



Comprehensive Phytochemical Characterization and *In Vitro/In Vivo* Anti-inflammatory Activity of *Chrysanthemum macrocarpum* Hydro-methanolic Extract: A First Insight into an Algerian Saharan Endemic

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Abstract

Introduction: *Chrysanthemum macrocarpum*, an Algerian Saharan endemic traditionally used in folk medicine, remains poorly explored beyond its essential oil. This study aims to characterize, for the first time, the phytochemical composition, phenolic profile, and biological activities of the hydro-methanolic extract of *C. macrocarpum*.

Materials and Methods: Aerial parts were extracted with 80:20 methanol:water. Phytochemical screening was performed for secondary metabolites, and phenolic compounds were identified by HPLC-PDA. Antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP (ferric ion reducing antioxidant potential), and CUPRAC (cupric reducing antioxidant capacity) assays, while anti-inflammatory potential was tested *in vitro* (BSA protein denaturation) and *in vivo* (carrageenan-induced mouse paw edema).

Results: Screening revealed abundant (+++) tannins, flavonoids, alkaloids, coumarins, and anthocyanins, with moderate quinones (+). Extraction yield was 18.36%, with phenolic and flavonoid contents of 106.93 mg GAE/g and 44.57 mg QE/g, respectively. HPLC-PDA identified five major phenolics, dominated by chicoric acid (10.104 mg/g), kaempferol-3-O-glucoside (7.325 mg/g), and chlorogenic acid (4.738 mg/g). The extract displayed dose-dependent antioxidant activity with IC₅₀ values of 0.392 mg/ml (DPPH) and 0.299 mg/ml (ABTS). Reducing power assays further confirmed this activity, with values of 0.277 mmol FeSO₄/g extract (FRAP) and 0.48 mmol TEAC/g extract (CUPRAC). *In vitro*, HME-CM inhibited BSA protein denaturation with an IC₅₀ of 70.92 µg/ml. Moreover, *in vivo*, HME-CM significantly reduced carrageenan-induced paw edema, showing a similar temporal pattern of anti-inflammatory activity to diclofenac, although diclofenac consistently produced a higher percentage of inhibition.

Conclusions: *C. macrocarpum* represents a promising source of natural antioxidants and anti-inflammatory agents, supporting its ethnomedicinal use and pharmacological potential.

Keywords: *Chrysanthemum macrocarpum*, HPLC-PDA, Algerian Saharan Flora, Phenolic Compounds, Antioxidant Activity, Anti-inflammatory Effect

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Introduction

Oxidative stress plays a pivotal role in the pathophysiology of numerous disorders, including neurodegenerative diseases (e.g., Alzheimer's), cancers, inflammatory and autoimmune conditions, viral infections, and cardiovascular disorders. It primarily arises from an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms,

leading to extensive cellular and tissue damage.^{1,2} The severe adverse effects of conventional therapies, such as hepatotoxicity, have intensified interest in natural bioactive molecules, particularly those derived from medicinal plants, which are considered promising alternatives due to their potential efficacy and improved safety profile.³

Algeria, characterized by exceptional floristic diversity, harbors a wide range of medicinal plants with high therapeutic potential. This richness is especially remarkable in Saharan endemic species, which are often abundant in phenolic compounds.⁴ Among plant families, *Asteraceae* is the largest, comprising more than 24,000 species distributed worldwide, except in Antarctica. In Algeria, fifteen species of the genus *Chrysanthemum* have been recorded,⁵ including *Chrysanthemum macrocarpum*, locally known as "Bouchicha".⁶ The term "Guertoufa" is also used to refer to a group of Saharan herbs that includes *C. macrocarpum* alongside other species such as *Cotula cinerea*, *C. fuscum*, *C. trifurcatum*, *C. denticolum*, and *Matricaria pubescens*.⁷ *C. macrocarpum* (Sch. Bip.) Coss. & Kralik ex Batt., synonym *Endopappus macrocarpus* (Coss. & Kralik ex Batt. & Trabut) Sch. Bip., is a small annual herb (under 20 cm) characterized by an initially prostrate, later erect stem, and fleshy, bright green, pinnatisect leaves. Its inflorescence is a capitulum with yellow tubular florets surrounded by white ligules. It typically grows in sandy-clayey depressions and wadi beds, emerging mainly after rainfall.^{5,8} Traditionally, this endemic Saharan herb is widely employed in folk medicine for managing fever, migraine, arthritis, vertigo, hypertensive symptoms, and infectious diseases such as pneumonia, colitis, stomatitis, as well as intestinal infections.⁹ Additionally, Touareg communities utilize this plant for culinary purposes and as an herbal tea.¹⁰

Recent studies have demonstrated that species within the *Chrysanthemum* genus exhibit diverse pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties,^{11,12} as well as antitumor, antiviral, anti-allergic, hepatoprotective, nephroprotective, and insecticidal effects.^{13,14} In Algeria, only a few studies have focused on *C. macrocarpum*, mainly addressing the composition of its essential oil^{7,9} and the antibacterial and cytotoxic activities of certain triterpenic diesters.¹⁰ In contrast, several investigations have concentrated on assessing the biological activities of *C. coronarium*, another species belonging to the same genus as *C. macrocarpum*.^{15,16}

To the best of our knowledge, this is the first study to characterize the phytochemical profile, perform HPLC-PDA analysis, and assess the antioxidant and anti-inflammatory (*in vitro* and *in vivo*) activities of Algerian *C. macrocarpum*. In Algeria, this work contributes to the valorization of Algerian flora and the search for novel bioactive compounds from natural sources.

Materials and Methods

Plant Collection

The aerial parts (stems and flowers) of *C. macrocarpum* were collected from Hassi Messaoud, Ouargla Province, Algeria (31°40'57" N, 6°04'21" E) in April 2024.

Taxonomic identification was carried out by a botanist at the Department of Biology, University of Boumerdes based on the reference flora of.⁵

Experimental Animals

Male NMRI mice (20–30 g) were obtained from the animal facility of the Pasteur Institute, Algeria and housed under standard laboratory conditions (25 °C; natural light/dark cycle) with free access to food and water. Animals were fasted for 12 h prior to the experiment, with free access to water. All experimental procedures were conducted in compliance with the guidelines of the Institutional Animal Care Committee of the Algerian Ministry of Higher Education and Scientific Research, under Agreement Number 45/DGLPAG/DVA.SDA.14.

Phytochemical Screening

The phytochemical tests performed on *C. macrocarpum* were based on a series of colorimetric methods used to detect the presence or absence of secondary metabolites in the plant powder or its 5% infusion. The targeted metabolites included total tannins, gallic tannins, catechin tannins, alkaloids, quinones, flavonoids, free anthracenic compounds, anthocyanins, coumarins, leucoanthocyanins, sennosides, steroids, saponins, iridoids, and mucilage.^{17,18} The abundance of each metabolite was estimated using the key +, ++, +++, corresponding to low, moderate, and high levels, respectively.

Hydromethanolic Extraction

After drying in a well-ventilated area protected from light, the plant material was ground into a fine powder to increase the extraction surface area. A quantity of 20 g of the powdered plant was macerated at room temperature in 100 ml of a hydroalcoholic mixture (methanol:water, 80:20 v/v). After 24 hours of maceration, the mixture was filtered and the filtrate was evaporated under reduced pressure using a rotary evaporator at a temperature not exceeding 45 °C, until a dry extract was obtained. The extraction yield was determined by relating the mass of the obtained extract to the initial mass of the plant material.¹⁹

Determination of Total Polyphenol Content

The total polyphenol content was determined spectrophotometrically using the Folin–Ciocalteu colorimetric method, as described by Singleton et al.²⁰ Briefly, 0.5 ml of the extract was mixed with 5 ml of distilled water, followed by the addition of 0.5 ml of Folin–Ciocalteu reagent. After thorough mixing, the mixture was left to stand for 3 minutes before adding 0.5 ml of 20% sodium carbonate (Na₂CO₃). The solution was then incubated for 1 hour at room temperature, protected from light. The absorbance was measured at 760 nm against a blank. Results were expressed as milligrams of gallic acid equivalents per gram of dry

matter (mg GAE/g extract $\pm$ SD), based on a calibration curve prepared with gallic acid.

Determination of Total Flavonoid Content

The flavonoid content was determined spectrophotometrically based on the formation of a complex between the extract and aluminum chloride (AlCl_3), as described by Ayoola et al.²¹ Briefly, 1 ml of the extract was mixed with 1 ml of 2% AlCl_3 solution. The mixture was incubated for 10 minutes at room temperature, and the absorbance was measured at 430 nm against a blank. Results were expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/g DM $\pm$ SD), based on a calibration curve prepared with quercetin.

High-performance Liquid Chromatography (HPLC-PDA)

Quantitative analysis of phenolic compounds was performed using an HPLC system equipped with a PDA detector (Shimadzu Nexera-i LC-2040C 3D). Separation was carried out on a phenylhexyl reverse-phase column (3 μm , 4.6 $\times$ 150 mm; GL Sciences InterSustain). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile; Merck, HPLC grade), delivered at a flow rate of 1 ml/min. The gradient elution program was: 0–5 min: 5% B; 5–20 min: 5–25% B; 20–30 min: 25–50% B; 30–35 min: 50–90% B; 35–40 min: 90% B; 40–45 min: return to 5% B and equilibrate for 5 min before the next run. The injection volume for both samples and standards was 10 μl , and the column temperature was maintained at 30 $^\circ\text{C}$. Stock solutions of standards and extracts were prepared at a concentration of 1000 mg/L. The phenolic profile was determined using 15 reference phenolic standards. Quantification was based on external calibration curves of these standards, and results were expressed as milligrams per gram of extract (mg/g extract).

Determination of Antioxidant Activity

Free Radical Scavenging Activity (DPPH Assay)

The free radical scavenging activity was evaluated by DPPH according to the method described by Blois.²² Samples at different concentrations (0.05, 0.1, 0.25, 0.5 mg/ml) and standard solutions were taken and made up to 3 ml with ethanol. Then DPPH $^\bullet$ solution (1.0 ml, 0.26 mM) was added. The reaction mixture was vortexed thoroughly, left in the dark at room temperature for 30 minutes and measured at 517 nm. The activity was calculated by the following equation:

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

Where A_{control} and A_{sample} are the DPPH radical and DPPH samples absorbance, respectively. The inhibitory concentration (IC_{50}) of the samples which

needed to inhibit 50% of the DPPH radicals obtained from the standard curve was compared to that of standard antioxidant (Trolox).

Cation Free Radical Scavenging Activity (ABTS Assay)

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) cation radical scavenging activity was determined according to the method described by Re et al.²³ Treatment of ABTS (2.0 mM) prepared in phosphate buffer (0.1 M, pH 7.4) with potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) in a ratio 1:2 (2.45 mM) gave the ABTS $^{+\bullet}$, which was kept at dark at room temperature for 6 h. ABTS $^{+\bullet}$ - $\text{K}_2\text{S}_2\text{O}_8$ solution (1.0 ml) was added to each standard and extract solution at different concentrations. After 30 minutes of incubation, the inhibition was calculated for each concentration relative to a blank absorbance at 734 nm. Experiments were performed in triplicate. Cation free radical scavenging activity was calculated as percentage inhibition by the following equation:

$$\text{Activity \%} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_{control} and A_{sample} are the ABTS solution and samples absorbance, respectively. The results were plotted against the concentration activity. The IC_{50} was calculated by using the slope equation.

Ferric Reducing Antioxidant Power (FRAP Assay)

The determination of the reduction capacity of Iron (III) is based on the increase in absorbance values resulting from the reduction of the orange-light brown $[\text{Fe}(\text{III})\text{-TPTZ}]$ complex, formed by the reaction of $\text{Fe}(\text{III})$ with 2,4,6-tripyridyl-S-triazine (TPTZ), to the dark blue-purple $\text{Fe}(\text{II})\text{-TPTZ}$ complex in the presence of antioxidants.²⁴ The FRAP reagent was prepared by mixing 1 ml of 10 mM 2,4,6-Tripyridyl-S-triazine solution, prepared in 40 mM HCl, with 1 ml of 20 mM FeCl_3 solution, prepared in water, and 10 ml of 300 mM acetate buffer (pH 3.6), followed by heating at 37 $^\circ\text{C}$. Iron sulphate (FeSO_4) solutions of varying concentrations were prepared as a positive control. After adding 10 μl of each extract at different concentrations and the standards into the wells, 300 μl of freshly prepared FRAP reagent was added. The reaction mixtures were incubated at 37 $^\circ\text{C}$ for 4 minutes. Absorbance values were measured at 593 nm. The reducing power of the plant extracts was calculated as mmol FeSO_4 equivalents per gram of extract (mmol FeSO_4/g extract) using the calibration equation derived from the standard iron sulphate solutions at different concentrations.

Copper (II) Ion Reducing Antioxidant Capacity Activity (CUPRAC)

The reducing antioxidant capacity activity of Copper (II) ions is based on the reduction of a blue complex formed by

the reaction of Cu^{2+} ions with Neocuproine to a yellow complex in the presence of antioxidants.²⁵ For the experiment, a 7.5 mM Neocuproine (2,9-dimethyl-1,10-phenanthroline) solution was prepared in ethanol, a 10 mM Copper (II) chloride solution was prepared, and a 1 M ammonium acetate buffer at pH 7.0 was prepared with water. For the positive control, Trolox standards at different concentrations were prepared. To each well, 50 μl of copper (II) solution, 50 μl of neocuproine solution, and 50 μl of ammonium acetate buffer were added. The mixtures were incubated at 37 °C for 15 minutes. Then, 100 μl of plant extract and standard solutions at varying concentrations were added to the wells. The mixtures were incubated at 37 °C in the dark. The activity resulted in the formation of an orange coloured complex. At the end of the incubation, absorbance values were measured at 450 nm, with the reference solution (without the sample) serving as the baseline. The reducing power capacity of the plant extracts was calculated as mmol Trolox equivalents per gram of extract (mmol TEAC/g extract) using the calibration equation derived from the different concentrations of the standard Trolox solutions.

In vitro and In vivo Determination of Anti-inflammatory Effect BSA Denaturation Assay for In vitro Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity was evaluated *in vitro* using the BSA denaturation method²⁶ with minor modifications. Reaction mixtures included: a negative control (0.45 ml of 0.5% BSA + 0.05 ml methanol), a standard (0.45 ml of 0.5% BSA + 0.05 ml diclofenac at 50, 100, and 150 $\mu\text{g}/\text{ml}$), and test samples (0.45 ml of 0.5% BSA + 0.05 ml plant extract at the same concentrations). After incubation at 37 °C for 20 minutes and heating at 57 °C for 3 minutes, 2.5 ml of phosphate buffer (pH 6.6) was added. Absorbance was recorded at 660 nm (Shimadzu UV-Vis spectrophotometer). Results represent the mean of three replicates, and inhibition of protein denaturation was calculated relative to the negative control using the following formula:

$$\text{Activity (\%)} = \frac{A_c - A_e}{A_c} \times 100$$

Where A_c and A_e are the control and extract absorbance, respectively. The results were plotted against the concentration activity. The IC_{50} was calculated by using the slope equation.

Carrageenan Induced Mouse Paw Edema Method for In vivo Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity of the HME-CM was investigated on carrageenan induced inflammation in mouse paw following the method of.²⁷ The mice were randomly divided into three groups ($n = 4$ each): Group I received distilled water (1 ml/100 g); Group II received diclofenac

sodium (100 mg/kg); and Group III received the plant extract (200 mg/kg). Administrations were given by buccal-esophageal gavage. One hour later, inflammation was induced by subplantar injection of 0.05 ml of 1% carrageenan into the right hind paw. Paw diameter was measured hourly for 6 h using a digital caliper (Holex) at the metatarsal level. The anti-inflammatory activity was expressed as the percentage inhibition of edema using the following formula:

$$\text{Inhibition of paw edema (\%)} = \frac{C - C_1}{C} \times 100$$

Where C and C_1 mean increase in paw circumference of control and treated animal respectively.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation (SD). Statistical differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Scheffé, Tukey–Kramer, and Fisher post hoc tests for multiple comparisons. A significance level of $p < 0.05$ was considered statistically significant. Different letters indicate significant differences between groups. All statistical analyses were performed using OriginPro 2018 (64-bit).

Results

Phytochemical Screening

The phytochemical results for all the targeted metabolites are given in Table 1. Several secondary metabolites were detected in the aerial parts (flowers and stems) of *C. macrocarpum*. The results indicated a high abundance (+++) of total tannins, gallic tannins, alkaloids, flavonoids, coumarins, leucoanthocyanins, sennosides, mucilages, anthocyanins and a presence (+) of quinones. Metabolites like catechin tannins, free anthracenic compounds, iridoids, and steroids were completely absent (-).

Determination of Yield of Extraction, Total Polyphenols and Flavonoids Content

The HME-CM extract exhibited a dark brown, caramel-like appearance, with an extraction yield of $18.36 \pm 1.12\%$. Its total polyphenol and flavonoid contents were 106.93 ± 4.15 mg GAE/g extract and 44.57 ± 2.29 mg QE/g extract, respectively.

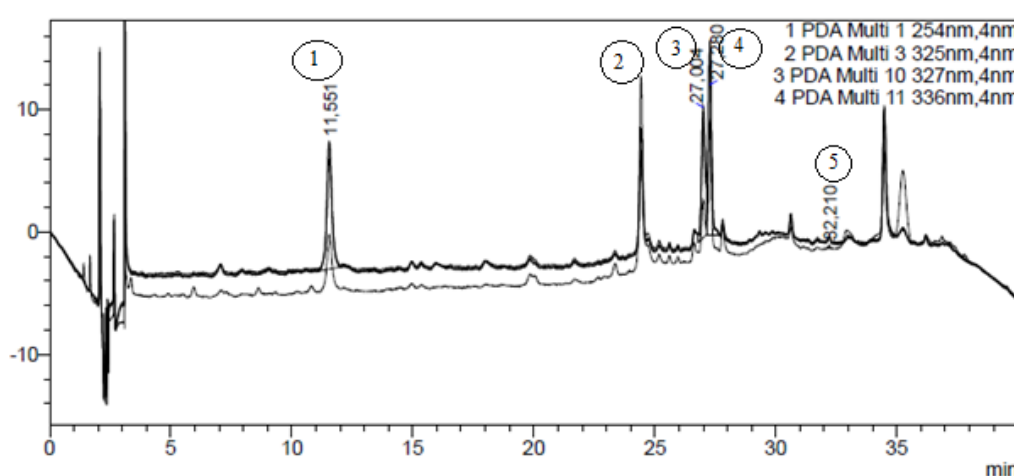
HPLC-PDA Analysis

Individual phenolic compound contents of the hydro-methanolic extract of *C. macrocarpum* (HME-CM) were screened against 15 standard phenolic compounds. The HPLC-PDA chromatogram of the HME-CM is presented in Figure 1, while quantitative results for the identified compounds are summarized in Table 2.

Table 1. Phytochemical Screening Results of the Aerial Parts (flowers and stems) of *Chrysanthemum macrocarpum*

Phytochemicals	Abundance
Total tannins	+++
Gallic tannins	+++
Alkaloids	+++
Flavonoids	+++
Coumarins	+++
Leucoanthocyanins	+++
Sennosides	+++
Mucilages	+++
Anthocyanins	+++
Quinons	+
Catechin tannins	-
Free anthraquinones	-
Iridoids	-
Steroids	-

(+) presence; (++) abundant; (+++) highly abundant; (-) absence.

**Figure 1.** HPLC-PDA chromatogram of the HME-CM. 1: Chlorogenic acid; 2: Kaempferol-3-O-glucoside; 3: Chicoric acid; 4: Apigenin-7-O-glucoside; 5: Quercetin.**Table 2.** Quantitative Analysis of Major Phenolic Compounds Identified in the HME-CM Extract by HPLC-PDA

Compounds	Retention time (minutes)	Concentration (mg/g extract)
Chlorogenic Acid	11.551	4.738
Kaempferol-3-O-glucoside	24.479	7.325
Chicoric Acid	27.004	10.104
Apigenin-7-O-glucoside	27.280	3.243
Quercetin	32.210	0.061

Chromatographic analysis of HME-CM revealed several peaks, among which only five were identified. Among the detected phenolics, chicoric acid exhibited the highest concentration (10.104 mg/g extract), followed by kaempferol - 3-O-glucoside (7.325 mg/g extract) and chlorogenic acid (4.738 mg/g extract). Apigenin-7-O-glucoside (3.243 mg/g extract) was also identified, whereas quercetin was present at a much lower level (0.061 mg/g extract). These findings indicate that the extract is particularly rich in phenolic acids (notably chicoric and chlorogenic acids) and glycosylated flavonoids (kaempferol-3-O-glucoside and apigenin-7-O-glucoside), suggesting a strong potential for antioxidant and anti-inflammatory activities.

Antioxidant Activity

The antioxidant activity of the HME-CM was evaluated using four complementary *in vitro* assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay, the FRAP (Ferric Reducing Antioxidant Power) assay, and the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay, with Trolox serving as the reference standard (Table 3).

Our data revealed that the HME-CM exerted a clear dose-dependent antioxidant activity in all four assays. In the DPPH radical scavenging assay, inhibition increased progressively from $15.34 \pm 0.27\%$ at the lowest tested

Table 3. Results of the Antioxidant Assays (DPPH, ABTS, FRAP, CUPRAC) of HME-CM Compared to Trolox

Method	HME-CM			Trolox		
	Equation	R	IC ₅₀	Equation	R	IC ₅₀
DPPH°	$y = 100.8x + 10.409$	0.9794	0.392 mg/ml	$y = 301.39x + 19.146$	0.968	0.102 mg/ml
ABTS	$y = 167.03x + 0.005$	0.8999	0.299 mg/ml	$y = 344.29x + 6.0506$	0.9952	0.127 mg/ml

Reducing power assays			
Method	Equivalent values	Equation	R
FRAP	0.277 mmol FeSO ₄ /g extract	$y = 0.7058x - 0.0341$	0.9582
CUPRAC	0.48 mmol TEAC/g extract	$y = 2.5452x + 0.4277$	0.9234

HME-CM: hydro-methanolic extract of *Chrysanthemum macrocarpum*; IC₅₀: Inhibitory Concentration 50, R²: coefficient of determination.

concentration (0.05 mg/ml) to $62.32 \pm 0.74\%$ at the highest concentration (0.5 mg/ml). A similar response was observed in the ABTS radical cation decolorization assay, with inhibition values ranging from $34.81 \pm 0.47\%$ to $67.35 \pm 1.13\%$ for the same concentrations. Notably, the higher inhibition percentages obtained in the ABTS assay suggest a greater sensitivity of this method toward the antioxidant compounds present in HME-CM.

For the DPPH assay, HME-CM exhibited an IC₅₀ value of 0.392 mg/ml, while Trolox showed a markedly lower IC₅₀ of 0.102 mg/ml, indicating a higher radical scavenging efficiency of the standard. Similarly, in the ABTS assay, the extract displayed an IC₅₀ of 0.299 mg/ml, whereas Trolox demonstrated stronger activity with an IC₅₀ of 0.127 mg/ml. The reducing power of HME-CM, assessed by the FRAP method, was 0.277 mmol FeSO₄/g extract, compared with the standard curve obtained for Trolox. Likewise, the CUPRAC assay revealed a value of 0.48 mmol TEAC/g extract, confirming the extract's significant reducing capacity.

In vitro and In vivo Results of Anti-inflammatory Effect

In vitro Results of Anti-inflammatory Activity

The variations in the percentage inhibition of BSA protein denaturation by HME-CM and diclofenac at different concentrations compared to the control group are presented in Figure 2. The results indicate that the HME-CM extract exhibits a highly significant anti-inflammatory effect ($F(2,6) = 184717.14706$; $p < 0.0001$; $R^2 = 0.99998$). At concentrations of 50, 100, and 150 µg/ml, the extract achieved inhibition percentages of $46.29 \pm 2.92\%$, $55.29 \pm 2.13\%$, and $62.98 \pm 1.14\%$, respectively. In contrast, significantly higher inhibition values were recorded for diclofenac, reaching $57.18 \pm 1.08\%$, $63.42 \pm 1.35\%$, and $74.85 \pm 1.22\%$ at the corresponding concentrations ($F(2,6) = 93859.36364$; $p < 0.0001$; $R^2 = 0.99997$). A clear dose-dependent inhibition of BSA protein denaturation was observed for HME-CM, with a 50% inhibition (IC₅₀) reached at 70.92 µg/ml ($y = 0.1669x + 38.163$; $R^2 = 0.998$), indicating a significant anti-inflammatory potential. However, diclofenac exhibited greater potency, with a lower IC₅₀ of 14.82 µg/ml ($y = 0.1767x + 47.48$; $R^2 = 0.972$).

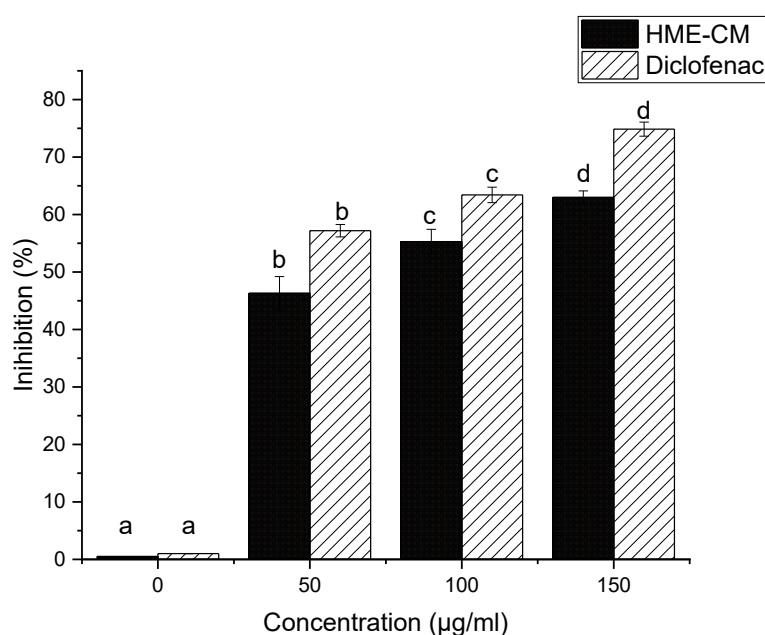


Figure 2. Anti-inflammatory Activity of HME-CM and Diclofenac Evaluated by the BSA Denaturation Assay. HME-CM: hydro-methanolic extract of *Chrysanthemum macrocarpum*. Values with different superscript letters among the bars within each treatment show highly statistically significant differences according to Scheffé, Tukey–Kramer, and Fisher tests ($p < 0.0001$).

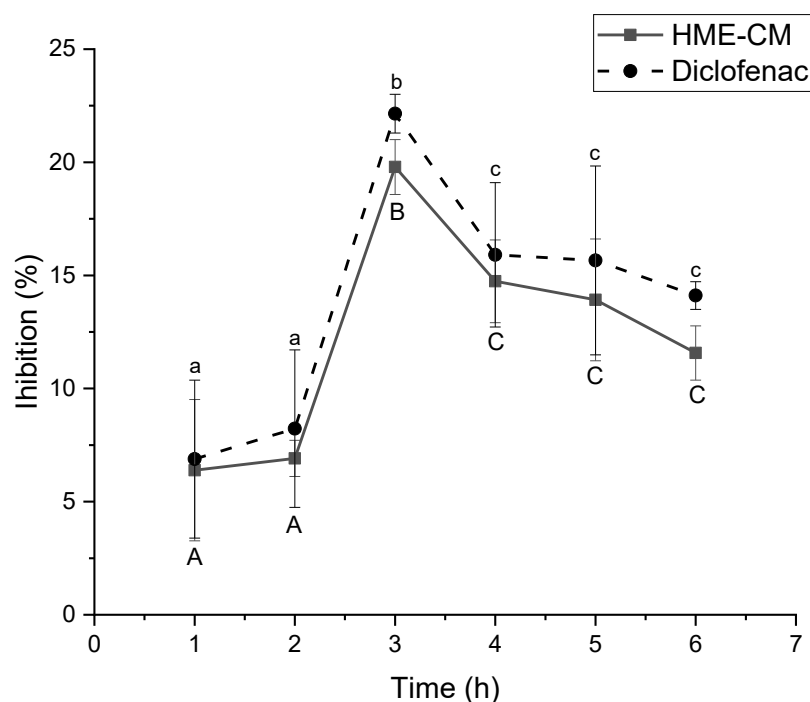


Figure 3. Time-dependent Effect of HME-CM and Diclofenac on Carrageenan-induced Paw Edema. Data are expressed as mean $\pm$ SD ($n = 5$) compared to negative control. Values with different superscript letters among the curves within each treatment show statistically significant differences according to Scheffé, Tukey–Kramer, and Fisher tests ($p < 0.0001$). Capital letters refer to HME-CM; lowercase letters refer to diclofenac.

In vivo Results of Anti-inflammatory Activity

The *in vivo* anti-inflammatory effect of the HME-CM extract and standard (Diclofenac) are presented in Figure 3. In control animals, the subplantar injection of carrageenan produced a local edema. As shown in Figure 3, HME-CM exhibited an edema inhibition profile comparable to that of diclofenac over the 6-hour observation period. At 1 h, both treatments (HME-CM and Diclofenac) produced low inhibition rates, with no significant difference between them ($6.39 \pm 3.12\%$; $6.88 \pm 3.49\%$, respectively; $p = 0.859$). The maximal inhibitory effect was observed at 3 h, where HME-CM achieved $19.79 \pm 1.21\%$ inhibition compared to $22.15 \pm 0.85\%$ for diclofenac ($p = 0.0196$). A gradual decrease was then noted until 6 h ($11.57 \pm 1.20\%$ for HME-CM and $14.11 \pm 0.61\%$ for Diclofenac; $p = 0.0137$).

Discussion

Phytochemical screening constitutes a crucial preliminary step in phytopharmacological research, enabling the detection and assessment of the abundance of primary and secondary metabolites within plant extracts. These phytochemicals are biologically active chemical compounds, recognized for their diverse beneficial roles in both human health and supporting plant physiological functions.²⁸ The obtained results highlighted the richness of the extract in certain secondary and primary metabolites. These compounds provide scientific support for some of the plant's traditional

uses, particularly its antioxidant and anti-inflammatory activities, which are directly relevant to conditions such as arthritis and may indirectly contribute to other traditional uses. Other potential activities, including antimicrobial or antihypertensive effects, were not evaluated in this study and remain to be investigated.²⁹ Some of these metabolites were previously reported in a recent study by Belhachat et al.¹⁵ in *Chrysanthemum coronarium* (L.) collected from the Algiers region in northeastern Algeria. Organic extracts were obtained by Soxhlet extraction using water, methanol, and ethanol, with the methanolic extract showing the highest phenolic and flavonoid contents. The observed differences with our study may be due to species-specific variation, as *C. macrocarpum* and *C. coronarium* likely differ in secondary metabolite profiles, as well as differences in extraction conditions (solvent, time, temperature, plant part) and environmental factors (soil, climate, harvest time, developmental stage). Similar results have also been reported by Chae.²⁸ A methanol:water mixture (80:20, v/v) was selected to optimize the extraction efficiency of phenolic compounds, as methanol is recognized as one of the most effective solvents for recovering phenolic constituents from plant matrices.²⁹ To date, no studies have reported the extraction yield, total polyphenol content, or flavonoid content of *C. macrocarpum*. Most available data concern other species of the same genus. It is important to note that the composition of the hydromethanolic extract

of *C. macrocarpum* may vary substantially in both quantity and quality due to the influence of biotic and abiotic factors on secondary metabolite biosynthesis. Environmental conditions such as temperature, humidity, light intensity, and the availability of water, minerals, and CO₂ significantly affect plant growth and metabolite production.³⁰ In addition, factors such as plant age, harvest season, plant organ used, sample preparation, analytical method, and solvent polarity also influence the phytochemical profile.^{31,32}

For instance, Soxhlet extraction of *C. coronarium* using methanol yielded $30.14 \pm 0.02\%$, demonstrating the high efficiency of this method for recovering bioactive compounds.¹⁵ The total phenolic content reached 208.50 ± 0.25 mg GAE/g extract, nearly double the value observed in the present work. Conversely, the flavonoid content reported by the same authors (23.46 ± 0.08 mg QE/g extract) was significantly lower than that obtained in our study (44.57 ± 2.29 mg QE/g extract). This distribution is consistent with that observed in many *Chrysanthemum* species, where derivatives of caffeic acid (chlorogenic, dicaffeoylquinic, and chicoric acids) and glycosylated flavones/flavonols constitute major metabolites in floral and foliar organs. For example, in *C. morifolium*, targeted quantification has revealed high levels of caffeoylquinic acids and the presence of apigenin-7-O-glucoside in several cultivars, confirming the predominance of phenolic acids and O-glycosylated flavonoids in this genus.^{33,34}

In terms of biological relevance, the major identified compounds are associated with well-documented pharmacological properties. Chicoric acid exhibits strong antioxidant and anti-inflammatory effects through Nrf2/HO-1 activation and NF- κ B inhibition.³⁶ Chlorogenic acid, also abundant in HME-CM, shares similar mechanisms, enhancing antioxidant defenses via Nrf2/HO-1 activation, metal chelation, and radical scavenging, while attenuating inflammatory signaling (TLR4/NF- κ B and JNK), thereby supporting endothelial protection.³⁷

Similarly, kaempferol-3-O-glucoside (K3G) also displays antioxidant and anti-inflammatory potential by modulating ROS-dependent MAPK and NF- κ B pathways, reducing cytokines (IL-6, IL-1 β), nitric oxide, and PGE₂ production. In addition, kaempferol and its derivatives inhibit aldose reductase and show antiviral (anti-HIV-1) activity.³⁸ Furthermore, apigenin-7-O-glucoside, a common flavonoid in Asteraceae, contributes antispasmodic, antioxidant, and anticancer effects.³⁹ Quercetin further complements this phytochemical profile with potent antioxidant, anti-inflammatory, and cardioprotective properties by reducing oxidative stress, inflammatory mediators, and improving endothelial function.⁴⁰ Taken together, the abundance of phenolic acids and glycosylated flavonoids in HME-CM strongly supports its high antioxidant and anti-inflammatory potential. The variations observed when compared to

other *Chrysanthemum* species may be attributed to genetic differences, environmental conditions, and extraction parameters, as previously reported for this genus.³⁵ DPPH, ABTS, FRAP and CUPRAC assays are often used simultaneously to evaluate the *in vitro* antioxidant activities of extracts or active substances.³⁶ Overall, the results indicate that although Trolox consistently exhibited higher antioxidant activity across all assays, HME-CM still exhibits a considerable antioxidant potential. This activity can be attributed to the high content of phenolic acids (notably chicoric and chlorogenic acids) and flavonoids (such as kaempferol-3-O-glucoside and apigenin-7-O-glucoside) identified in the extract by HPLC-PDA (Figure 1, Table 2). Several studies have demonstrated a strong relationship between phenolic content and antioxidant activity.

For example, Nguyen et al.³⁷ have reported that phenolic compounds play a vital role in the antioxidant activity in *C. morifolium*. Additionally, when conducted a correlation study between the phenolic compounds and the antioxidant capacities of *Chrysanthemum* species³⁸ show that the caffeoylquinic acids and flavonoids exhibit a good correlation with DPPH, ABTS, and FRAP values.

According to Lim et al.³⁹ samples with an IC₅₀ value below 50 μ g/ml are considered very strong antioxidants, those between 50 and 100 μ g/ml are classified as strong antioxidants, values between 101 and 150 μ g/ml indicate moderate antioxidant activity, and IC₅₀ values exceeding 150 μ g/ml correspond to weak antioxidant activity. Higher IC₅₀ values were reported by Belhachat et al.¹⁵ when assessing antioxidant activity using three methods: DPPH (4.72 ± 0.06 mg/L), ABTS (2.49 ± 0.01 mg/L), and reducing power (2.43 ± 0.02 mg/L), indicating that the HME-CM exhibited a superior antioxidant effect. The scavenging activity of antioxidants against the DPPH[•] radical is mainly driven by their capacity to donate a hydrogen atom, which results in the loss of the radical's characteristic violet color. In contrast, the neutralization of the ABTS^{•+} radical occurs predominantly through a single electron transfer mechanism, although hydrogen atom transfer may also contribute.⁴⁰ Among the methods employed, the ABTS assay appeared to be the most sensitive for evaluating the antioxidant capacity of HME-CM, as it provided a lower IC₅₀ value (0.299 mg/ml) compared to DPPH. This can be explained by the fact that the ABTS radical is soluble in both aqueous and organic media, allowing it to interact with a broader spectrum of hydrophilic and lipophilic antioxidant compounds present in the extract, thereby offering a more comprehensive assessment of its antioxidant potential.⁴¹

In the present study, the reducing power of HME-CM, evaluated by the FRAP and CUPRAC methods, confirms its strong electron-donating capacity. These findings are in line with previous reports on *Chrysanthemum* species. For instance, an ethanolic extract of *C. morifolium* leaves exhibited

a FRAP IC₅₀ value of 100.388 µg/ml, indicative of strong reducing activity.⁴² Similarly, a study on *C. indici flos* using an on-line OGE-HPLC-FRAP system identified 18 antioxidant compounds capable of efficiently reducing Fe³⁺ to Fe²⁺.⁴³ Both FRAP and CUPRAC assays are based on single electron transfer (SET) mechanisms, measuring the ability of antioxidants to reduce metal ions (Fe³⁺ to Fe²⁺ in FRAP and Cu²⁺ to Cu⁺ in CUPRAC). High values in these assays reflect the presence of effective electron donors that can neutralize oxidized intermediates generated during lipid peroxidation, thereby acting as primary or secondary antioxidants. Overall, the strong reducing power observed for HME-CM aligns with the antioxidant potential reported in other *Chrysanthemum* extracts, further supporting its relevance as a potent source of natural antioxidants. Although no specific study on *Chrysanthemum* using the CUPRAC assay was identified in this research, the method remains highly relevant for quantifying reducing capacity through electron donation. The CUPRAC method stands out for its simplicity, reagent stability, reproducibility, and the ability to perform measurements at physiological pH. It is suitable for both hydrophilic and lipophilic antioxidants, ensures rapid reactions for most flavonoids, and enables selective oxidation without interference from sugars or citric acid, while also quantifying compounds containing -SH groups.⁴¹

To date, few studies have investigated the anti-inflammatory activity of *Chrysanthemum* extracts using the BSA denaturation assay. However, research on *Matricaria pubescens* (Asteraceae) has demonstrated a dose-dependent and effective inhibition of thermal albumin denaturation, comparable to diclofenac, thereby confirming its anti-inflammatory potential.⁴⁴ The anti-inflammatory effect observed for the HME-CM extract may result from the action of a single active constituent or from the synergistic interaction of multiple secondary metabolites, such as flavonoids and tannins. Notably, some flavonoids exhibit strong inhibitory activity against various enzymes, including protein kinase C, protein tyrosine kinase, and phospholipase A2.⁴⁵

These data suggest that HME-CM exhibits significant *in vivo* anti-inflammatory activity, with an action profile similar to that of diclofenac up to 3 hours. This behavior is consistent with reports on *Chrysanthemum* extracts in other experimental models, notably in zebrafish, where stem and leaf extracts of *C. morifolium* modulated inflammatory processes through their flavonoids, phenolic acids, and polysaccharides.⁴⁶ Other studies have confirmed the *in vivo* anti-inflammatory efficacy of *C. indicum*, likely mediated by the inhibition of COX-2/NO pathways and pro-inflammatory cytokines (TNF-α, IL-1β).⁴⁷ The gradual reduction in inhibitory effect after the 3-hour peak likely results from metabolic degradation of HME-CM's active constituents or their clearance from the target tissue.⁴⁸ Notably, these *in vivo*

observations align with the *in vitro* findings: HME-CM demonstrated significant inhibition of BSA protein denaturation, indicating its capacity to stabilize inflammatory proteins and potentially reduce tissue damage during inflammation.

Conclusion

This study provides the first contribution to the knowledge of the phytochemical composition, HPLC-PDA analysis, and biological potential of *C. macrocarpum* from the Algerian Sahara. The extract exhibited interesting antioxidant and anti-inflammatory activities, probably related to its richness in phenolic acids and flavonoids. These findings provide a scientific basis for the traditional uses of this species and open new perspectives for its pharmacological valorization.

Authors' Contributions

All authors participated equally in the design, data collection, and analysis of the study. All authors critically reviewed the manuscript and approved its final version for publication.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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