman–Keuls test (Stat Box 6.4). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

This test allows a qualitative analysis using specific reagents that induce coloration and/or precipitation reactions.
Preliminary phytochemical analyses of hydromethanolic extracts obtained from the leaves and rhizomes of *C. caeruleus* (L.) revealed the presence of flavonoids, tannins, terpenes, mucilages, and alkaloids. Rhizome extracts appeared to be richer in tannins compared to other constituents, while leaf extracts showed a notable abundance of terpenes and flavonoids. Alkaloids were detected in leaf extracts but were absent in rhizomes. Our results are consistent with previous phytochemical studies on the rhizome of *C. caeruleus* L. (**Ouda et *al.*, 2021)**.

Bioactive content

Quantitative analysis of bioactive compounds revealed significant differences between the two plant parts. The leaf extracts exhibited significantly higher levels of total phenolic compounds and flavonoids compared to the rhizome extracts ($p < 0.05$). In contrast, the rhizome extracts showed a significantly greater content of condensed tannins than the leaves ($p < 0.05$) (Table 1).
These results are consistent with the findings of **Saffidine (2018)**, who also reported higher concentrations of phenolic and flavonoid compounds in the leaves compared to the rhizomes. The differences in secondary metabolite profiles between the two plant parts may reflect variations in their physiological roles and environmental exposure.

Identification of phenolic compounds

Phenolic compounds were identified using HPLC by comparing the retention times of the phenolic compounds present in the plant samples with those of standard compounds analyzed under identical conditions using an HPLC-DAD system.
Twelve phenolic compounds were identified in *C. caeruleus* L., including three flavonoids (quercetin, hesperidin, and epicatechin) and nine phenolic acids (3-hydroxybenzoic acid, 4-hydroxybenzoic acid, benzoic acid, chlorogenic acid, caffeic acid, coumaric acid, rosmarinic acid, sinapinic acid, and *trans*-cinnamic acid) (Table 2).
In the leaf extract, quercetin, hesperidin and epicatechin were the predominant components. In addition, other phenolic compounds such as 3-hydroxybenzoic acid, benzoic acid, 4-hydroxybenzoic acid and t-cinnamic acid were present in this extract. Compared to the leaf extract, the rhizome extract displayed a diversity of phenolic compounds, with dominance of quercetin, epicatechin, hesperidin, and rosmarinic acid. The minor identified compounds in the rhizome extract include 3-hydroxybenzoic acid, chlorogenic acid, syringic acid, caffeic acid, t-cinnamic acid, 4-hydroxybenzoic acid, p-coumaric acid and sinapinic acid.
To the best of our knowledge, the present study is the first research devoted to the detection of phenolic compounds in *C. caeruleus* using HPLC.
A qualitative study of the phenolic compounds in *C. tinctorius* revealed the presence of flavonoids (apigenin) and phenolic acids (gallic acid), which are recognized as important natural products with valuable medicinal properties **(Zhang et *al.*, 1997)**. In the seeds of the same species, epigallocatechin was identified as the major phenolic compound, while gallocatechins were the predominant components. Furthermore, HPLC analysis demonstrated the presence of various flavonoid compounds, including kaempferol, rutin hydrate, luteolin, and quercetin hydrate **(Yu et *al.*, 2013)**.
According to **Iacopini et *al.* (2008)**, phenolic compounds such as quercetin and epicatechin have been extensively studied and are recognized as potent antioxidants with significant biological, pharmacological, and medicinal properties. These compounds effectively protect human low-density lipoproteins (LDL) from oxidative damage, thereby providing stronger cardioprotective effects than α-tocopherol. Additionally, quercetin has been shown *in vitro* to inhibit human platelet aggregation, suggesting potential anticancer activity.
Hesperidin, another key flavonoid, exhibits strong antioxidant properties, including significant reducing power, radical scavenging capacity, and iron-chelating ability **(Zanwar et *al.*, 2014).** Studies conducted on both humans and animals have provided evidence of hesperidin's ability to regulate inflammatory and oxidative states, along with its capacity to enhance aerobic capacity (**Martínez-Noguera et *al.*, 2019).** These compounds represent promising candidates for future therapeutic applications and biomedical research.

Table1 Bioactive contents and antioxidant activity of hydro-methanolic extracts of rhizomes and leaves of *C. caeruleus* (L).

Plants part	Phenolic content (µg.GAE mg^{-1})	Flavonoid content (µg.QE mg^{-1})	Tannins content (µg.CE mg^{-1})	DPPH (IC_{50}=µg/mL)	β-carotene (%)	FRAP (EC_{50}=mg/mL)
Rhizome	245.48 ± 0.22^{b}	77 ± 0.14^{b}	28.2 ± 0.13^{a}	545± 35.3^{b}	23.6 ± 0.62^{b}	0.59 ± 0.005^{b}
Leaves	416.83 ± 1.59^{a}	305.75 ± 0.24^{a}	2.007 ± 0.006^{b}	11.3 ± 0.5^{a}	74.94 ± 0.5^{a}	0.58 ± 0.076^{a}
BHT	-	-	-	8.5 ±1.9^{c}	86.43 ± 0.39^{c}	0.45 ± 0.045^{c}

(µg.GAE mg-1)–µg Gallic acid equivalents/mg of extract, (µg.QE mg-1) – µg Quercetin equivalents/mg of extract, (µg.CE mg-1) – µg Catechin equivalents/mg (µg GAE/mg) – micrograms of gallic acid equivalents per milligram of extract; (µg QE/mg) – micrograms of quercetin equivalents per milligram of extract; (µg CE/mg) – micrograms of catechin equivalents per milligram of extract. Letters a–d represent values (mean ± standard deviation, n = 3); different letters indicate significant differences ($p < 0.05$). (IC_{50}, EC_{50}) – half-maximal inhibitory concentration. Antioxidant activities are expressed as IC_{50} (µg/mL) for the DPPH assay and EC_{50} (mg/mL) for the FRAP assay. BHT (butylated hydroxytoluene) was used as a positive control.

Hemolytic activity

The hemolysis test was performed to assess the potential hemolytic activity of the plant, even though it possesses strong antioxidant properties. Indeed, the traditional use of this plant in medicine and drug preparation would be impossible if it exhibited hemolytic effects, which indicate cytotoxicity.

The results reveal a dose-dependent relation ship between extract concentration and hemolytic activity. Upon incubation of human erythrocytes with varying concentrations (12.5, 25, and 50 mg/mL) of leaf and rhizome extracts at 37°C for 60 minutes, the rhizome extract exhibited minimal hemolytic activity, with values not exceeding 3% even at the highest concentration (50 mg/mL). In contrast, the leaf extract demonstrated a modest hemolytic effect, reaching 14.45% at 50 mg/mL. At lower concentrations (12.5 and 25 mg/mL), hemolysis percentages for the rhizome extract were 0.8% and 1.5%, respectively, while the leaf extract induced hemolysis of 2.86% and 8.33%, correspondingly. **Dahmani et *al.* (2018)** reported that no mortality was observed in albino mice when testing the rhizome extract of *C. caeruleus* L. *in vivo*. However, variations in the mice's body weight compared to the controls were observed. It should be noted that the IC_{50} or EC_{50} values obtained during the assessment of the antioxidant activity of both extracts did not exceed 1 mg/mL.

Antioxidant activity

The free radical scavenging capacities of BHT and plant extracts were assessed using the IC_{50} value in µg/mL, representing the concentration of extract required to achieve 50% free radical scavenging. This index is inversely proportional to the antioxidant potential. IC_{50} values for *C. caeruleus* rhizome and leaf extracts are presented in (Table 2).

The leaf extract demonstrated significant antioxidant activity, although its potency was lower than that of BHT (Table 2). In contrast, the rhizome extract showed low activity. Phenolic compounds in the leaf extract exhibited higher antioxidant activity than those in the rhizome extract.

In the β-carotene bleaching test, the antioxidant capacity of the extracts was measured by the inhibition of β-carotene oxidative degradation (bleaching) caused by linoleic acid oxidation products. Regarding the influence of different plant organs, the leaf extract exhibited the highest potency ($p < 0.05$), with an inhibition rate more than three times higher than that of the rhizome extract. None of the plant extracts showed a percentage inhibition as strong as the synthetic antioxidant BHT ($p < 0.05$).

The presence of reducing agents in plant extracts leads to the reduction of the Fe^{3+} ferricyanide complex to its Fe^{2+} ferrous form. Furthermore, the ferric reducing power of the leaf extract was significantly higher than that of the rhizome extract ($p < 0.05$). This effect is expressed as an EC_{50} value; however, it remains lower than that of the BHT standard (Table 2).

The superior antioxidant activity observed in the leaf extracts may be attributed to their higher content of phenolic compounds. As noted by **Habellah et *al.* (2016)**, the antioxidant potential of plant extracts is strongly influenced by both the quantity and quality of their phenolic composition. Similarly, **Cai et *al.* (2004)** reported that the antioxidant capacity of medicinal plants is significantly affected by their phenolic content. Additionally, some phenolic compounds contributing to antioxidant activity may not have been detected by HPLC due to limitations in the identification process. Furthermore, the differences in compound concentrations between leaves and rhizomes may be explained by the heterogeneous distribution of secondary metabolites within plant tissues, both at the cellular and subcellular levels **(Vladimir-Knežević et *al.*, 2012)**. These variations may also be linked to the presence of natural pigments in the leaves, which are key secondary metabolites playing essential roles throughout the plant's life cycle and are well known for their potent antioxidant activity **(Lu et *al.*, 2021)**. Our results corroborate previous work by **Saffedine (2018)** on *C. caeruleus* L. extracts from Setif, Algeria, where leaf extracts exhibited markedly higher antioxidant activity compared to rhizomes. Specifically, the IC_{50} for the aqueous leaf fraction was 9.6 ± 0.26 µg/mL, whereas the petroleum ether rhizome fraction showed a much higher IC_{50} of 439.89 ± 5.82 µg/mL.

Table2 Chemical composition of extracts from the rhizomes and leaves of *C. caeruleus* (L).

Phenolic Compounds	Correlation (r^2)	Λ (nm)	RT (min)	Rhizomes (ppm)	Leaves (ppm)
3-Hydroxy Benzoic Acid	0.99928	287	22.545	1.847	1.236
4- Hydroxy Benzoic Acid	0.99994	278	17.647	0.495	0.509
Benzoic Acid	0.99986	278	47.629	N.D.	1.631
Catechin Hydrate	0.99906	278	11.499	N.D.	N.D
Chlorogenic Acid	0.99970	330	16.239	1.298	N.D
Caffeic Acid	0.99892	330	21.476	0.972	N.D
Epicatechin	0.99879	278	20.169	4.761	2.249
Gallic Acid	0.99966	278	5.912	N.D.	N.D.
Hesperidin	0.99705	287	65.989	3.656	3.549
P-Coumaric Acid	0.99982	330	33.597	0.354	N.D.
Quercetin	0.99962	254	76.313	6.43	4.624
Rosmarinic Acid	0.99907	330	70.655	3.564	N.D
Sinapinic Acid	0.99925	330	37.264	0.197	N.D
Syringic Acid	0.99839	278	22.628	1.176	N.D
t-Cinnamic Acid	0.99998	278	75.207	0.719	0.309
t-Ferulic Acid	0.99993	330	37.202	N.D	N.D

Results are expressed in ppm– mg/kg or mg/L equivalent. Compounds were identified by comparison of retention times (RT) and UV absorption maxima (Λ) with those of authentic standards. The correlation coefficient (r^2) refers to the linearity of calibration curves. N.D – Not detected.

Antibacterial activity

Antibacterial activity of the extracts was assessed through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, with the corresponding results detailed in Table 3. Both extracts exhibited diverse antibacterial effects against all tested organisms. MIC values ranged from 0.6 mg/mL to 5 mg/mL. The lowest MIC value (0.6 mg/mL) was observed against *Enterococcus faecalis* for the leaf extract. While activity against *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* was comparatively lower, the leaf extract demonstrated strong activity against both Gram-positive and Gram-negative bacteria. Conversely, the MBC values suggest that, at these concentrations, the extracts' activity is likely bacteriostatic rather than bactericidal. Similar to the antioxidant activities, the results indicate a highly promising antibacterial potential for the leaf extract, in which quercetin is one of the major components. This key plant flavonoid possesses a broad range of pharmacological activities and has been shown to inhibit the growth of various viruses, fungi, and

both Gram-positive and Gram-negative bacteria. Its antimicrobial effects are mediated through multiple mechanisms, including disruption of the cell membrane, altered membrane permeability, inhibition of protein and nucleic acid synthesis, reduced expression of virulence factors, mitochondrial dysfunction, and prevention of biofilm formation **(Nguyen & Bhattacharya, 2022)**. **Shu and colleagues (2011)** further reported that quercetin exhibits antibacterial activity against a variety of bacterial strains, including those affecting the respiratory, gastrointestinal, urinary, and integumentary systems.

Table3 MIC and MBC values of hydromethanolic extracts from *Carthamus caeruleus* against clinical bacteria.

					Positive Control	
	Rhizomes		**Leaves**		**Ampicillin (10mg/mL)**	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria						
Escherichia coli	10	>10	5	>10	<0.15	<0.15
Klebsiella pneumoniae	5	>10	1.25	>10	10	>10
Morganella morganii	10	>10	1.25	>10	>10	>10
Proteus mirabilis	>10	>10	5	>10	<015	<0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10
Gram-positive bacteria						
Enterococcus faecalis	2.5	>10	0.6	>10	<0.15	<0.15
Listeria monocytogenes	>10	>10	>10	>10	<0.15	<0.15
MRSA	2.5	>10	2.5	>10	<0.15	<0.15

MIC- minimal inhibitory concentration, MBC – minimal bactericidal concentration.

CONCLUSION

This study represents the first comprehensive phytochemical investigation of *Carthamus caeruleus* L., including both leaves and rhizomes. The findings reveal a rich array of bioactive compounds responsible for notable antioxidant and antibacterial properties. Leaf extracts exhibited the highest levels of phenolic and flavonoid compounds, while rhizome extracts—traditionally used in the treatment of burns and skin disorders—also exhibited notable bioactivities. Antioxidant and antibacterial assays highlight the potential of *C. caeruleus* L. extracts for combating oxidative stress and bacterial infections, making them promising for the development of novel therapeutic agents, such as rhizome-based topical formulations for wound healing and skin protection, and leaf-based natural products or herbal supplements with both antioxidant and antibacterial properties. Nonetheless, further research is warranted to elucidate the precise roles and mechanisms of individual phytochemicals in driving these bioactivities.

Acknowledgments: Special thanks to Prof. Omar Kharoubi for providing the instruments used to conduct the antioxidant and hemolytic activity assessments.

REFERENCES

Cai, Y., Luo, Q., Sun, M., and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life sciences*, 74(17), 2157-2184. https://doi.org/10.1016/j.lfs.2003.09.047

Caponio, F., Alloggio, V., and Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chemistry*, 64(2), 203-209. https://doi.org/10.1016/S0308-8146(98)00146-0

Dahmani, M. M., Laoufi, R., Selama, O., and Arab, K. (2018). Gas chromatography coupled to mass spectrometry characterization, anti-inflammatory effect, wound-healing potential, and hair growth-promoting activity of Algerian *Carthamus caeruleus* L (*Asteraceae*). *Indian Journal of Pharmacology,* 50(3), 123. http://doi.org/10.4103/ijp.IJP_65_17

Djamilatou, Z. S., Djibo, A. K., and Seini, B. S. S. H. (2021). Screening phytochimique, dosage des polyphénols et détermination de l'activité antioxydante de deux plantes antihypertensives du Niger. *European Scientific Journal*, 17(17), 335-349. https://doi.org/10.19044/esj.2021.v17n17p335

El Cadi, H., El Cadi, A., Kounnoun, A., Oulad El Majdoub, Y., Palma Lovillo, M., Brigui, J., Dugo, P., Mondello, L., & Cacciola, F. (2020). Wild strawberry (*Arbutus unedo*): Phytochemical screening and antioxidant properties of fruits collected in northern Morocco. *Arabian Journal of Chemistry*, 13(8), 6299–6311. https://doi.org/10.1016/j.arabjc.2020.05.022

Fedoul, F. F., Meddah, B., Larouci, M., Tir Touil, A., Merazi, Y., Bekhti, N., Piras, A., Falconieri, D., and Cakmak, Y. S. (2022). Medicinal applications, chemical compositions, and biological effects of Algerian *Ocimum basilicum* L. var genovese with the conversion of experimental doses to humans. *Journal of Applied Biotechnology Reports* ,9(2), 671–683 https://doi.org/10.30491/jabr.2021.290237

Gheffour, K., Boucherit, K., and Boucherit-Otmani, Z. (2015). Étude phytochimique et évaluation de l'activité antioxydante des extraits d'*Echinopsspinosus*. *Phytothérapie*, 5(13), 288-294. https://doi.org/10.1007/s10298-015-0917-8

Habellah, R. M., Karoune, S., Kechebar, M. S. A., & Bounab, H. (2016). Étude des composés phénoliques et des activités antioxydantes de l'Acacia ehrenbergiana de la région de Tindouf. *Journal Algérien des Régions Arides*, 13(1), 27–34.

Haddouchi, F., Chaouche, T. M., and Halla, N. (2018). Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d'Algérie. *Phytotherapy*, 16(S1), S254–S262. https://doi.org/10.1007/s10298-016-1086-8

Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S., and Belmalha, S. (2020). Phytochemical screening,polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon*, 6(11). https://doi.org/10.1016/j.heliyon.2020

Hosni, H., Salama, A., Abudunia, A., Cherrah, Y., Ibrahimi, A., and Alaoui, K. (2020). Toxicité aiguë, cytotoxicité et effet antiradicalaire de l'extrait méthanolique des feuilles de l'asphodèle, *Asphodelus microcarpus. Phytotherapy*, 18(5), 284–290. https://doi.org/10.3166/phyto-2019-0136

Iacopini, P., Baldi, M., Storchi, P., and Sebastiani, L. (2008). Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions. *Journal of Food Composition and Analysis*, 21(8), 589–598. https://doi.org/10.1016/j.jfca.2008.03.011

Khémiri, I., Essghaier, B., Sadfi-Zouaoui, N., and Bitri, L. (2020). *Carthamus tinctorius* L. in the management of skin injuries. *Oxidative Medicine and Cellular Longevity*, 2020, 1–12. https://doi.org/10.1155/2020/4103418

Lu, W., Shi, Y., Wang, R., Su, D., Tang, M., Liu, Y., and Li, Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *International Journal of Molecular Sciences*, 22(9), 4945. https://doi.org/10.3390/ijms22094945

Martínez-Noguera, F. J., Marín-Pagán, C., Carlos-Vivas, J., Rubio-Arias, J. A., and Alcaraz, P. E. (2019). Acute effects of hesperidin on oxidant/antioxidant state markers and performance in amateur cyclists. *Nutrients*, 11(8), 1898. https://doi.org/10.3390/nu11081898

Mayouf, N., Charef, N., Saoudi, S., Baghiani, A., Khennouf, S., and Arrar, L. (2019). Antioxidant and anti-inflammatory effect of *Asphodelus microcarpus* methanolic extracts. *Journal of Ethnopharmacology*, 239, 111914. https://doi.org/10.1016/j.jep.2019.111914

Merouane, A., Fellag, S., and Noui, A. (2020). Variation of phenolic content and antioxidant activity in organs and populations of *Phlomis crinita* L. *Revista Cubana de Plantas Medicinales*, 25(4). https://doi.org/10.1016/j.indcrop.2018.01.081

Nguyen, T. L. A., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: An approach to its mechanistic principle. *Molecules*, 27(8), 2494. https://doi.org/10.3390/molecules27082494

Ouda, A. N., Fatiha, M., Sadia, M., Zohra, S. F., & Noureddine, D. (2021). In vivo anti-inflammatory activity of aqueous extract of *Carthamus caeruleus L.* rhizome against carrageenan-induced inflammation in mice. *Jordan Journal of Biological Sciences*, 14(3), 529–535. https://doi.org/10.54319/jjbs/140319

Pires, T. C., Dias, M. I., Barros, L., Alves, M. J., Oliveira, M. B. P., Santos-Buelga, C., and Ferreira, I. C. (2018). Antioxidant and antimicrobial properties of dried Portuguese apple variety (*Malus domestica* Borkh. cv Bravo de Esmolfe). *Food Chemistry*, 240, 701–706. https://doi.org/10.1016/j.foodchem.2017.08.010

Saffidine, K. (2018). Étude analytique et biologique des flavonoïdes extraits de *Carthamus caeruleus* L. et de *Plantago major* L. (Doctoral dissertation, University of Setif). http://dspace.univsetif.dz:8888/jspui/handle/123456789/1680

Shu, Y., Liu, Y., Li, L., Feng, J., Lou, B., Zhou, X., and Wu, H. (2011). Antibacterial activity of quercetin on oral infectious pathogens. *African Journal of Microbiology Research*, *5*(30), 5358-5361 https://doi.org/10.5897/AJMR11.849

Taskova, R., Mitova, M., Mikhova, B., and Duddeck, H. (2003). Bioactive phenolics from *Carthamus lanatus* L. *Zeitschrift für Naturforschung C*, 58(9–10), 704–707. https://doi.org/10.1515/znc-2003-9-1019

Toubane, A., Rezzoug, S. A., Besombes, C., and Daoud, K. (2017). Optimization of accelerated solvent extraction of *Carthamus caeruleus* L.: Evaluation of antioxidant and anti-inflammatory activity of extracts. *Industrial Crops and Products*, 97, 620–631. https://doi.org/10.1016/j.indcrop.2016.12.002

Vladimir-Knežević, S., Blaženković, B., Bival Štefan, M., & Babac, M. (2012). *Plant polyphenols as antioxidants influencing human health.* In V. Rao (Ed.), *Phytochemicals as nutraceuticals – Global approaches to their role in nutrition and health* (pp. 155–180). InTech. https://doi.org/10.5772/2742

Yu, S. Y., Lee, J. J., Kim, J. D., Kang, S. N., Lee, S. K., Jang, J. Y., Lee, H. K., Lim, J. H., and Lee, O. H. (2013). Phenolic composition, antioxidant activity and anti-adipogenic effect of hot water extract from safflower (*Carthamus tinctorius* L.) seed. *Nutrients*, 5(12), 4894–4907. https://doi.org/10.3390/nu5124894

Zanwar, A. A., Badole, S. L., Shende, P. S., Hegde, M. V., & Bodhankar, S. L. (2014). *Cardiovascular effects of hesperidin: A flavanone glycoside*. In Ronald Ross Watson, Victor R. Preedy, & Sherma Zibadi (Eds.), *Polyphenols in Human Health and Disease* (Vol. 2, pp. 989–992). Academic Press. https://doi.org/10.1016/B978-0-12-398456-2.00076-1

Zhang, H. L., Nagatsu, A., Watanabe, T., Sakakibara, J., & Okuyama, H. (1997). Antioxidative compounds isolated from safflower (*Carthamus tinctorius* L.) oil cake. *Chemical and Pharmaceutical Bulletin*, 45(12), 1910–1914. https://doi.org/10.1248/cpb.45.1910