Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 08 May 2026
Revised 07 June 2026
Accepted 20 June 2026

Natural Resources for Human Health 2026, Vol. 6 (5): 1010–1024

KEYWORDS:
Oregano, Zaâta, Algeria, plant, Anti-Inflammatory, carrageenan.

Assessment of the Antioxidant, Anti-Inflammatory, and Acute Toxicity Properties of an Aqueous Extract of Origanum vulgare L

Saliha Torche[1*], Lynda Aïnouz[2,3], Selma Benhizia[4], Katiba Beroual[5], Fatma Amira Hani[6], Mohamed Zaouani[2]

[1]Gestion de la Santé et Productions Animales Research Laboratory (GSPA), Institute of Veterinary Sciences, University Constantine 1Frères Mentouri, 25000 Constantine, Algeria.
[2]Laboratory of Food Hygiene and Quality Assurance System (HASAQ), Higher National Veterinary School, Oued-Smar, 16000 Algiers, Algeria.
[3]Laboratory of Biology and Animal Physiology, ENS-Kouba, 16000 Algiers, Algeria.
[4]Animal Pathology Research Laboratory (PADESCA), Institute of Veterinary Sciences, University Constantine 1Frères Mentouri, 25000 Constantine, Algeria.
[5]Laboratory of Biotechnologies related to Animal Reproduction (LBRA), Institute of Veterinary Sciences, Blida University 1, Blida, Algeria.
[6]Research Laboratory Animal Health and Production (SPA), Higher National Veterinary School, Abbes Street, Oued-Smar, 16000 Algiers, Algeria.
* Corresponding author's email: saliha.torche@umc.edu.dz,
ORCID: https://orcid.org/0000-0002-1065-0766

ABSTRACT: Oregano (Origanum vulgare L., "Zaâtar") is among the plants most frequently cited in Algerian traditional medicine, where it is taken almost exclusively as an aqueous decoction. The aim of this study was to characterize chemically the aqueous decoction of Origanum vulgare aerial parts and to determine its antioxidant capacity in vitro, its acute oral toxicity and its anti-inflammatory activity in vivo. The decoction contained 388.15 ± 1.92 µg gallic acid equivalents, 104.81 ± 1.71 µg quercetin equivalents and 53.26 ± 1.71 µg catechin equivalents per mg of dry extract. It scavenged the DPPH and ABTS radicals with IC 50 values of 9.44 ± 0.33 and 6.02 ± 0.02 µg/mL, and reduced cupric and ferric ions with A 0.50 values of 9.50 ± 0.26 and 18.97 ± 0.56 µg/mL. A single oral ose of 2000 mg/kg caused no mortality, no clinical sign and no change in body-weight gain. Given orally at 200 and 400 mg/kg, the decoction reduced carrageenan-induced paw edema at every time point (P < 0.001); repeated-measures analysis of variance showed effects of treatment ($F(3,20) = 482.74$), of time ($F(3,60) = 111.57$) and of their interaction ($F(9,60) = 15.43$), all with P < 0.001. Mean inhibition over 4h reached 52.77 ± 0.77% at 400 mg/kg, a value indistinguishable from diclofenac (55.82 ± 2.03%; P = 0.270), whereas 200 mg/kg was less effective (48.03 ± 0.81%; P = 0.003). The traditional Algerian decoction is therefore innocuous, rich in phenolic compounds and as effective as diclofenac at 400 mg/kg.

1. INTRODUCTION

Oxidative stress and inflammation are two closely coupled processes, and plant phenolics remain of interest because they can act on both at once (Sripoo et al. 2022; Sun et al. 2024). Traditional pharmacopoeias are a productive starting point for identifying such compounds, provided that the preparation studied in the laboratory is the one actually used by patients.

Origanum vulgare L. (Lamiaceae) is an aromatic perennial of Mediterranean origin (Touaitia et al. 2026). In Algeria it is known as "Zaâtar" and ranks among the species most frequently cited in ethnobotanical surveys, where it is used against bronchial complaints, as a digestive and an antiseptic, and for colds and stomach upsets (Gniezdilova et al. 2024; Soltani et al.2025). It is also employed in food, in agriculture and in veterinary practice (Belkacem et al. 2025; Tao et al. 2025). In Algerian households, however, the plant is prepared almost exclusively as an aqueous decoction of the dried flowering aerial parts, and never as an essential oil or an alcoholic extract.

The aerial parts contain essential oils, phenolic acids, flavonoids, tannins, sterols and terpenoids (Pezzani et al. 2017; Moghrovyan et al. 2022), in proportions that vary with chemotype, provenance and harvest date. Results obtained on one population therefore cannot be transferred to another without verification. The extraction procedure matters just as much: Martins et al. (2014) showed that the decoction and the infusion of *Origanum vulgare* L. outperform the hydroalcoholic extract in several antioxidant assays, and that the most active antioxidants of oregano are water soluble.

Despite this, the published pharmacology of *Origanum vulgare* L. rests overwhelmingly on essential oils and organic extracts (Avola et al. 2020; Moghrovyan et al. 2022, 2023), and the few studies devoted to aqueous preparations were carried out *in vitro* only (Kaurinovic et al. 2011; Skotti et al. 2014). No work has yet combined the chemical characterization of an Algerian oregano decoction with an evaluation of its antioxidant capacity, its acute oral toxicity and its anti-inflammatory activity in a whole animal.

The objectives of this study were to determine the qualitative composition through phytochemical screening, to assess acute toxicity, and to evaluate the antioxidant and anti-inflammatory activity in vitro and in vivo, of the aqueous extract prepared from an aqueous decoction of the aerial parts of *Origanum vulgare* L., harvested in the Jijel region in north-eastern Algeria.

2. MATERIALS AND METHODS

2.1. Plant material

Flowering aerial parts of *Origanum vulgare* L. were collected in May 2025 in the municipality of Erraguene, in the south-west of the wilaya of Jijel, which is situated in north-eastern Algeria (Fig. 1). The specimen was authenticated at the Laboratory of Toxicology and Pharmacology, Institute of Veterinary Sciences, University Constantine 1 Frères Mentouri, where a voucher specimen was deposited. The material was washed to remove impurities, then air-dried at room temperature in the darkto preserve their phytochemical integrity. After drying, they were ground into a fine powder and stored in sealed containers.

2.2 Dry extract preparation

The flowering aerial parts of *Origanum vulgare* L. decoction was prepared by boiling 100 g of powder in 1000 mL of sterile distilled water for 15 min (Bamba et al. 2021), then cooled, filtered through Whatman No. 1 paper, and evaporated to dryness in an oven at 40 °C. The extraction yield was 6 % (w/w).Water was the only extraction solvent used and the resulting aqueous extract of aerial parts of *Origanum vulgare* L. (ADOV) was stored in airtight containers at 4 °C until further analysis.

2.3 Chemical reagents

The chemicals and reagents used in this study were of high analytical quality and were from Sigma (St. Louis, MO), Merk (Mannheim, Germany), Biochemical (Germany) and (Troikaa Pharmaceuticals, India). Biochemical parameters were measured by using standard and colorimetric commercial kits (SPINREACT, Sant Esteve De Bas (GI). SPAIN), following the manufacturer's protocols.

2.4Phytochemical screening

Preliminary phytochemical screening of the plant extract was carried out using standard qualitative methods in order to identify the presence of major secondary metabolites. Phenols, alkaloids, flavonoids, tannins, terpenoids, sterols, coumarins, quinones, saponins and anthocyanins were screened by standard qualitative procedures were based on characteristic color changes and precipitation reactions specific to each class of bioactive compounds (Trease and Evans 2002).

2.5. Phenolics quantification

Total phenolic content was determined in a 96-well microplate by the Folin–Ciocalteu method (Bakhouche et al. 2021): 20 µL of extract (1 mg/mL) were mixed with 100 µL of Folin–Ciocalteu reagent (1:10) and 75 µL of sodium carbonate (7.5 %,

w/v), and the absorbance was read at 765 nm after 2 h in the dark. Results are expressed as µg gallic acid equivalents (GAE) per mg of dry extract, from a gallic acid curve ($y = 0.006x + 0.182$; $R^2 = 0.993$). Flavonoids were measured according to Topcu et al. (2007), with aluminium nitrate (10 %, w/v) and potassium acetate (1 M), reading at 430 nm, and are expressed as µg quercetin equivalents (QE) per mg ($y = 0.010x - 0.002$; $R^2 = 0.999$). Condensed tannins were quantified by the vanillin–HCl method (Saci et al. 2020), reading at 500 nm, and are expressed as µg catechin equivalents (CE) per mg, from a catechin curve (0–300 µg/mL; $R^2 = 0.999$). All determinations were performed in triplicate.

2.6.Antioxidant assays

DPPH radical scavenging was assessed by mixing 160 µL of DPPH reagent (0.1 mM) with 40 µL of extract (1.5625–100 µg/mL) and reading at 517 nm after 30 min (Bakhouche et al. 2021); BHA and BHT served as standards. The $ABTS^{\bullet+}$ cation was generated with potassium persulfate, diluted to an absorbance of 0.7 at 734 nm, and 160 µL were added to 40 µL of sample; the absorbance was read at 734 nm after 10 min (Re et al. 1999), with Trolox and ascorbic acid as standards. Both assays are expressed as IC_{50}, the concentration scavenging 50 % of the radical. Cupric ion reducing antioxidant capacity was measured with $CuCl_2$ (10 mM), neocuproine (7.5 mM) and ammonium acetate (1 M), reading at 450 nm after 40 min (Apak et al. 2004), with BHA and BHT as standards. Ferric reducing power was determined with potassium ferricyanide and ferric chloride, reading at 700 nm (Saci et al. 2020), with ascorbic acid and α-tocopherol as standards. Both reducing assays are expressed as $A_{0.50}$, the concentration giving an absorbance of 0.50; a lower $A_{0.50}$ denotes a stronger reducing capacity. Organic solvents were used only as analytical reagents.

2.6.1.In vitro Anti-inflammatory activity(Inhibition of albumin denaturation)

The method given by Govindappa et al. (2011) with slight modification was used. The reaction mixture contained 500 µl of 1% aqueous solution of bovine serum albumin fraction prepared in phosphate buffer saline of pH 6.4 and extract at concentrations 100, 200 and 400µg/mL. The reaction mixture was incubated at 37°C for 20 min and then heated to 50°C for 20 min, reaction mixtures were cooled at room temperature and turbidity was measured spectrophotometrically at 660 nm. Diclofenac was used as the comparative standard (Pungle et al, 2018). The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

% Inhibition= [(Abs control- Abs sample) / Abs control] ×100

Where, Abs control is the absorbance of the bovine serum albumin fraction, Abs sample is the absorbance of bovine serum albumin fraction with extract/standard (Govindappa et al., 2011)

2.8. Animals and housing

Male and female mice were obtained from the animal breeding division of Pasteur Institute (IPA) of Kouba, (Algiers, Algeria). The mice were housed in an animal facility (ENSV Oued Smar, Algiers) in separate cages under controlled conditions of humidity, lighting, and temperature (50 ± 10% RH, 12-h light/dark, 23 ± 2 °C). They were given water and standard diet *ad libitum* throughout the experiment. All in vivo experiments commenced after a 7-day adaptation period and were carried out in compliance with the institutional guidelines for animal care as well as the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) following approval by the local Ethical Committee of the Houari Boumediene University of Sciences and Technology (USTHB), Algeria (approval number 45/DGLPAG/DVA.SDA.14).

2.9Acute oral toxicity

Acute oral toxicity was assessed following OECD guideline 423 (OECD 2001). Mice were allocated to two groups of three. The treated group received a single oral dose of 2000 mg/kg body weight of ADOV by gavage; the control group received distilled water. The animals were observed continuously for 1 h, intermittently over the following 4 h and daily for 14 days, for mortality, behavioral changes and signs of toxicity. Body weight was recorded on days 0, 7 and 14.

2.10.In vivo Anti-inflammatory activity(Carrageenan-induced paw edema)

Mice were assigned to four groups of six. ADOV (200 or 400 mg/kg) was given orally and diclofenac (10 mg/kg) subcutaneously 1 h before the induction of inflammation; control animals received distilled water. Edema was induced by injecting 0.1 mL of carrageenan (1 %, w/v, in saline) into the sub-plantar tissue of the right hind paw (Moghrovyan et al. 2023). Paw thickness was measured with a digital vernier caliper 1, 2, 3 and 4 h after the injection and is expressed in mm.

Inhibition of edema was calculated for each animal as $[(E_c - E_t) / E_c] \times 100$, where E_c is the mean paw thickness of the control group and E_t that of the treated animal at the corresponding time.

2.7 Statistical analysis

In vitro assays were run in triplicate (n = 3); the toxicity study included three mice per group and the anti-inflammatory experiment six mice per group. Normality and homogeneity of variances were verified with the Shapiro–Wilk and Levene tests. IC_{50} and $A_{0.50}$ values of the extract and of the standards were compared by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. Body weights were compared at each time point by Student's t test with Welch's correction, and body-weight gain by two-way repeated-measures ANOVA (group × time). Paw edema was analyzed by a two-way repeated-measures ANOVA (treatment × time); sphericity was checked with Mauchly's test and the Greenhouse–Geisser correction applied when it was violated, followed by Tukey's HSD test at each time point. Effect sizes are given as partial eta squared (η_p^2). The level of significance was set at $P < 0.05$. Computations were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1 Phytochemical screening and quantification of phenolics

The phytochemical screening of ADOV revealed phenols, flavonoids, tannins, terpenoids, sterols, coumarins and quinones, whereas saponins, anthocyanins and alkaloids were not detected (Table 1). The decoction was rich in phenolic compounds and flavonoids, and also contained an appreciable amount of condensed tannins; the three determinations were highly reproducible, with coefficients of variation of 0.50, 1.63 and 3.21% respectively (Table 2).

3.2 Antioxidant assays

In the four antioxidant assays the response was concentration-dependent. ADOV scavenged the DPPH radical (fig.2; Tab. 3)with an IC_{50} of 9.44 ± 0.33 µg/mL, a value significantly lower than that of BHT (22.32 ± 0.10 µg/mL; $P < 0.001$) but higher than that of BHA (5.78 ± 0.12 µg/mL; $P < 0.001$). In the ABTS assay (fig.3; Tab. 3),the IC_{50} was 6.02 ± 0.02 µg/mL, approximately twice the values of Trolox and ascorbic acid ($P < 0.001$ in both cases), which did not differ from each other ($P = 0.453$). The cupric ion reducing capacity (fig. 4; Tab. 3)of ADOV ($A_{0.50}$ = 9.50 ± 0.26 µg/mL) did not differ from that of BHT (9.62 ± 0.19 µg/mL; $P = 0.747$), and its ferric reducing power ($A_{0.50}$ = 18.97 ± 0.56 µg/mL) was stronger than that of α-tocopherol ($P < 0.001$) but weaker than that of ascorbic acid ($P < 0.001$) (fig.5; Tab. 3).

3.3 Acute oral toxicity

A single oral dose of 2000 mg/kg of ADOV caused no mortality, no behavioral or autonomic change and no visible pathological alteration during the 14-day observation period, so the LD_{50} exceeds 2000 mg/kg. The two groups differed slightly in body weight on day 0, and body-weight gain was therefore taken as the endpoint of toxicological relevance: it was virtually identical in treated and control animals over the 14 days ($P = 0.960$), and the repeated-measures ANOVA showed no group × time interaction ($F(2,8) = 1.35$; $P = 0.312$) (Table 4).

3.4 Anti-inflammatory activities

3.4.1. In vitro inhibition of albumin denaturation

ADOV was tested for anti-inflammatory activity using an in vitro method. The test was carried out in comparison with diclofenac sodium as the reference standard. The results showed acceptable anti-inflammatory activity (table5).

The in vitro anti-inflammatory activity of the ADOV extract and the reference drug (diclofenac) was assessed at concentrations of 100, 200 and 400 µg/mL (Table 5). Diclofenac exhibited strong dose-dependent inhibition, ranging from 81.61 ± 1.79 per cent at 100 µg/mL to 91.95 ± 0.89 per cent at 400 µg/mL. ADOV exhibited moderate anti-inflammatory activity at 100 µg/mL (43.22 ± 0.18 per cent) and at 200 µg/mL (42.76 ± 0.31 per cent), which increased significantly to 58.85 ± 1.53 per cent at 400 µg/mL. A one-way ANOVA revealed that the inhibitory effect of diclofenac was significantly higher than that of the extract at all concentrations ($p < 0.001$).

3.4.2 In vivo Carrageenan-induced paw edema

Sub-plantar carrageenan produced an edema that remained between 6.13 ± 0.08 and 6.28 ± 0.06 mm over the 4 h of observation in control mice. Both doses of ADOV and diclofenac reduced this edema (Table 6, Fig.6). The two-way repeated-measures ANOVA revealed an effect of treatment ($F(3,20) = 482.74$; $P < 0.001$; $\eta_p^2 = 0.986$) and of time ($F(3,60) = 111.57$; $P < 0.001$; $\eta_p^2 = 0.848$; Greenhouse–Geisser corrected, $\varepsilon = 0.509$; Mauchly's $W = 0.206$, $P < 0.001$), together with a treatment × time interaction ($F(9,60) = 15.43$; $P < 0.001$; $\eta_p^2 = 0.698$). At every time point both doses differed from the control ($P < 0.001$). Inhibition of the edema rose with time and peaked at the fourth hour, at 66.00 ± 0.83% for 200 mg/kg and 69.90 ± 0.71% for 400 mg/kg (Table 7). Averaged over the whole period, 400 mg/kg inhibited the edema by 52.77 ± 0.77%, a value indistinguishable from diclofenac (55.82 ± 2.03%; $P = 0.270$), whereas 200 mg/kg was less effective (48.03 ± 0.81%; $P = 0.003$). The 400 mg/kg dose could not be distinguished from diclofenac at any of the four time points ($P \geq 0.450$), while 200 mg/kg fell below the reference drug at the third hour ($P < 0.001$).

4. DISCUSSION

The phenolic content measured in the ADOV extract (388.15 ± 1.92 µg GAE/mg of dry extract) is high for an aqueous preparation. Expressed in the same units, it is close to twice the 207.64 ± 0.69 µg GAE/mg reported by de Torre et al. (2022) for an ethanolic extract of *Origanum vulgare* L. from Navarra, Spain, and clearly above the values obtained by Parra et al. (2021) for a Chilean Andean population. Moreover, water extracts the phenolics of this species efficiently, in agreement with Martins et al.(2014), who found that the decoction outperforms hydroalcoholic extraction and that the most active antioxidants of oregano are water soluble. The condensed tannin content deserves mention because tannins are rarely quantified in oregano and almost never in aqueous preparations, and the absence of alkaloids, saponins and anthocyanins allows the activities reported here to be attributed to the phenolic fraction.

The DPPH IC50 value for ADOV indicates that the dry extract obtained by decoction of the plant under study possesses very potent antioxidant properties. Its efficacy is 2.4 times greater than that of BHT ($p < 0.001$); however, it remains less potent than BHA. The ABTS test revealed that ADOV's antioxidant capacity is approximately half that of Trolox and ascorbic acid. Studies describing preparations of raw oregano as comparable to Trolox must be interpreted whilst taking this difference in potency into account. Metal reduction tests provide further insight into the mechanism of action: in the CUPRAC test, ADOV and BHT were indistinguishable ($p = 0.747$), whilst in the FRAP test, ADOV proved more effective than α-tocopherol. The reduction of cupric ions is facilitated by ortho-dihydroxy (catechol) groups, which are precisely present in rosmarinic and caffeic acids, the major phenolic compounds of *Origanum vulgare* L. (De Torre et al. 2022). The strong CUPRAC response therefore indicates a composition rich in catechol, which is consistent with the observation by Tusevski et al. (2014) that, amongst twenty-seven medicinal plants, *Origanum vulgare* L. exhibited the highest copper-reducing capacity, and with the ranking of oregano amongst the most active aqueous extracts tested by Skotti et al. (2014).The ADOV dry extract is therefore a potent free radical scavenger, but is not equivalent to a pure reference antioxidant. These results thus establish the decoction of oregano leaves, frequently used in Algeria, as the most appropriate means of achieving antioxidant effects.

No deaths, clinical signs or changes in body weight were observed following administration of a single oral dose of 2,000 mg/kg, indicating that ADOV is non-toxic at this dose. This result is reassuring, although predictable for a preparation that has been traditionally consumed for centuries; its practical significance lies in the fact that it places effective anti-inflammatory doses, which are five to ten times lower, within a comfortable safety margin for patients.

The assessment of anti-inflammatory activity in vitro using the BSA (bovine serum albumin) denaturation method is based on a substance's ability to prevent changes in protein structure caused by heat. In this study, we tested the anti-inflammatory effect of ADOV in comparison with diclofenac sodium, used as a reference, at concentrations of 100, 200 and 400 µg/mL. The results showed acceptable anti-inflammatory activity. Indeed, diclofenac exhibited strong dose-dependent inhibition, ranging from 81.61 ± 1.79 per cent at 100 µg/mL to 91.95 ± 0.89 per cent at 400 µg/mL. In contrast, ADOV exhibited moderate anti-inflammatory activity at 100 µg/mL (43.22 ± 0.18 per cent) and at 200 µg/mL (42.76 ± 0.31 per cent), which increased significantly to 58.85 ± 1.53 per cent at 400 µg/mL. The bovine serum albumin (BSA) denaturation assay is a widely established in vitro model for screening anti-inflammatory agents based on their capacity to protect proteins from heat-induced structural damage (Belahcene et al. 2023). Non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac

sodium are frequently employed as positive reference standards in many assays due to their potent, dose-dependent inhibition profile (Tabassum et al. 2023).When comparing the anti-inflammatory efficacy of ADOV to botanical extracts from *Origanum vulgare* L.(oregano) evaluated using the bovine serum albumin (BSA) denaturation assay, similar moderate to high protective trends emerge. Studies assessing *Origanum vulgare* L. essential oils and aqueous by products (such as hydrolates) demonstrate notable protein stabilizing capacity, often attributing their performance to bioactive phenolic constituents like carvacrol, thymol, and beta-caryophyllene (Moghrovyan et al.2022; Naccari et al. 2025).

Carrageenan-induced paw edema is a biphasic response: the early phase (0–2 h) is driven by histamine and serotonin, a plateau around the third hour is maintained by kinin-like substances, and the late phase is sustained by prostaglandins released after cyclooxygenase induction (Cadirci et al. 2012; Zadeh-Ardabili and Rad 2019; Kulshreshtha et al.2025).The observed temporal profile is consistent with the biphasic nature of carrageenan-induced inflammation. At 1 and 2 hours, no significant difference was observed between the two doses of ADOV administered and of diclofenac, nor between the two doses themselves. A dose-dependent effect only became apparent from the third hour onwards. This corresponds to the prostaglandin-dependent phase of the inflammatory response. Indeed, at a dose of 200 mg/kg, the results obtained with ADOV showed a significantly ($p < 0.001$) lower inhibitory effect than those obtained with diclofenac. By contrast, at a dose of 400 mg/kg, no significant difference ($p = 0.474$) was observed between the response obtained with diclofenac and that obtained with ADOV. At the fourth hour, the results obtained with ADOV at a dose of 400 mg/kg induced a slightly greater inhibition of paw oedema than that of diclofenac. These results indicate that ADOV may exert its anti-inflammatory effect primarily during the late phase of carrageenan-induced inflammation, which is largely associated with prostaglandin production, thus suggesting a synergistic effect. This interpretation is consistent with the known constituents of oregano: flavonoids inhibit prostaglandin production and target the late phase of acute inflammation (Mansour et al. 2014), carvacrol reduces oedema and gastric lesions in rodents (Silva et al. 2012), and monoterpenes suppress nitric oxide formation in the same model (Peana et al. 2006).

The results obtained highlight a correlation between in vitro and in vivo anti-inflammatory activity. Indeed, a strong correlation was observed between the in vitro inhibition of BSA denaturation and the in vivo inhibition of carrageenan-induced paw oedema at the 3-hour mark. These results suggest that the in vivo anti-inflammatory activity of the extract is based, at least in part, on the stabilisation of membrane proteins and theprevention of the release of prostaglandins, kinins, and lysosomal enzymes, along with neutrophil infiltration (Bekkouch et al. 2023). Plant-derived anti-inflammatory agents (polyphenols and flavonoids) bind to the heat-sensitive sites of bovine serum albumin (BSA). This binding stabilises the tertiary structure, thereby preventing denaturation and coagulation induced by heat, chemicals or stress (Benmohamed et al. 2023).Although the standard drug Diclofenac consistently showed superior efficacy due to its pure synthetic nature and high specificity, the plant extract demonstrated a concentration-dependent inhibitory response, surpassing 58 % inhibition at 400 µg /mL. This moderate-to-strong activity at higher concentrations suggests the presence of bioactive secondary metabolites (such as polyphenols, flavonoids, or terpenes) capable of stabilizing proteins or inhibiting inflammatory cascades.

The point of clinical interest is a different one. The decoction studied here contains no essential oil, and yet it reproduces the effect obtained with oregano essential oils and hydro-ethanolic extracts (Avola et al. 2020; Moghrovyan et al. 2022, 2023) and with the aqueous oregano formulation of Sripoo et al. (2022). The water-soluble phenolic fraction is therefore sufficient on its own, and the volatile terpenes usually credited with the activity are not indispensable, an argument in favour of the traditional preparation, which is also the safest and the least expensive. The dose–response relationship deserves emphasis, because it distinguishes a pharmacological effect from a non-specific one: doubling the dose raised the mean inhibition from 48.03 to 52.77 % and, more tellingly, abolished the gap with diclofenac at the third and fourth hours. A single-dose design could not have made that distinction.

Three limitations must be taken into account. The assessment of acute toxicity follows the limit test protocol set out in OECD Guideline 423 and is therefore based on a limited number of animals in order to comply with animal welfare standards. It would be useful to supplement this toxicological study with haematological tests. Antioxidant capacity was measured exclusively in acellular chemical systems, which reflect the ability to donate electrons and hydrogen, but not bioavailability. Finally, the individual phenolic compounds in the decoction were not identified. This study therefore constitutes a necessary step in the preclinical phase, but one that should be supplemented before any potential therapeutic use.

5. CONCLUSION

In conclusion, the aqueous extract obtained by decoction of the aerial parts of *Origanum vulgare L.* harvested in the Erraguen region of Jijel, a preparation traditionally used in Algerian medicine to reduce inflammation, is rich in phenolic compounds, flavonoids and condensed tannins. It's copper (I) ion-reducing activity is comparable to that of BHT, and its DPPH scavenging activity is 2.4 times higher. This extract shows no acute toxicity up to 2,000 mg/kg and inhibits both BSA denaturation and carrageenan-induced paw oedema in a dose-dependent manner. These anti-inflammatory effects are comparable to those of diclofenac sodium, particularly at a dose of 400 mg/kg. These results provide the first experimental evidence of the efficacy of the traditional Algerian use of oregano decoction and demonstrate that the water-soluble phenolic fraction alone reproduces the anti-inflammatory effect generally attributed to the essential oil. The chromatographic identification of the individual phenolic compounds in this fraction and the elucidation of their action on the cyclooxygenase pathway are the priority areas for future research.

Acknowledgments: The authors thank the Institute of Veterinary Sciences of Constantine, the Higher National Veterinary School of Algiers and the Biotechnology Research Centre (CRBT) for providing the laboratory facilities used in this work.

Abbreviations: ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, ADOV: aqueous decoction of the dried oregano flowering aerial parts, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, b.wt: body weight, CUPRAC: Cupric-reducing antioxidant capacity, DPPH: 1,1-Diphenyl,2 picrylhydrazyl; IC50: Half-maximal inhibitory concentration

Conflict of interest: The authors declare that they have no personal, commercial, governmental or technological relationship that could have influenced the objectivity of this research. This work received no specific financial support.

Author contributions: S.T., M.Z. and K.B. conceived and designed the study; L.A. and M.Z. carried out the acute toxicity and anti-inflammatory experiments; S.T. and S.Be. performed the phytochemical screening and the antioxidant assays; S.Bo. was responsible for data curation and statistical analysis; S.T. wrote the original draft; all the authors participated in the critical revision and approved the final version of the manuscript.

Funding: The authors received no specific funding for this work.

Ethical considerations: All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14). Animals were allocated to the experimental groups at random, and paw measurements were performed by an operator unaware of the treatment allocation.

Data availability: The original data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Apak R, Güçlü K, Özyürek M, Karademir SE (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agric. Food Chem. 52: 7970-7981. https://doi.org/10.1021/jf048741x

[2] Avola R, Granata G, Geraci C, Napoli E, Graziano ACE, Cardile V(2020). Oregano (Origanumvulgare L.) essential oil provides anti-inflammatory activity and facilitates wound healing in a human keratinocytes cell model. Food Chem. Toxicol.144: 111586. https://doi.org/10.1016/j.fct.2020.111586

[3] Bakhouche I, Aliat T, Boubellouta T, Gali L, Şen A, Bellik Y. Phenolic contents and in vitro antioxidant, anti-tyrosinase and anti-inflammatory effects of leaves and roots extracts of the halophyte Limoniumdelicatulum. S. Afr. J. Bot.2021; 139: 42-49. https://doi.org/10.1016/j.sajb.2021.01.030

[4] Bekkouch O, Zengin G, Harnafi M, Touiss I, Khoulati A, Saalaoui E, Amrani S (2023). Anti-inflammatory study and phytochemical characterization of Zingiberofficinale Roscoe and Citrus limon L. juices and their formulation. ACS omega. 8(30), 26715-26724.https://pubs.acs.org/10.1021/acsomega.2c04263

[5] Bamba B, Benie CKD, Ouattara A, Doukourou DN, Kamou RK, Ouattara K. Teneurs en phénols totaux, activités antioxydantes des macérés et décocté des feuilles de Uvariachamae P. Beauv. (Annonaceae) (2021). Int. J. Biol. Chem. Sci. 15: 54-67. https://doi.org/10.4314/ijbcs.v15i1.6

[6] Belahcene S, Kebsa W, Omoboyowa DA, Alshihri AA, Alelyani M, Bakkour Y, Leghouchi E (2023). Unveiling the chemical profiling antioxidant and anti-inflammatory activities of Algerian Myrtus communis L. essential oils, and exploring molecular docking to predict the inhibitory compounds against cyclooxygenase-2. Pharmaceuticals. 16(10), 1343.https://doi.org/10.3390/ph16101343

[7] Benmohamed M, Guenane H, Messaoudi M, Zahnit W, Egbuna C, Sharifi-Rad, M.,Yousfi M (2023). Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of Pistaciaatlantica. Molecules. 28(1), 349.https://doi.org/10.3390/molecules28010349

[8] Belkacem M, Bouguerra A, Benmahioul B (2025). Bioactivity of Thymus algeriensis and Origanum essential oils: applications in eco-friendly pest management and veterinary medicine. North-West. J. Zool.; 21: 45-53. https://doi.org/10.1080/0972060x.2025.2582627

[9] Cadirci E, Süleyman H, Gürbüz P, Uz A, Güvenalp Z, Demirezer LÖ (2012). Anti-inflammatory effects of different extracts from three Salvia species. Turk. J. Biol. 36: 59-64. https://doi.org/10.3906/biy-1001-17

[10] De Torre MP, Cavero RY, Calvo MI(2022).Anticholinesterase activity of selected medicinal plants from Navarra region of Spain and a detailed phytochemical investigation of Origanum vulgare L. ssp. vulgare. Molecules. 27: 7100. https://doi.org/10.3390/molecules27207100

[11] Gniezdilova V, Mylenka M, Kozak I. Origanum vulgare L. (2024). Botanical features, use in folk medicine, and significance in the cultures of other countries. J. VasylStefanykPrecarpath. Natl. Univ. Biol. 11: 195-202. https://doi.org/10.15330/jpnubio.11.195-202

[12] Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP (2011). Antimicrobial, antioxidant and in vitro antiinflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L.). Journal of Pharmacognosy and Phytotherapy 3(3):43-51. t http://www.academicjournals.org/JMPR

[13] Kaurinovic B, Popovic M, Vlaisavljevic S, Trivic S. (2011).Antioxidant capacity of Ocimumbasilicum L. and Origanumvulgare L. extracts. Molecules. 16: 7401-7414. https://doi.org/10.3390/molecules16097401

[14] Mansour S, Djebli N, Ozkan EE, Mat A. (2014). In vivo anti-inflammatory activity and chemical composition of Hypericum scabroides. Asian Pac. J. Trop. Med. 7 (Suppl 1): S514-S520. https://doi.org/10.1016/s1995-7645(14)60283-2

[15] Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira ICFR (2014). Decoction, infusion and hydroalcoholic extract of Origanum vulgare L.: different performances regarding bioactivity and phenolic compounds. Food Chem.158: 73-80. https://doi.org/10.1016/j.foodchem.2014.02.099

[16] Moghrovyan A, Parseghyan L, Sevoyan G, Darbinyan A, Sahakyan N, Gaboyan M, Karabekian Z, Voskanyan A. (2022). Antinociceptive, anti-inflammatory and cytotoxic properties of Origanumvulgare essential oil, rich with beta-caryophyllene and beta-caryophyllene oxide. *Korean J. Pain* 35: 140-151. https://doi.org/10.3344/kjp.2022.35.2.140

[17] Moghrovyan A, Ginovyan M, Avtandilyan N, Parseghyan L, Voskanyan A, Sahakyan N, Darbinyan A (2023). The possible anti-inflammatory properties of hydro-ethanolic extract of oregano. Funct. Foods Health Dis. 13: 476-486. https://doi.org/10.31989/ffhd.v13i10.1211

[18] Naccari C, Ginestra G, Micale N, Palma E, Galletta B., Costa R, Cristani M. (2025). Binary combinations of essential oils: Antibacterial activity against Staphylococcus aureus, and antioxidant and anti-inflammatory properties. Molecules, 30(3), 438. https://doi.org/10.3390/molecules30030438

[19] OECD. Guidance document on acute oral toxicity testing. Organisation for Economic Co-operation and Development, Paris. 2001. https://www.oecd.org/chemicalsafety/testing/

[20] Parra C, Muñoz P, Bustos L, Parra F, Simirgiotis MJ, Escobar H. UHPLC-DAD characterization of Origanum vulgare L (2021). from Atacama Desert Andean region and antioxidant, antibacterial and enzyme inhibition activities. Molecules. 26: 2100. https://doi.org/10.3390/molecules26072100

[21] Peana AT, Marzocco S, Popolo A, Pinto A.(2006) (-)-Linalool inhibits in vitro NO formation: probable involvement in the antinociceptive activity of this monoterpene compound. Life Sci. 78: 719-723.Https//doi.org/10.1016/j.lfs.2005.05.065

[22] Pungle R, Tambe A, More A, Kharat A. (2018) Anti-inflammatory and antioxidant potentiality of Solanum xanthocarpum. African Journal of Biotechnology. 37:1188-1195 https://doi.org/10.5897/AJB2018.16560

[23] Pezzani R, Vitalini S, Iriti M. (2017). Bioactivities of Origanum vulgare L.: an update. Phytochem. Rev. 16: 1253-1268. https://doi.org/10.1007/s11101-017-9535-

[24] Kulshreshtha, S, Tandalekar, Y B, Goyal, A, Srivastava, A K, Jachak S. (2025). Selected Indian Medicinal Plants Exhibit Anti-Inflammatory Activity by Modulating Pro-Inflammatory Cytokines In Vitro and in Carrageenan-Induced Rat Paw Edema." Chemistry & Biodiversity 22, no. 11: e03382. https://doi.org/10.1002/cbdv.202403382

[25] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C.(1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

[26] Saci F, Bachirbey M, Louaileche H, Gali L, Bensouici C. (2020).Changes in anticholinesterase, antioxidant activities and related bioactive compounds of carob pulp (Ceratonia siliqua L.) during ripening stages. *J. Food Meas. Charact.*14: 937-945. https://doi.org/10.1007/s11694-019-00344-9

[27] Soltani Y, Benyamina A, Toumi M N, Mansouri A, Bouzidi MA, Toumi F (2025). Medicinal plants used in the treatment of asthma in western Algeria: an ethnobotanical survey in Sidi Bel Abbès, Oran, and Mascara. Ethnobotany Research and Applications, 32. https://doi.org/10.32859/era.32.18.1-21

[28] Silva FV, Guimarães AG, Silva ERS, Sousa-Neto BP, Machado FDF, Quintans-Júnior LJ, Arcanjo DDR, Oliveira FA, Oliveira RCM.(2012). Anti-inflammatory and anti-ulcer activities of carvacrol, a monoterpene present in the essential oil of oregano. J. Med. Food . 15: 984-991. https://doi.org/10.1089/jmf.2012.0102

[29] Skotti E, Anastasaki E, Kanellou G, Polissiou M, Tarantilis PA.(2014). Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. Ind. Crops Prod. 53: 46-54. https://doi.org/10.1016/j.indcrop.2013.12.013

[30] Sripoo AR, Maheswari UTN, Rajeshkumar S. (2022).Preparation of oregano, coffee and black cumin aqueous formulation and its anti-inflammatory activity. Int. J. Health Sci. 6 (S3): 288-295. https://doi.org/10.53730/ijhs.v6ns3.5184

[31] Sun S, Liu Z, Lin M, Gao N, Wang X.(2024). Polyphenols in health and food processing: antibacterial, anti-inflammatory and antioxidant insights. Front. Nutr. 11: 1456730. https://doi.org/10.3389/fnut.2024.1456730

[32] Tabassum S, Ahmad S, Ali B, Usman F, Jabeen Q, Sajid-ur-Rehman M, Basit A. (2023). Chemical profiling and evaluation of toxicological, antioxidant, anti-inflammatory, anti-nociceptive and tyrosinase inhibitory potential of Portulacariaafra using in-vitro, in-vivo and in-silico studies. Arabian Journal of Chemistry, *16*(6), 104784.https://doi.org/10.1016/j.arabjc.2023.104784

[33] Tao L, Liang Y, Xia Z, Wang X, Wang X, Chao Z, Guo J.(2025). Antibacterial activities of oregano essential oils and their active components. Front. Pharmacol. 16: 1579283. https://doi.org/10.3389/fphar.2025.1579283

[34] Topcu G, Ay M, Bilici A, Sarikurkcu C, Ozturk M, Ulubelen A. (2007).A new flavone from antioxidant extracts of Pistacia terebinthus. Food Chem. 103: 816-822. https://doi.org/10.1016/j.foodchem.2006.09.028

[35] Touaitia R, Gheraibia S, Boufahja F, Jouini A Z, Khezami L, Idres T. (2026). Phytochemical profiling, antioxidant, and broad-spectrum antimicrobial activities of Origanumvulgare essential oil with molecular docking and ADME insights. ACS omega. 11(8), 13629-13640. https://doi.org/10.1021/acsomega.5c10975

[36] Trease GE, Evans WC.(2002). Pharmacognosy. 15th ed. London: W.B. Saunders.

[37] Tusevski O, Kostovska A, Iloska A, Trajkovska L, GadzovskaSimic S.(2014). Phenolic production and antioxidant properties of some Macedonian medicinal plants. Cent. Eur. J. Biol. 9: 888-900. https://doi.org/10.2478/s11535-014-0322-1

[38] Zadeh-Ardabili PM, Rad SK.(2019). Anti-pain and anti-inflammation like effects of Neptune krill oil and fish oil against carrageenan induced inflammation in mice models: current status and pilot study. Biotechnol. Rep. 22: e00341. https://doi.org/10.1016/j.btre.2019.e00341

Table 1. Phytochemical screening of the aqueous decoction of ADOV.

Chemical class	Result's
Phenols	+
Flavonoids	+
Tannins	+

Chemical class	Result's
Terpenoids	+
Sterols	+
Coumarins	+
Quinones	+
Saponins	−
Alkaloids	−
Anthocyanins	−
+ : detected	− *: not detected*

.

Table 2. Total phenolic, flavonoid and condensed tannin contents of ADOV.

	Total phenolics (µg GAE/mg)	Flavonoids (µg QE/mg)	Condensed tannins (µg CE/mg)
ADOV	388.15 ± 1.92	104.81 ± 1.71	53.26 ± 1.71

Means ± standard deviation of three determinations. GAE, gallic acid equivalents; QE, quercetin equivalents; CE, catechin equivalents.

Table 3. Antioxidant capacity of ADOV and of the reference standards.

Tested product	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	CUPRAC A0.50 (µg/mL)	FRAP A0.50 (µg/mL)
ADOV	9.44 ± 0.33 a	6.02 ± 0.02 a	9.50 ± 0.26 a	18.97 ± 0.56 a
BHA	5.78 ± 0.12 b	*NT*	3.64 ± 0.01 b	*NT*
BHT	22.32 ± 0.10 c	*NT*	9.62 ± 0.19 a	*NT*
Trolox	*NT*	3.23 ± 0.15 b	*NT*	*NT*
Ascorbicacid	*NT*	3.13 ± 0.06 b	*NT*	6.77 ± 0.25 b
α-Tocopherol	*NT*	*NT*	*NT*	34.93 ± 0.11 c

Means ± standard deviation of three measurements. Within a column, values without a common superscript letter differ (ANOVA followed by Tukey's HSD test, P < 0.05). ANOVA: DPPH, F(2,6) = 5261.3; ABTS, F(2,6) = 947.6; CUPRAC, F(2,6) = 1011.1; FRAP, F(2,6) = 4619.7; all P < 0.001. nt, not tested. A lower value denotes a stronger activity.

Natural Resources for Human Health
eISSN: 2583-1194

Table 4. Body weight and body-weight gain of mice given a single oral dose of 2000 mg/kg of ADOV.

	Controlgroup	ADOV group at 2000 mg/kg	*P* value
Period	**Body weight (g)**		
Day 0	20.38 ± 0.08	22.17 ± 1.03	0.225
Day 7	23.53 ± 0.67	24.40 ± 0.72	0.430
Day 14	24.43 ± 0.23	26.27 ± 0.35	0.017
Period	**Body-weight gain (g)**		
Day 0 – Day 7	3.15 ± 0.60	2.23 ± 0.32	0.267
Day 0 – Day 14	4.05 ± 0.16	4.09 ± 0.69	0.960

Means ± standard error of the mean (n = 3 per group). Groups were compared at each time point by Student's t test with Welch's correction; exact p values are given. The higher body weight of the treated group on day 14 reflects the difference already present on day 0.

Table5: Inhibition percentage of albumin denaturation (%)

	Inhibition % at 100µg /mL	Inhibition % at 200 µg /mL	Inhibition % at 400µg /mL
Diclofenac	81,61± 1,79	85,06± 2,36	91,95±0,89
ADOV	43,22± 0,18	42,76± 0,31	58,85±1,53
***P* value ADOV Vs Diclofenac**	0,0046 (***)	0,0039 (***)	0,00030(***)

*Data are expressed as Means ± standard error of the mean (n = 3). *p ≤ 0,05 significant; **, ≤ 0,01 very significant; ***, ≤ 0,001 highly significant, ADOV versus diclofenac at different concentration).*

Table 6. Paw edema after sub-plantar carrageenan injection in mice [Mice PESPCarInj, (mm)]pre-treated with diclofenac and ADOV at different concentration.

Treatment	Mice PESPCarInj (mm) at 1 h	Mice PESPCarInj (mm) at 2 h	Mice PESPCarInj (mm) at 3 h	Mice PESPCarInj (mm) at 4 h
Water	6.13 ± 0.08	6.21 ± 0.16	6.24 ± 0.07	6.28 ± 0.06
Diclofenac10 mg/kg	3.93 ± 0.24*	2.86 ± 0.23*	2.23 ± 0.09*	1.95 ± 0.07*
ADOV 200mg/kg	4.34 ± 0.23*	3.49 ± 0.16*	2.92 ± 0.03*	2.13 ± 0.05*
ADOV 400mg/kg	4.19 ± 0.09*	3.22 ± 0.11*	2.42 ± 0.15*	1.89 ± 0.04*

Natural Resources for Human Health
eISSN: 2583-1194

*Means ± standard error of the mean (n = 6). * P < 0.001 versus the carrageenan control group at the same time point (repeated-measures ANOVA followed by Tukey's HSD test). ANOVA at each time point: 1 h, F(3,20) = 31.72; 2 h, F(3,20) = 81.97; 3 h, F(3,20) = 401.60; 4 h, F(3,20) = 1513.61; all P < 0.001.*

Table 7. Inhibition (%) of carrageenan-induced paw edema by ADOV, with diclofenac as reference.

Treatment	Inhibition % at 1 h	Inhibition % at 2 h	Inhibition % at 3 h	Inhibition % at 4 h	Inhibition % at Mean over 4 h
Diclofenac10 mg/kg	36.00 ± 4.00	54.03 ± 3.72	64.33 ± 1.45	68.95 ± 1.04	55.82 ± 2.03
ADOV 200mg/kg	29.18 ± 3.74	43.77 ± 2.53	53.16 ± 0.45*	66.00 ± 0.83	48.03 ± 0.81*
ADOV 400mg/kg	31.76 ± 1.51	48.20 ± 1.80	61.20 ± 2.39	69.90 ± 0.71	52.77 ± 0.77

*Means ± standard error of the mean (n = 6). * P < 0.05 versus the diclofenac-treated group (ANOVA followed by Tukey's HSD test); the absence of an asterisk indicates the absence of a difference from diclofenac.*

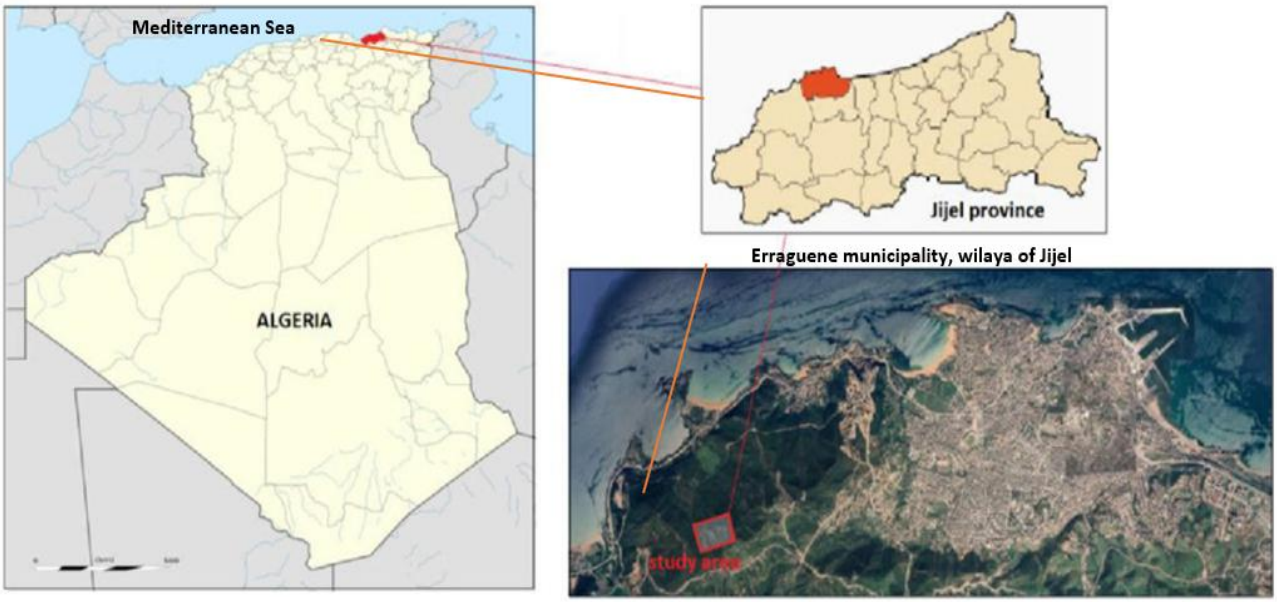

Figure1. Erraguene municipality, wilaya of Jijel, Algeria.

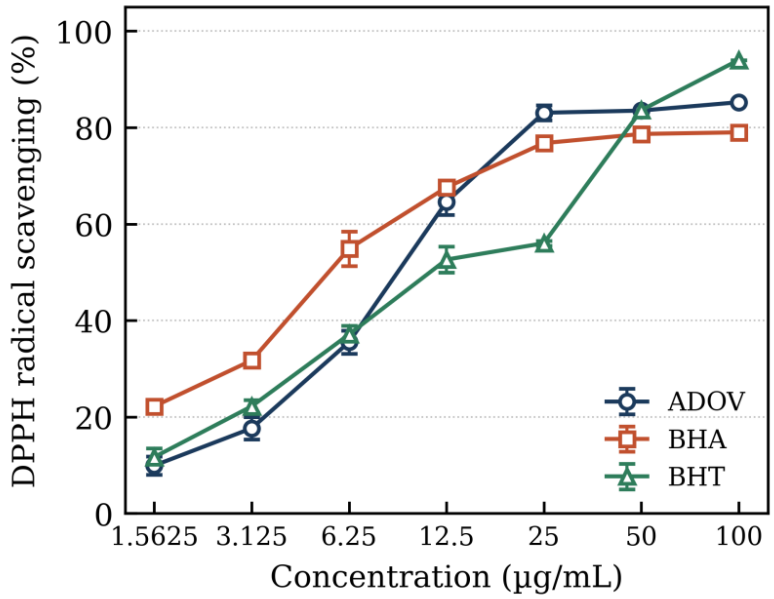

Figure2. DPPH radical scavenging activity (%) of the aqueous decoction of *Origanumvulgare* L. aerial parts (ADOV) and of the standards BHA and BHT. Means ± standard deviation (n = 3).

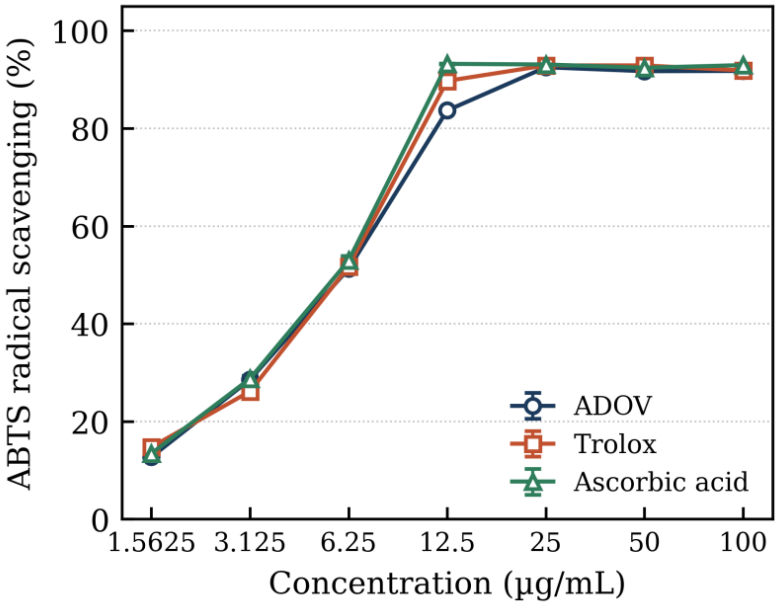

Figure 3. ABTS radical cation scavenging activity (%) of ADOV, Trolox and ascorbic acid. Means ± standard deviation (n = 3).

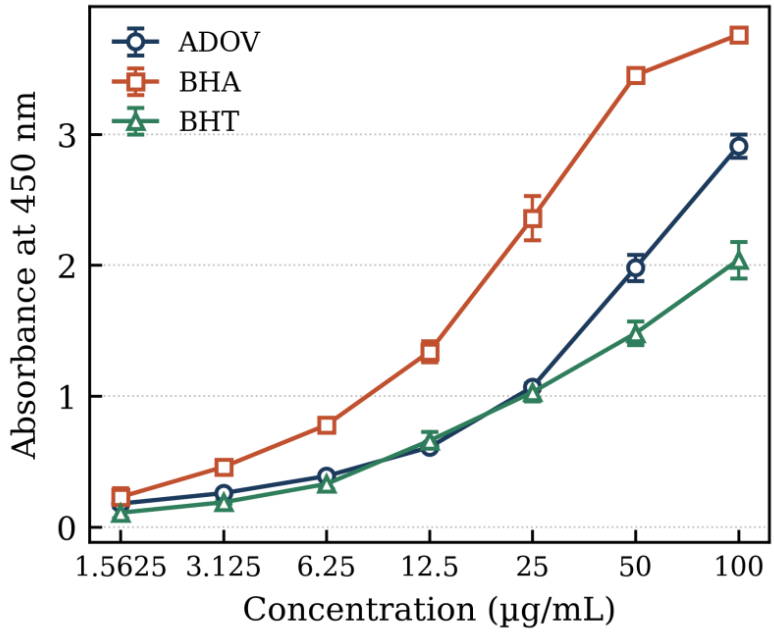

Figure 4. Cupric ion reducing capacity (absorbance at 450 nm) of ADOV, BHA and BHT. Means ± standard deviation (n = 3).

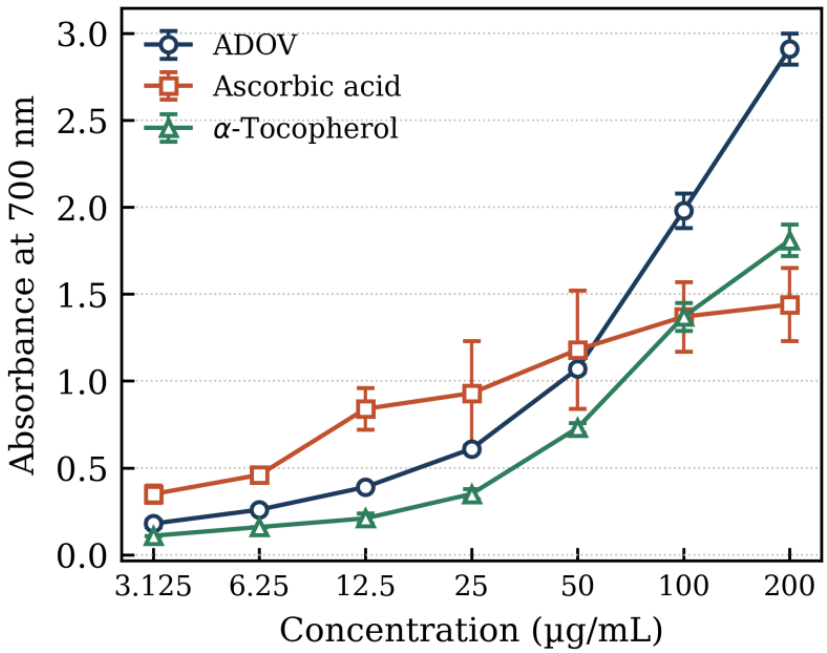

Figure 5. Ferric reducing power (absorbance at 700 nm) of ADOV, ascorbic acid and α-tocopherol. Means ± standard deviation (n = 3).

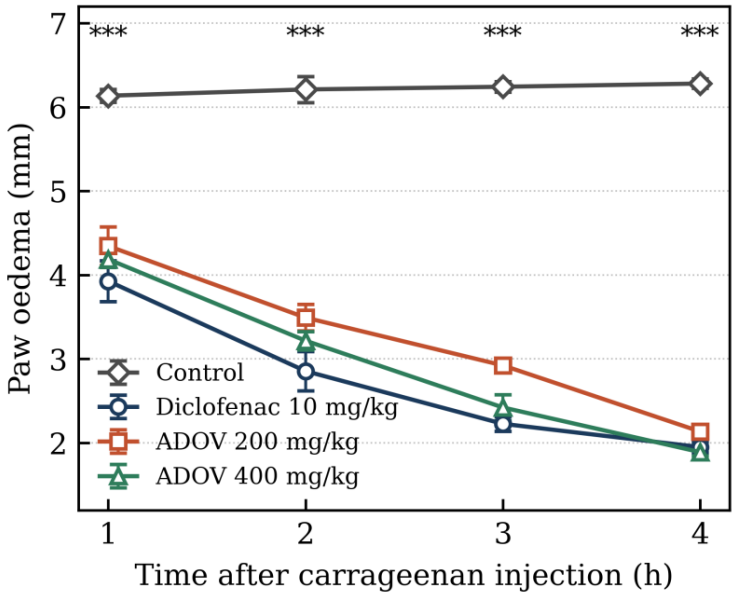

Figure 6. Time course of carrageenan-induced paw edema in mice pre-treated with ADOV (200 and 400 mg/kg, orally) or diclofenac (10 mg/kg, subcutaneously). Means ± standard error of the mean (n = 6). *** $P < 0.001$ versus the control group at the corresponding time point.