Arch Biol Sci. 2024;76(2):161-174 https://doi.org/10.2298/ABS240122011M

Assessment of antioxidant and antiinflammatory activities and acute toxicity of the aqueous extract from a mixture of leaves and flowers of *Anabasis articulata* (Forssk.) Moq.

Yasmina Makhlouf[1], Amel Bouaziz[1,*], Nabil Benazi[2], Saliha Djidel[1], Assia Bentahar[1], Nihed Barghout[1], Seddik Khennouf[1] and Saliha Dahamna[1]

[1]*Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1*, Setif, Algeria

[2]*Institut Pasteur Algeria, Antenna M'sila*, M'sila, Algeria

Corresponding author: a.bouaziz@univ-setif.dz

Received: January 22, 2024; **Revised**: March 14, 2024; **Accepted**: March 16, 2024; **Published online**: April 19, 2024

Abstract: Colometric assays were used to quantify the secondary metabolites obtained by a decoction of the extract of *Anabasis articulata* (DEAA) flowers and leaves. Antioxidant activity was examined using several methods: total antioxidant capacity, the 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging assay, and the β-carotene bleaching assay. Single oral doses of 2000 and 5000 mg/kg body weight were administered to albino mice to assess acute toxicity. *In vitro* antiinflammatory activity was determined using the egg albumin denaturation test, and the in vivo inflammatory effect was assessed utilizing carrageenan, croton oil, and xylene-induced edema tests. Results showed that DEAA contained high amounts of polyphenols, flavonoids, and tannins and exhibited antioxidant activity in all tested assays. DEAA inhibited protein denaturation and did not cause any mortality or adverse effects. Oral administration of 200 mg/kg DEAA significantly reduced the edema induced by carrageenan, croton oil, and xylene. This study highlights the use of *Anabasis articulata* (Forssk.) Moq. in traditional herbal medicine. It possesses antioxidant activity and can be considered safe for oral consumption; it also has potential beneficial effects in treating diseases associated with inflammation and pain.

Keywords: *Anabasis articulata* (Forssk.) Moq.; polyphenols; antioxidants; acute toxicity; anti-inflammatory activity

INTRODUCTION

Medications designed to treat pain and inflammation often come with side effects and offer limited capacity, especially in the treatment of chronic conditions[1]. Inflammation is the reactive condition characterized by hyperemia and exudation from blood vessels, resulting in redness, heat, swelling, and pain within a tissue in reaction to physical or chemical damage or bacterial invasion [2]. It is the body's tissue response to injury and involves a complex cascade of enzyme activation, mediator release, fluid extravasations, cell migration, tissue disintegration, and repair [3]. Both steroidal antiinflammatory and nonsteroidal antiinflammatory drugs (NSAIDs) are used to mitigate inflammation. Steroids have a distinct function in the treatment of inflammatory illnesses. However, due to their toxicity, steroids are typically prescribed for short durations, except in highly critical instances where the risks are considered acceptable. Long-term use of NSAIDs is also linked to serious adverse effects, most notably bleeding in the gastrointestinal tract [4]. Natural products exhibit high effectiveness with few adverse effects as alternatives to pharmaceutical therapies. One promising approach to finding antiinflammatory medications is to research plants utilized in conventional medicine as antiinflammatory compounds [5].

Anabasis articulata (Forssk) Moq., commonly known as "ajrem" in Algeria, belongs to the Chenopodiaceae family and is a plant widely used in traditional medicine to treat fever, headaches, diabetes, and skin disorders, including eczema and lice [6]. The plant is a dwarf shrub, 35-110 cm in height. Stems are woody for about half the length or more, erect, or twisted. Branches have equal internodes and oppose each other; they are brittle, with mature ones displaying bark that splits and peels; the leaves are reduced to small 2-lobed cupules, villous, with

solitary flowers measuring up to 5 mm [7]. The plant is consumed orally after decoction in water, either by itself or in combination with other medicinal herbs [8].

A. articulata has been reported to have medicinal properties due to its antioxidant and antimicrobial activities [9], antiangiogenic activity [10], antiarthritic effect [11],and antidiabetic activity [8,12,13]. Researchers have evaluated the effect of another genus of the same family. Evaluations of the antioxidant and antibacterial activities were carried out on Moroccan *Anabasis aretioïdes* Coss. &Moq. [14]. *Atriplex Halimus* Sub. Sp. *Schwein furthii* from Algeria was assessed for its *in vitro* antioxidant activity [15]; *Agatophora alopecuroide*, *Hammada elegans, Salsola baryosma*, and *Salsola vermiculata* were evaluated for their antioxidant, and α-amylase- and α-glycosidase-inhibiting activities [13]. Phytochemical studies of *A. articulata* revealed a variety of secondary metabolites, including phenolics [16], alkaloids [17], and saponins such as 3-O-glucopyranosyl of stigmasterol, ß-sitosterol, sitostanol, 3-O-[ß-D-the glucopyranosyl] oleanolic acid, 3-O-[ß-D-glucopyranosyl -28-O-ß-D-xylopyranosyl] oleanolic acid, in addition to proceric acid [12].

No scientific investigations have been performed into the antiinflammatory effect and toxicity of the aqueous extract of the *A. articulata* leaf and flower mixture obtained by decoction. In this study, we assessed the total phenolic, total flavonoid, and total tannin contents in this extract andtheir safety by examining acute toxicity. In addition, we examined the *in vitro* and *in vivo* antiinflammatory effects.

MATERIALS AND METHODS

Ethics statement

All experimental studies were approved by the Committee of the Algerian Association of Sciences in Animal Experimentation(http://aasea.asso.dz/articles/) under Law No.8808/1988, associated with veterinary medical activities and protection of animal health (N°JORA: 004/1988).Permission for experimental use was obtained from the Faculty of Nature and Life Sciences, Ferhat Abbas University of Setif 1. All procedures were performed in compliance with laws and institutional guidelines.

Nomenclature

The medicinal plant used in the present study was *A. articulata* (Forssk.) Moq., commonly called "El Ajrem", belongs to the Chenopodiaceae family. The plant was identified by Professor B.Oujhih, an expert taxonomist at the Institute of Nutrition and Agronomy, University of Batna (Algeria). A voucher specimen was deposited at the laboratory under the number SNV 0045–2020.

Chemicals and instruments

All analytical grade chemicals utilized in this study were procured from E. Merck, Germany, including the Folin-Ciocalteu reagent, sodium bicarbonate (Na_2CO_3), gallic acid, aluminum trichloride ($AlCl_3$), quercetin, tannic acid, 2.2-diphenyl-1-picrylhydrazyl(DPPH), butylated hydroxytoluene (BHT), sulfuric acid, sodium phosphate, molybdate, ascorbic acid, β-carotene, linoleic acid, Tween 40, Tris-HCl buffer, aspirin, phosphate-buffered saline, carrageenan, indomethacin, croton oil, xylene.

Plant material

A. articulata was harvested in October-November 2020 from the El Mergueb natural reserve of M'sila province (North Algeria) at 35° 42' N latitude and 4° 32' E longitude. The flowers and leaves of *A. articulata* were air-dried in shade, crushed, and then powdered using an electric grinder.

Extraction procedure

The flower and leaves mixture of *A. articulata* powder (100 g) was extracted by decoction using 1000mL of boiled distilled water according to the method of Ferreira et al. [18]. The resulting mixture was subjected to magnetic agitation for 20 min. After filtration, the filtrate was evaporated to dryness to obtain DEAA. The residue was weighed to obtain the extractive yield and stored in an airtight bottle at 4°C.

Animal material

Albino mice and rats (weighing between 18-30 and 120-200 g, respectively) were keptin polycarbonate cages for7 days in standard laboratory conditions (12 h

light/dark cycle, 23±2°C with free access to food and water) before the beginning of the experiment.

Quantitative phytochemical analysis

Determination of total phenolic content

The content of phenolic compounds in the extracts was estimated spectrophotometrically using the Folin-Ciocalteu reagent method described by Li et al. [19]. The procedure entails the addition of 500 µL of Folin-Ciocalteu reagent (diluted 10 times) to 100 µL of the extract or gallic acid. After 4 min, 400 µL of 7.5% sodium bicarbonate solution (Na_2CO_3) was added. After 90 min of incubation in the dark at room temperature, the absorbance was read at 765 nm. The concentration of total polyphenols was calculated from the regression equation of the gallic acid calibration curve at different concentrations. The results are expressed in mg equivalent of gallic acid per g of dry extract (mg EAG/g DE).

Determination of flavonoid content

Quantitative determination of flavonoids was performed using the aluminum trichloride method [20]. Briefly, 1 mL of the $AlCl_3$solution (2%) was added to 1 mL of the extract solution at different prepared concentrations in the appropriate solvent. At 430 nm, the absorbance was measured after 10 min of incubation. The concentration of flavonoids in the extracts was deduced from a calibration line established with quercetin and expressed in milligram equivalents of quercetin per gram of dry extract (mg EQ/g DE).

Determination of total tannin content

The total content of tannins in the extract was estimated using the Folin-Ciocalteu reagent and tannic acid as standard, according to the method described by Prasanth et al. [21]. The reaction mixture was made by combining 0.5mL of extract, 2.5 mL of 10% Folin-Ciocalteu reagent, and 2.5 mL of 7.5% Na_2CO_3. The samples were incubated at 45C° for 45 min in darkness. Absorbance was then measured at 765 nm, and the calibration curve was established using different concentrations of tannic acid (25-300 µg/mL). Total tannin content was expressed as mg equivalent of tannic acid per g of dry extract (mg TAE/g DE).

Estimation of *in vitro* antioxidant activity

Total antioxidant capacity

Total antioxidant capacity (TAC) was evaluated with an assay based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH [22]. An aliquot (0.1 mL) of plant extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95C° for 90 min in darkness. The mixture was allowed to cool to room temperature, and the absorbance was measured at 765 nm against a blank prepared with one mL of the reagent solution and the appropriate volume of the sample's solvent. It was then incubated in the same manner as the other samples.

DPPH radical scavenging assay

The extract's free radical scavenging activity was measured using DPPH with the modified method of Shimamura et al. [23]. Briefly, a 0.4-mM solution of DPPH was prepared in 100mL methanol, and 800 µL of this solution was added to 200 µL of the sample at different concentrations. The absorbance was determined at 517 nm after 30 min. BHT was used as standard.

$(Abs_{control} - Abs_{sample} / Abs_{control})$ is the percentage of radical scavenging activity;

$Abs_{control}$: Absorbance of the control (without sample).

Abs_{sample}: Absorption of the extract with the reagent.

β-Carotene bleaching assay

This assay is based on the capacity of antioxidant molecules to inhibit β-carotene oxidative degradation caused by linoleic acid oxidative compounds according to the method of Kartal et al. [24]. A mixture of 0.5 mg of beta-carotene, 1 mL of chloroform, 25 µL of linoleic acid, and 200 mg of Tween 40 was used to create a ß-carotene/linoleic acid emulsion. Chloroform was evaporated at 40°C using a vacuum evaporator. Afterward, 100 mL of distilled oxygenated water was added while the mixture was vigorously shaken. To an aliquot of 2.5 mL of this

emulsion, 350 µL of DEAA or the reference antioxidant (BHT) was added and well mixed. The absorbance was recorded after 0, 1, 2, 4, 6, and 24 h at 490 nm. A negative control consisted of 2.5 mL distilled water or methanol instead of extract or reference antioxidant. All samples were assayed in triplicate. The percentage of inhibition was determined using the equation below:

$$\text{Inhibition \%} = \text{Abs}_{(t)} / \text{Abs}_{(t0)} \times 100.$$

where $\text{Abs}_{(t)}$ was the absorbance of the test sample at the given time t, and $\text{Abs}_{(t0)}$ was the absorbance of the test sample at a time t_0.

Estimation of *in vitro* antiinflammatory activity

The protein denaturation experiment was carried out, as reported by Bouaziz et al. [23]. The volume of egg white was adjusted with a buffer solution of 20 mM Tris-HCl (pH6.8) to obtain a dilution solution of 1:100. The solution was gently stirred for 10 min, followed by filtration. Identical volumes were transferred to tubes, followed by the addition of DEAA or aspirin, and the tubes were incubated at 74°C for 15 min. The absorption was measured at 650 nm, and the following % inhibition of protein denaturation was calculated:

$$\text{inhibition \%} = [(\text{Abs control} - \text{Abs treated}) / \text{Abs treated}] \times 100.$$

***In vivo* investigations**

Acute toxicity study

The acute toxicity was assessed on mice according to the Organization of Economic Co-operation and Development guidelines 423 (OECD 423) [26]. The animals were randomly divided into three groups containing five male mice, each weighing between 18-30 g. Two treated groups were orally given a single dose of DEAA at 2000mg/kg and 5000mg/kg body weight, while the control group was given only distilled water. The first day of the treatment was taken as Day 0,and the day of sacrifice was Day 14. Individual lots of mice were monitored for 4 h after treatment to detect behavioral and physiological changes in comparison to the control. Gross behavioral and toxic effects were observed at short intervals for 24 h, then daily for the next 14 days to detect late effects.

The body weight of each mouse was measured during the study period, on days 0, 7, and 14. All mice were killed on day 14. The kidney, liver, heart, and spleen were weighed to determine relative organ weights and to observe macroscopic lesions. The relative organ weight of each animal was then determined using the formula:

$$\text{Relative organ weight} = (\text{absolute organ weight (g)} / \text{body weight of mouse on sacrifice day (g)}) \times 100.$$

For histological examination, the liver and kidney were fixed in 10% formalin, dehydrated in ascending degrees of ethanol (70-100%), cleared in xylene, and finally embedded in paraffin. Afterward, thin sections of 5 µm were stained with hematoxylin-eosin and examined with a light microscope. Digital images recorded at 20×magnification.

Antiinflammatory activity

Carrageenan-induced paw edema in rats

The antiinflammatory effect of DEAA against carrageenan-induced acute paw edema in rats was assessed according to the method described by Tsai and Lin [27]. Five groups of 5 rats each were treated with DEAA (50, 100, and 200 mg/kg *per os*), indomethacin (10 mg/kg *per os*), and distilled water (10mL/kg *per os*). One h after administration, acute inflammation was produced by the subplantar injection of 0.1 mL of (1%carrageenan in saline) in the left hind paw. The thickness of each paw was measured using a digital caliper. Measurement was carried out at 0 h (V_0: before carrageenan injection) and 1, 2, 3, 4, 5, and 6 h after the induction of inflammation (V_T). The difference between V_T (1, 2, 3, 4, 5, and 6 h) and V_0 was taken as the edema volume value. The percentages of increase were calculated according to the following formula:

$$\text{increase \%} = (D_n - D_0) / D_0 \times 100.$$

Croton oil-induced ear edema in mice

The mouse ear edema was induced by topical application of croton oil according to the method described in [28] with slight modifications. Croton oil was dissolved in a 5% acetone solution (v/v), and 10 µL of the solution were applied to both the anterior and posterior

surfaces of the right ear with a micropipette. The left ear (control) received the vehicle (acetone 80, distilled water 20 v/v). Groups of mice (n=5) received oral doses of DEAA (100 and 200 mg/kg), vehicle (distilled water), or indomethacin (10 mg/kg) 1 h before croton oil application. Six h later, the thickness of each ear was measured using a digital caliper, and the antiinflammatory activity was expressed as the percentage of edema inhibition using the following formula:

$$\text{Inhibition \%} = 100 \times (D_n - D)/D_n,$$

where D is the difference in ear edema thickness in the treated group, and D_n is the difference in ear edema thickness in the negative group.

Xylene-induced ear edema in mice

The xylene-induced ear edema test was carried out according to the method of Manouze et al. [29] with slight modifications. Briefly, each group (5animals per group) was given by gavage one dose (100 or 200 mg/kg) of DEAA, indomethacin (10 mg/kg), or vehicle (10 mL/kg) 1 h. The right ear's inner and outer surfaces were treated topically with 0.02 mL of xylene to cause ear edema. The left ear was used as a control. One h after xylene application, the thickness of the ear was measured with a digital caliper. The following formula was used to calculate the inhibition percentage of ear edema:

$$\text{Inhibition \%} = 100 \times (D_n - D)/D_n,$$

where D is the difference inear edema thickness in the treated group, and D_n is the difference inear edema thickness in the negative group.

Statistical analysis

Statistical comparisons were performed using Graph Pad Prism (version 7.00 for Windows). One-way analysis of variance (ANOVA) followed by Dunnet's test was used for all assays except for the *in vitro* antiinflammatory effect, where the Student's t-test was used. Statistical data are presented as the mean±standard deviation (SD) with n=3, while for *in vivo* experiments data are presented as the mean±standard error of the mean (SEM) with n=5. A difference was considered significant when the P value was less than 0.05.

RESULTS

Quantitative phytochemical analysis

The mixture of the flowers and leaves of *A. articulata* extracted by decoction gave a high yield percentage (10.7%). The quantitative assessment of polyphenols, flavonoids, and tannins were estimated at 210.20±0.565mg GAE/g DE, 13±0.055mg QE/g DE and 138.27±1.442mg TAE/g DE respectively.

Estimation of *in vitro* antioxidant activity

For this test, DEAA had an EC_{50} of 0.4093±0.0058 mg/mL compared to the reference antioxidant; ascorbic acid was noted, which proved to be more effective ($P<0.001$) with an EC_{50} of 0.002±0.003mg/mL. In the DPPH test, DEAA possesses a good scavenging activity (IC_{50} =0.170±0.0006) compared to BHT (IC_{50} = 0.026±0.0006 mg/mL). Fig. 1 shows the results of the antioxidant activity of DEAA measured by the β-carotene bleaching test. DEAA effectively prevented the bleaching of β-carotene (79.833±1.03%) compared to the standard BHT (98.11±1.05%). Results obtained from the evaluation of the antioxidant activity of DEAA indicate that the extract contains reductants capable of conferring stability to the oxidant molecules.

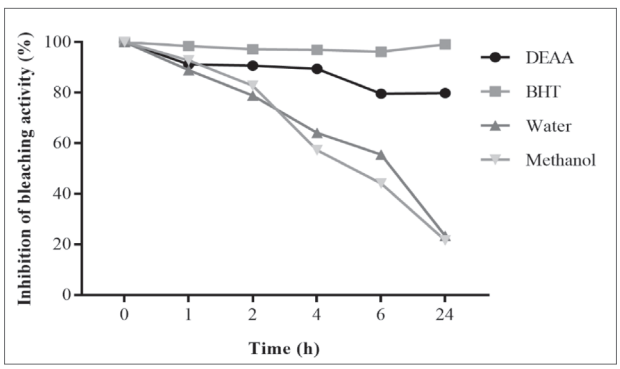

Fig. 1. Antioxidant activity of the decocted extract of *A. articulata* measured by β-carotene bleaching test. Bleaching kinetics of β-Carotene in the presence of DEAA, methanol, water, and standard BHT during 24 h. Values are presented as the mean ±SD. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction; BHT –butylated hydroxytoluene.

Estimation of *in vitro* antiinflammatory activity

DEAA was able to prevent denaturation of albumin in a concentration-dependent way. The percentage of

inhibition was within arange from 29.71% to 92.17% at the concentration range of 0.0625to 0.5mg/mL (Fig. 2). Aspirin as a reference drug exhibited a higher inhibition capacity (96.03%) at the higher concentration for the denaturation of egg test.

In vivo investigation

Acute toxicity study

The results of the acute toxicity study showed that after 14 days of observation DEAA given to mice in single doses of 2000 and 5000 mg/Kg body weight (b.w.) resulted in no mortality or morbidity. Moreover, the animals did not exhibit any toxic effects or behavioral or morphological changes.

Effect on body weight and organ relative weight

The changes in the body weight of control and mice treated with the extract of *A. articulata* obtained by decoction during the 14 days of treatment are presented in Fig. 3. The body weight gain showed no significant difference (P>0.05) between the control and treated groups. However, the body weight of mice at 24, 48, 72 h, and 7 days (P>0.05) after administration of DEAA at 5000mg/kg exhibited a decrease but was statistically insignificant compared to the time of initial treatment. No significant changes were observed in the body weights in the treated groups compared to that of the control group, suggesting that DEAA had no effect on the growth of mice at the oral doses administered.

The vital organs (liver, kidneys, heart, and spleen) of mice were weighed after 14 days of administration at doses of 2000 and 5000 mg/kg (Fig. 4). The liver and kidney of the group treated with 5000 mg/kg showed a statistically significant increase in weight (P<0.001 and P<0.01, respectively) compared to the control group. However, the heart and spleen showed no alteration in weight compared to the control group (Fig. 4).

Histological analysis

The histopathological study of the selected organs in the acute toxicity test in mice showed no remarkable change when comparing the treated group that received 2000 mg/kg of DEAA and the control group. Fig. 5 shows a normal hepatic lobule composed of

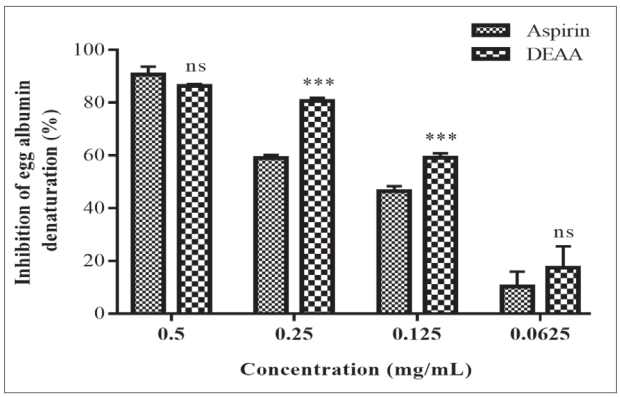

Fig. 2.*In vitro* antiinflammatory effect of DEAA according to the egg albumin denaturation test. Aspirin was used as a reference drug. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are presented as the mean±SD compared to aspirin. ns – no significant difference, *** P<0.001.

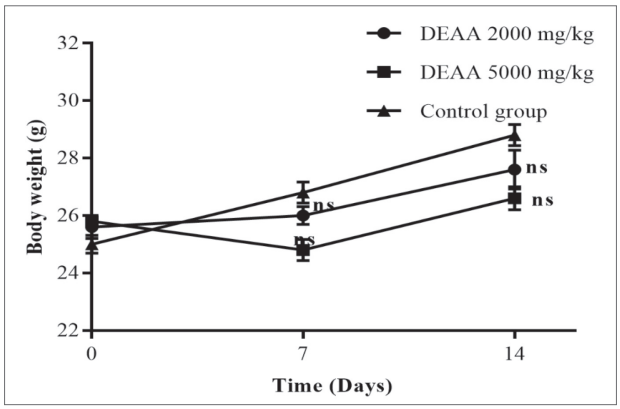

Fig. 3. Body weight changes in the treated mice and their controls at days 0, 7,and 14in the acute toxicity experiment of DEAA. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Results are expressed as the mean±SEM(n=5). ns–no significant difference.

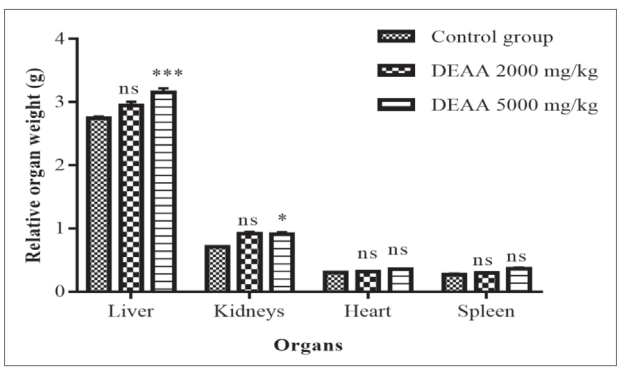

Fig. 4. Relative organ weights of mice receiving DEAA after 14 days of acute toxicity experiment. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Results are expressed as the mean ±SEM (n = 5). ns – no significant difference; *P<0.05, *** P<0.001

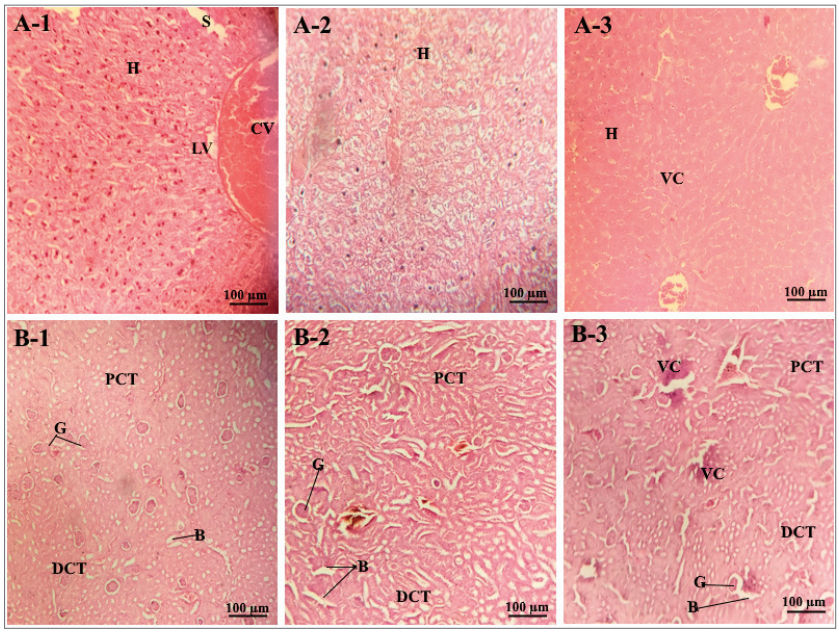

Fig. 5. Histopathological study of liver (**A**) and kidney (**B**) tissues of mice treated with a single dose of 2000 and 5000 mg/kg of DEAA. H & E stain (×20), (scale bars=100 µm). H and E – hematoxylin-eosin; **A-1**– Section of the liver of the control;**A-2**– Section of the liver of a 2000 mg/kg DEAA-treated animal;**A-3**– Section of the liver of a 5000 mg/kg DEAA treated representative wascharacterized byvascular congestions in the entirety of the structure of the liver; **B-1**– Section of the kidney of the control; **B-2**– Section of the kidney of a 2000 mg/kg DEAA-treated animal; **B-3**– Section of the kidney of a 5000 mg/kg DEAA treated animal where vascular congestion was observed. CV –central vein; H – hepatocyte; S – sinusoids; VC – vascular congestions; LV – lymphatic vessels; G – glomerulus; B – Bowman's space; PCT – proximal collecting tubules; DCT – distal collecting tubules; VC – vascular congestions.

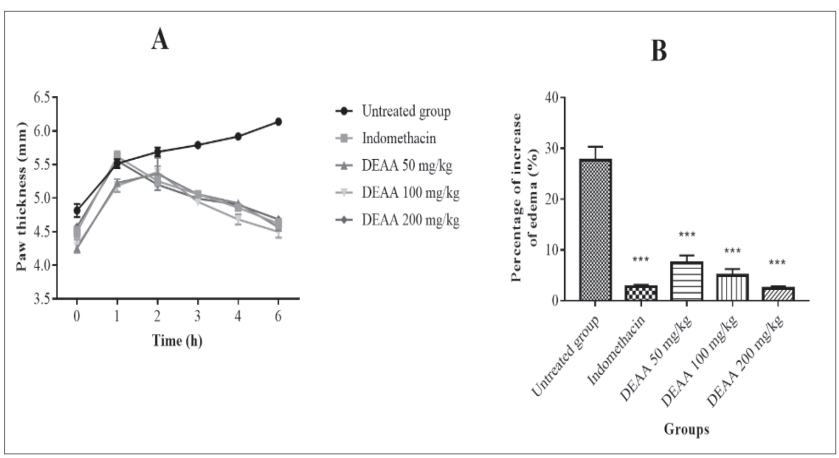

Fig. 6. Antiinflammatory effect of DEAA on carrageenan-induced paw edema in rats. **A** – Changes in paw thickness (mm). **B** – Percentage of increase of edema. DEAA – extract of the flowers and leaves of *A.articulata* obtained by decoction. Results are expressed as the mean±SEM (n = 5). ns – no significant difference, ***P<0.01 were considered significant when compared with the untreated group.

hepatocytes arranged in hepatic cords separated by sinusoids that drain into central vein lymphatic vessels. The same was observed for kidneys, normal glomerulus, Bowman's space, proximal collecting tubules, and distal collecting tubules (Fig. 5). However, in the group treated with5000 mg/kg of DEAA, the liver and kidney showed normal structures, but with vascular congestions.

Invivo antiinflammatory activity

Carrageenan-induced paw edema

Three doses of 50, 100, and 200 mg/kg were tested to evaluate the antiinflammatory activity of DEAA. The results obtained were compared with those of the standard indomethacin (10 mg/kg). Fig. 6A shows the variations in paw thickness (mm) concerning carrageenan-induced paw edema over6 h following the administration of standard and three doses of DEAA. In carrageenan-administered animals, the paw edema increased gradually and reached a peak at6 h (5.98±0.18 mm). However, in animals treated with DEAA doses, the carrageenan-induced inflammation was reduced compared to the control group. The administration of indomethacin significantly prevented the progression of the inflammation at the plantar level of the rat's paw at 2 h and presented the lowest edema compared to the control at 6h (4.72 ±0.12mm). Fig. 6B illustrates that DEAA caused a dose-dependent decrease in the percentage of paw edema. The percentage of edema increase after 6h in rats receiving DEAA showed a similar effect at an oral dose of 200

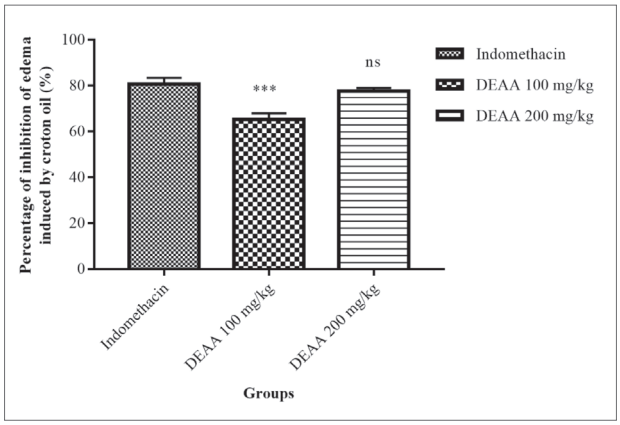

Fig. 7. Antiinflammatory effect of DEAA on croton oil-induced ear edema in mice. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are expressed as means±SEM. ns – no significant difference, *** P<0.001 were considered significant when compared with indomethacin.

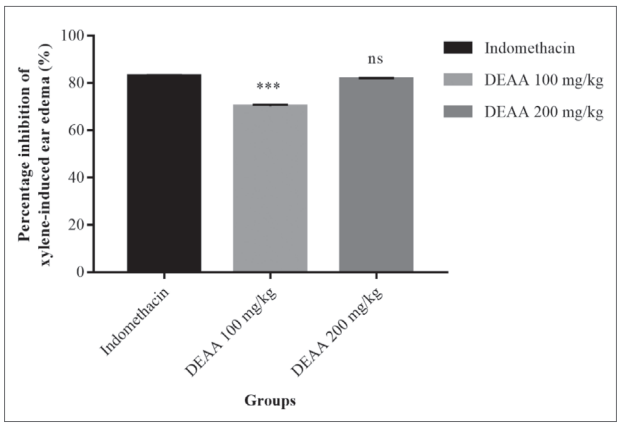

Fig. 8. Antiinflammatory effect of DEAA on xylene-induced ear edema in mice. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are expressed as means±SEM (n=5). ns – no significant difference, *** P<0.001 were considered significant when compared with indomethacin.

mg/kg (2.41±0.48%) compared to indomethacin-treated group (2.77±0.37%). The effect was less noticeable (7.49±1.43%) at 50 mg/kg.

Croton oil-induced ear edema in mice

DEAA at doses of 100 and 200 mg/kg caused inhibition of edema induced by croton oil in mice at 6 h with percentages of inhibition of 68.59±0.84%and 77.58±0.64%, respectively, while 80.68±1.25%inhibition was recorded with the nonsteroidal antiinflammatory drug indomethacin (Fig. 7).

Xylene-induced ear edema in mice

Fig. 8. shows that DEAA at doses of 100 and 200 mg/kg effectively reduced the edema to 70.25±0.59% and 81.74±0.36%, respectively. Indomethacin used as a positive control promoted an antiinflammatory reduction to 82.98±0.36%.

DISCUSSION

Medicinal plants are widely acknowledged as a significant source of bioactive compounds. Due to limited research in the literature, this study investigates the extract of *A. articulata* obtained by decoction. Here, we provide experimental findings related to acute toxicity and antiinflammatory and antioxidant properties. Phytochemical analysis revealed the existence of several classes of secondary metabolites in *A. articulata* leaf and flower extracts obtained by decoction. The yield of DEAA extraction was 10.7%. Compared to other species of the Chenopodiaceae family, this value was lower than the yield of 19.87% reported for *Atriplex halimus*[30]and *Arthrophytum scoparium* by Dehimi et al. [15]. Many studies point out that the percentage of extraction yield depends mainly on the extraction procedure, in particular, the temperature used during the extraction, the polarity of the compounds of the extract, the solvent ratio, and the methods of extraction (maceration, decoction, evaporation) [31,32].

The present study revealed that the DEAA exhibited a high concentration of phenolic compounds (TPC=210.20±0.565 GAE/g DE).This finding was better than that of Benhammou et al. [33], who utilized methanolic maceration extract from *A. articulata* stems (25.48±3.83 mg EAG/g DE) and roots (19.85±7.52 mg GAE/g DE). These results were consistent with those reported by El-Haci et al. [34] for the endemic Algerian *A. aretioides* ethanolic maceration extract of leaves (231.85±20.59 mg EAG/g DE). Concerning the total flavonoid content, the *A. articulata* decoction revealed13.01±0.055mg QE/g DE. Benhammou et al. [33] reported a lower total flavonoid content for stem and root extracts (3.08±0.20 and 3.80±0.06 mg QE/g DE, respectively).In contrast, the ethanolic and methanolic maceration extracts of the aerial part (leaves) of *A. aretioides* showed a high amount of flavonoid content, 132.8±24.58 and 46.68±0.74 mg

CE /g DE, respectively [34]. The total tannins in our study accounted for 138.27mg TAE/g, an amount low compared to the Moroccan *A. aretioides* Coss. &Moq. that had 2.56±1.54 mg TAE/g DE and 5.93±2.42 mg TAE/g DE for the methanolic extracts of the aerial part and roots, respectively[35].

Research on the antioxidant capacity and identification of secondary metabolites from Saharan Algerian plants is scarce. Studies of the extract obtained by decoction of flowers and leaves of *A. articulata* have not been undertaken. The antioxidant capacities of plant extracts are influenced not only by their composition but also by the testing conditions used [36]. For this reason, the antioxidant capabilities of the studied extract were evaluated using different methods. The phosphomolybdate method was used to determine the total antioxidant capacity. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The study revealed that the antioxidant activity of the leaf and flower extract was 0.4093±0.006 mg/mL. Benhammou et al. [33] reported similar results in their investigation of *A. articulata* (stem and root) collected from the Bechar region (southern Algeria).

The DPPH radical scavenging method is a common spectrophotometric technique used to assess the antioxidant capacity of extracts. It relies on the ability of the DPPH radical to lose its color in the presence of antioxidants, which occurs when an antioxidant compound donates an electron or hydrogen to the DPPH radical[37]. The antioxidant activity of *A. articulata* was found to be strong, exhibiting significant scavenging activity against DPPH, with an IC_{50}=0.1695±0.00059 mg/mL. This value was higher than that reported by Hamdoon et al. [38] for an ethyl acetate extract and chloroform extract of *A. articulata* from Libya (246.38±4.01 µg/mL and 416.61±6.19 µg/mL, respectively). But, for the ethanolic extract, a similar scavenging activity with an $IC_{50}$of 149.26±3.23µg/mL was obtained. Bouaziz et al. [9] reported that extracts of the aerial part of Tunisian *A. oropediorum* exhibited a better scavenging efficiency against DPPH radicals with an IC_{50} of 0.70±0.80 µg/mL, 2.23±0.38 µg/mL 3.72±0.63 µg/mL, and 1.12±0.19 µg/mL for hexane, ethyl acetate, methanol, and water extracts, respectively.

The β-carotene bleaching test is based on proton transfer processes where linoleic acid, an unsaturated fatty acid, is oxidized by reactive oxygen species (ROS) created by oxygenation, which will cause discoloration. Antioxidants in the examined extracts reduce the degree of discoloration [39]. DEAA exhibited a notable ability to inhibit the bleaching of β-carotene over24 h when compared to the positive control.

Results from the assessment of DEAA antioxidant activities showed that the extract contains reductants that can provide ROS stability. For different types of plants, the extraction techniques and extraction solvents have different effects on the antioxidant activity of bioactive chemicals. The antioxidant activity of DEAA could be attributed to its flavonoid and phenolic contents that act as a strong natural free radical scavenger. Moreover, there is a positive correlation between antioxidant activity and the content of polyphenols [40,41].

Protein denaturation is the disruption of proteins' tertiary and secondary structures caused by external stressors such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. When denatured, biological proteins lose their biological function [42] and cause inflammatory diseases. The ability of the decoction extract of *A.articulata* to inhibit protein denaturation was evaluated through the egg albumin denaturation technique. DEAA was able to inhibit protein denaturation and had an effect close to the standard drug at a dose of 5 mL/mg. Several investigations have shown that the anti-denaturation effect of DEAA may be attributable to secondary metabolites [43,44]. Their active components, such as polyphenols, triterpenoids, alkaloids, steroids, sinapic acid, coumaric acid, vanillic acid, kaempferol, ferulic acid, and benzoic acid, exhibit antiinflammatory activity [42,45].

Bioactive compounds derived from medicinal plants exhibit a range of beneficial biological properties. However, they can induce toxic effects, resulting in systemic toxicity, nephrotoxicity, and hepatotoxicity. Consequently, clinical complications may arise, potentially leading to carcinogenesis and severe infections [46]. The majority of serious side effects result from the misuse of herbal medications[47]. There is a lack of toxicological research in the literature regarding the aqueous extract of *A. articulata*. Therefore, we are

interested in conducting DEAA acute toxicity studies to improve its safety in treating specific human disorders.

In the acute toxicity study by gavage, no death up to 14 days of the study period was observed. There were no physical signs of toxicity even at the highest dose of 5000 mg/kg b.w., as evidenced by normal breathing and the absence of tremors, convulsions, diarrhea, salivation, and paralysis in the treated animals. This suggests that DEAA was safe and non-toxic to mice during the monitoring period according to the OECD categorization, and consequently, it was calculated that the median lethal dosage (LD_{50}) of DEAA was greater than 5000 mg/kg b.w. It could be inferred that compounds from the decoction of flowers and leaves of *A. articulata* extract present no acute toxicity since no toxic effects were observed even after high-dose administration.

No significant difference ($P>0.05$) was observed in body weight gain between the control and treated groups following DEAA administration. The extract did not influence the mice's weight gain throughout the study, indicating that changes in body weight compared to the control group would have revealed any potential toxicity of the extract[48]. This may be partly explained by the fact that DEAA had no detrimental effects on appetite or food consumption.

Organ weights are important indicators of target organ injury and physiological disturbances [49,50]. The current study did not detect any notable alterations in organ weight concerning the heart and spleen. However, relative weights of the kidney and liver exhibited a gradual increase at 5000 mg/kg, with this rise proving significant compared to the control group. This increase in liver relative weight may be due to an increased metabolic response to the extract, which may have increased hepatic activity [51].

Histopathology examination of the liver and kidney revealed normal hepatocytes and glomeruli, no alteration in the structure in treated animals compared to control except for the vascular congestions noticed in liver and kidney tissues of animals treated with DEAA (5000mg/kg).The presence of congestion in the liver and kidneys may result from a vasoconstriction action induced by the extract on the wall of blood vessels [52].

The *in vivo* antiinflammatory activity of DEAA was investigated using carrageenan-induced paw edema, xylene-, and croton oil-induced ear edema models. These three approaches are widely acknowledged as pharmacological models for evaluating the acute antiinflammatory effects of natural medicines [53]. The edema produced in rat feet by injection of carrageenan is mediated by histamine and 5-hydroxytryptamine (5-HT) during the first hour, after which the enhanced vascular permeability is sustained by kinin release for the next hour. From 2 to 6 h, the mediator appears to be a prostaglandin, and its release is linked to leucocyte migration into the inflamed location [54]. Prostaglandins promote the production of inflammatory exudates during tenderness. The use of nonsteroidal antiinflammatory medicines (NSAID) such as indomethacin reduces the impact of edema formation by inhibiting cyclooxygenase activity and prostaglandin synthesis [55]. We showed that the DEAA at 100 and 200 mg/kg exhibited similar effects in the 2^{nd}phase (3-6 h), comparable to the standard drug indomethacin. The antiinflammatory activity of the extract might act through a mechanism that involves the inhibition of cyclooxygenase (COX) associated with the inflammatory cascade induced by carrageenan [56]. The inhibition of these proinflammatory mediators by flavonoids may be one of the most important mechanisms of their antiinflammatory activity [57]. This is also in agreement with several studies claiming that many plants that contain chemical compounds such as polyphenols have powerful antiinflammatory abilities that work by inhibiting prostaglandin pathways [58].

Croton oil is obtained from the seeds of *Croton tiglium*. Externally, the oil produces topical irritation and edema. In this test, the inflammatory response is typically evaluated by measuring the change in ear plug weight at a specific time point following the application of the irritating agent [59]. Xylene is a phlogistic agent that induces ear edema as it is sensitive to COX inhibitors and is used to assess the effect of NSAID substances that inhibit prostaglandin synthesis [60]. The two tests cause immediate irritation that leads to the infiltration of inflammatory cells, vasodilation, accumulation of fluid, and edema [61]. The decoction extract of *A. articulata* at an oral dose of 200 mg/kg produced significant inhibition of edema compared to indomethacin, which is related to the release of inflammatory mediators like substance P, histamine, kinin-like substances, and prostaglandins produced by increased levels of phospholipase A2 and COX-2 [49]. The antiinflammatory

activity of the extract in the two antiinflammatory ear edema models could be due to its phytochemical compounds, such as polyphenols. Several plant species rich in polyphenols, particularly flavonoids, are reported to possess important pharmacological actions, such as anti-inflammatory [62]. According to other studies, flavonoids like rutin, quercetin, luteolin, hesperidin, and bi-flavonoids yielded significant antiinflammatory activity [63, 64], and the inhibition of histamine may be important. Several studies have highlighted the efficacy of polyphenols in mitigating inflammation by reducing the release of potent inflammatory mediators and inhibiting crucial inflammatory enzymes like COX and 5-lipoxygenase. The inhibition of these mediators is crucial in resolving inflammation and treating chronic inflammatory conditions.[65].

CONCLUSIONS

Research has been conducted on *Anabasis articulata* using different parts, such as stems and roots, to extract with polar solvents. Nonetheless, this study marks the first investigation into the antioxidant effects, toxicological profile, as well as the *in vitro* and *in vivo* antiinflammatory activity of the decoction extract derived from the flowers and leaves. The findings from the antioxidant and antiinflammatory investigations validate the traditional use of *A. articulata*. The acute toxicity study suggests that the aqueous extract of the plant is safe up to a dose of 5 g/kg of mice body weight when consumed orally as a single administration. DEAA can be considered a potential source of natural compounds that can be incorporated into the human diet, alongside its potential applications as antioxidant and antiinflammatory agents.

Funding: This work was funded by the Algerian Ministry of Higher Education and Scientific Research (MESRS) and the General Directory of Scientific Research and Technologic Development (DGRSDT).

Acknowledgments: The authors wish to thank the Algerian Ministry of Higher Education and Scientific Research (MESRS) and the General Directorate of Scientific Research and Technological Development (DGRSDT) for supporting this research study.

Author contributions: All authors participated in the formation and design of this study. Yasmina MAKHLOUF: conceptualization, investigation, methodology, Formal analysis, and writing – original draft. Amel BOUAZIZ: supervision, proposed the subject, designed the study review & editing, validation, and approved the final version of this article. Nabil BENAZI: supervision, data curation, provided the reagents and materials. Saliha DJIDEL, Assia BENTAHAR, Nihed BARGHOUT: methodology conducted the experiments. Seddik KHENNOUF: supervision, review and editing, validation. Saliha DAHAMNA: supervision, review, editing. All authors have read and approved the final version of this article.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: Data underlying the reported findings have been provided as a raw dataset, which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Makhlouf%20et%20al_Dataset.pdf

REFERENCES

1. Abdallaha HM, Abdel-Naimc AB, Ashourd OM, Shehataa IA, Abdel-Sattarb EA. Anti-inflammatory activity of selected plants from Saudi Arabia. Z Naturforsch C J Biosci. 2014;69(1-2):1-9. https://doi.org/10.5560/ZNC.2012-0168
2. Okoli CO, Akah PA, Nwafor SV. Anti-inflammatory activity of plants. J Nat Remedies. 2003;3(1):1-30.
3. Gong C, Hoff JT, Keep RF. Acute inflammatory reaction following experimental intracerebral hemorrhage in rat. Brain Res. 2000;871(1):57-65. https://doi.org/10.1016/S0006-8993(00)02427-6
4. Li RW, Myers SP, Leach DN, Lin GD, Leach G. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. J Ethnopharmacol. 2003;85(1):25-32. https://doi.org/10.1016/S0378-8741(02)00336-7
5. Calixto JB, Beirith A, Ferreira J, Santos ARS, Filho C, Yunes RA. Naturally occurring antinociceptive substances from plants. Phytother Res. 2000;14(6):401-18.
6. Hammiche V, Maiza K. Traditional medicine in Central Sahara: Pharmacopoeia of TassiliN'ajjer. J Ethnopharmacol. 2006;105(3):358-67. https://doi.org/10.1016/j.jep.2005.11.028
7. Khafagi A, Marei A, Mohamed S. Morphological and anatomical variations of *Anabasis articulata* ecotypes in Egypt. Arab Univ J AgricSci. 2005;13(2):233-48. https://doi.org/10.21608/ajs.2005.15350
8. Kambouche N, Merah B, Derdour A, Bellahouel S, Bouayed J, Dicko A, Younos C, Soulimani R. Hypoglycemic and antihyperglycemic effects of *Anabasis articulata* (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant. Afr J Biotechnol. 2009;8(20):5589-94.
9. Bouaziz M, Dhouib A, Loukil S, Boukhris M, Sayadi S. Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. Afr J Biotechnol. 2009;8(24):7017-27.
10. Abdulsahib WK, Abd AH, Qasim BJ, Sahib HB. Antiangiogenesis and antioxidant effect of *Anabasis articulata* stems extracts. Int J Pharm Sci Rev Res. 2016;41(2):88-94.
11. Abdulhusain ZH, Mahdi MA, Abdulsahib WK, Jasim LS. *Anabasis articulata* exerts an anti-arthritic effect on adjuvant-induced arthritis in rats. J Adv Pharm Technol Res. 2022;13(4):276-80.

12. Metwally NS, Mohamed AM, ELSharabasy FS. Chemical constituents of the Egyptian plant *Anabasis articulata* (Forssk) Moq and its antidiabetic effects on rats with streptozotocin-induced diabetic hepatopathy. J Appl Pharm Sci. 2012;2(4):54-65. https://doi.org/10.7324/JAPS.2012.2403
13. Djeridane A, Hamdi A, Bensania W, Cheifa K, Lakhdari I, Yousfi M. The *in vitro* evaluation of antioxidative activity, α-glucosidase and α-amylase enzyme inhibitory of natural phenolic extracts. Diabetes Metab Syndr. 2015;9(4):324-31. https://doi.org/10.1016/j.dsx.2013.10.007
14. Senhaji S, Lamchouri F, Toufik H. Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant *Anabasis aretioïdes*Coss. &Moq. (Chenopodiaceae). Biomed Res. Int. 2020;2020:1-16. https://doi.org/10.1155/2020/6152932
15. Dehimi K, Djoudi Z, Boulaouad A, Maadadi AR, Khennouf S. A Contribution to the Valorization of Two Medicinal Plants: *Atriplex Halimus* Sub. Sp. *Schweinfurthii* and *BuniumIncrassatum*, Growing in the Region of M'sila (North-East Algeria). IJNDD. 2020;12(4):208-16.
16. Gamal G, Abouelsaoud K, Elekhnawy E, Attia G. Anti-Biofilm Impact of Anabasis articulata (Forssk) Moq. Total Methanol Extract Against *Pseudomonas aeruginosa* Clinical Isolates. JAMPR. 2022;3(1):29-35. https://doi.org/10.21608/jampr.2022.131451.1027
17. Belyagoubi-Benhammou N, Belyagoubi L, Gismondi A, Di Marco G, Canini A, Atik Bekkara F. GC/MS analysis, and antioxidant and antimicrobial activities of alkaloids extracted by polar and apolar solvents from the stems of *Anabasis articulata*. Med Chem Res. 2019;28:754-767. https://doi.org/10.1007/s00044-019-02332-6
18. Ferreira A, Proença C, Serralheiro MLM, Araújo MEM. The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J Ethnopharmacol. 2006;108(1):31-7. https://doi.org/10.1016/j.jep.2006.04.010
19. Li H, Cheng K, Wong C, Fan K, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007;102(3):771-6. https://doi.org/10.1016/j.foodchem.2006.06.022
20. Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittelforschung. 1996;46(11):1086-9.
21. Prasanth DSNBK, Rao AS, Yejella RP. Phytochemical, *in vitro* antioxidant and antibacterial activities of *Argyreiapilosa* wight &arn. (whole plant). Int J Pharmacogn. 2017;4(4):109-17. https://doi.org/10.13040/IJPSR.0975-8232.IJP.4(4).109-117
22. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E.Anal Biochem. 1999;269(2):337-41.
23. Shimamura T, Sumikura Y, Yamazaki T, Tada A, Kashiwagi T, Ishikawa H, Matsui T, Sugimoto N, Akiyama H, Ukeda H. Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives-inter-laboratory evaluation study. Anal Sci. 2014;30(7):717-21. https://doi.org/10.2116/analsci.30.717
24. Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the antioxidant properties of *Ferulaorientalis* L. using a suitable extraction procedure. Food Chem. 2007;100(2):584-9. https://doi.org/10.1016/j.foodchem.2005.09.084
25. Bouaziz A, Djidel S, Bentaher A, Khennouf S. Polyphenolic content, Antioxidant and Anti-inflammatory activities of Melon (*Cucumis melo* L. var. inodorus) Seeds. JDDT. 2020;10(2-s):22-6.
26. OECD O. Guidelines for testing of chemicals, acute oral toxicity-fixed dose procedure. Published online 2001.
27. Tsai CC, Lin CC. Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on carrageenan- and adjuvant-induced paw edema in rats. J Ethnopharmacol. 1998;64(1):85-9. https://doi.org/10.1016/S0378-8741(98)00108-1
28. Dulcetti Junior O, Andreucci VC, Cunha IB da S, Araujo CEP, de Oliveira F, Marcucci MC. Investigation of the anti-inflammatory and analgesic activities of a sample of Brazilian propolis. Acta Farm Bonaerense. 2004;23(3):285-91.
29. Manouze H, Bouchatta O, Gadhi AC, Bennis M, Sokar Z, Ba-M'hamed S. Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of *Anacyclus pyrethrum* roots. Front pharmacol. 2017;8:598. https://doi.org/10.3389/fphar.2017.00598
30. Benhammou N, Bekkara FA, Panovska TK. Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. C R Chim. 2009;12(12):1259-66.
31. Markom M, Hasan M, Daud W, Singh H, Jahim J. Extraction of hydrolysable tannins from *Phyllanthus niruri*Linn.: Effects of solvents and extraction methods. Sep Purif Technol. 2007;52(3):487-96. https://doi.org/10.1016/j.seppur.2006.06.003
32. Wijngaard H, Hossain MB, Rai DK, Brunton N. Techniques to extract bioactive compounds from food by-products of plant origin. Food Res Int. 2012;46(2):505-13. https://doi.org/10.1016/j.foodres.2011.09.027
33. Benhammou N, Ghambaza N, Benabdelkader S, Atik-Bekkara F, Panovska FK. Phytochemicals and antioxidant properties of extracts from the root and stems of *Anabasis articulata*. Int Food Res J. 2013;20(5):2057-63.
34. El-Haci IA, Bekkara FA, Mazari W, Gherib M. Phenolics content and antioxidant activity of some organic extracts of endemic medicinal plant *Anabasis aretioides* Coss. &Moq. from Algerian Sahara. Pharmacogn J. 2013;5(3):108-12. https://doi.org/10.1016/j.phcgj.2013.05.004
35. Berrani A, Marmouzia I, Kharbach M, Bouyahya A, El Hamdani M, El Jemli M, Lrhorfi A, Benassaoui H, Zouarhi M, Larbi OM, El Abbes Faouzi M, Bengueddour R. *Anabasis aretioides*Coss. &Moq. phenolic compounds exhibit *in vitro* hypoglycemic, antioxidant and antipathogenic properties. JBCPP. 2019;30(2):251-7. https://doi.org/10.1515/jbcpp-2018-0154
36. Dudonne S, Vitrac X, Coutiere P, Woillez M, Mérillon JM. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J Agric Food Chem. 2009;57(5):1768-74. https://doi.org/10.1021/jf803011r
37. Bouaziz A, KhennoufS, ZargaMA, Abdalla S, Baghiani A, Charef N. Phytochemical analysis, hypotensive effect and

antioxidant properties of *Myrtus communis* L. growing in Algeria. Asian Pac J Trop Biomed. 2015;5(1):19-28. https://doi.org/10.1016/S2221-1691(15)30165-9
38. Mohammed H A, Alshalmani SK, Abdellatif AG.Antioxidant and quantitative estimation of phenolic and flavonoids of three halophytic plants growing in Libya. J Pharmacog Photochem. 2013;2(3):89-94.
39. Kamath V, Rajini PS. The efficacy of cashew nut (*Anacardium occidentale* L.) skin extract as a free radical scavenger. Food Chem. 2007;103(2):428-33. https://doi.org/10.1016/j.foodchem.2006.07.031
40. Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, Altman A, Kerem Z, Flaishman MA. Antioxidant activities and anthocyanin content of Fresh Fruits of Common Fig (*Ficus carica*L.). J Agric Food Chem. 2006;54(20):7717-23. https://doi.org/10.1021/jf060497h
41. Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. LWT - Food Sci Technol. 2008;41(3):385-90. https://doi.org/10.1016/j.lwt.2007.03.011
42. Kiranmayi GVN, Anusha V, Chandrika Y, Priya IVS. Preliminary phytochemical screening and in vitro evaluation of anti-inflammatory, antiarthritic, and thrombolytic activities of ethanolic leaf extract of *Bauhinia purpurea*. Int J Green Pharm. 2018;12(1):S241-7.
43. De P, Sarkar S, Mukhophadhyay MJ. Anti protein denaturation activity and bioactive compound screening of *Piper betel* aqueous and alcoholic leaf extract. J pharmacognphytochem. 2017;6(2):52-5.
44. Henneh IT, Ameyaw EO, Biney RP, Armah FA, Obese E, Konjah D, Teye E.*Ziziphusabyssinica* hydro-ethanolic root bark extract attenuates acuteinflammation possibly through membrane stabilization and inhibition of proteindenaturation and neutrophil degranulation. West Afr Pharm. 2018;29(2):81-94.
45. Mahdjar S, Bakka C, Dendougui H, Hadjadj M. Phytochemical profile and *in vitro* anti-inflammatory activity of *Anvillea radiata* (Coss and Dur) flowers extracts. AJRC. 2020;13(1):44. https://doi.org/10.5958/0974-4150.2020.00010.3
46. Guefack MGF, Damen F, Ngaffo CMN, Kuete V. Acute and sub-chronic toxicities assessment of methanol bark extract of *Hypericum roeperianum* in rats. S Afr J Bot. 2022;150:691-701. https://doi.org/10.1016/j.sajb.2022.08.006
47. George P. Concerns regarding the safety and toxicity of medicinal plants - An overview. J Appl Pharm Sci. 2011;1(6):40-4.
48. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N, Jaijoy K. Acute and subchronic toxicity study of the water extract from *Tiliacoratriandra* (Colebr.) Diels in rats. SJST. 2008;30 (5):611-9.
49. Righi N, Boumerfeg S, Deghima A, Fernandes PAR, Coelho E, Baali F, Cardoso SM, Coimbra MA, Baghiani A. Phenolic profile, safety assessment, and anti-inflammatory activity of *Salvia verbenaca* L. J Ethnopharmacol. 2021;272:113940. https://doi.org/10.1016/j.jep.2021.113940
50. Nouioura G, Tourabi M, Tahraoui A, El-yagoubi K, Maache S, Elfatemi H, Lyoussi B, Derwich EH. Assessment of the acute and subacute toxicity of the aqueous extract of Moroccan *Ferula communis* fruit in a mouse model. SPJ. 2023;31(8):101701. https://doi.org/10.1016/j.jsps.2023.101701
51. Aouachria S, Boumerfeg S, Benslama A, Benbacha F, Guemmez T, Khennouf S, Arrar L, Baghiani A. Acute, sub-acute toxicity and antioxidant activities (*in vitro* and *in vivo*) of *Reichardi apicroide* crude extract. J Ethnopharmacol. 2017;208:105-16. https://doi.org/10.1016/j.jep.2017.06.028
52. Grande D, Gioia MI, Terlizzese P, Iacoviello M. Heart Failure and Kidney Disease. In: Islam MdS, editor. Heart Failure: From Research to Clinical Practice. Vol. 3.Springer International Publishing; 2017. p. 219-38. (Advances in Experimental Medicine and Biology; Vol. 1067) https://doi.org/10.1007/5584_2017_126
53. Ben Khedir S, Mzid M, Bardaa S, Moalla D, Sahnoun Z, Rebai T. *In vivo* evaluation of the anti-inflammatory effect of *Pistacia lentiscus* fruit oil and its effects on oxidative stress. eCAM. 2016;2016:1-12. https://doi.org/10.1155/2016/6108203
54. Rosa MD, Willoughby DA. Screens for anti-inflammatory drugs. J Pharm Pharmacol. 1971;23(4):297-8. https://doi.org/10.1111/j.2042-7158.1971.tb08661.x
55. Boussouf L, Boutennoune H, Kebieche M, Adjeroud N, Al-Qaoud K, Madani K. Anti-inflammatory, analgesic and antioxidant effects of phenolic compound from Algerian *Mentha rotundifolia* L. leaves on experimental animals. S Afr J Bot. 2017;113:77-83. https://doi.org/10.1016/j.sajb.2017.07.003
56. Karbab A, Mokhnache K, Ouhida S, Charef N, Djabi F, Arrar L, Mubarak MS. Anti-inflammatory, analgesic activity, and toxicity of *Pituranthosscoparius* stem extract: An ethnopharmacological study in rat and mouse models. J Ethnopharmacol. 2020;258:112936. https://doi.org/10.1016/j.jep.2020.112936
57. Tunon M, Garcia-Mediavilla M, Sanchez-Campos S, Gonzalez-Gallego J. Potential of flavonoids as anti-inflammatory agents: Modulation of pro-inflammatory gene expression and signal transduction pathways. Curr Drug Metab. 2009;10(3):256-71. https://doi.org/10.2174/138920009787846369
58. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. Nutrients. 2018;10(11):1618. https://doi.org/10.3390/nu10111618
59. Al Amin M, Chowdhury IA, Mahbub KMM, Sattar M, Shahriar M, Kuddus MR, Rashid MA.Anti-inflammatory and Analgesic Activities of *Asteracantha longifolia*Nees. Bangla Pharma J. 2012;15(2):171-6. https://doi.org/10.3329/bpj.v15i2.12586
60. Sobeh M, Rezq S, Cheurfa M, Abdelfattah MAO, Rashied RMH, El-Shazly AM, Yasri A, Wink M, Mahmoud MF. *Thymus algeriensis* and *Thymus fontanesii*: Chemical composition, *in vivo* anti-inflammatory, pain killing and antipyretic activities: A comprehensive comparison. Biomolecules. 2020;10(4):599. https://doi.org/10.3390/biom10040599
61. Aham EC, Okagu IU, Nwodo FOC, Ndefo JC. Analgesic and anti-inflammatory activities of ethanol extract of *Hymenodictyonpachyantha* K. Krause stem bark. Comp Clin Pathol. 2019;28(6):1779-90. https://doi.org/10.1007/s00580-019-03019-5
62. Meotti FC, Missau FC, Ferreira J, Pizzolatti MG, Mizuzaki C, Nogueira CW, Santos AR.Anti-allodynic property of flavonoid myricitrin in models of persistent inflammatory and neuropathic pain in mice. BiochemPharmacol. 2006;72(12):1707-13. https://doi.org/10.1016/j.bcp.2006.08.028
63. Ramesh M, Nageshwar Rao1 Y, Appa Rao AVN, Prabhakar MC, Seshagiri Rao1 C, Muralidhar N, Madahava Reddy B. Antinociceptive and anti-inflammatory activity of a flavonoid isolated

from *Carallumaattenuata*. J Ethnopharmacol. 1998;62(1):63-6. https://doi.org/10.1016/S0378-8741(98)00048-8

64. Farahpour MR. Antioxidant activity, Antinociceptive and anti-inflammatory effects of Pot marigold hydroalcoholic extract on experimental animals. Inter J Pharm Tech Res. 2014;6(5):1640-6.65

65. Khouya T, Ramchoun M, Hmidani A, Bouhlali EDT, Amrani S, Alem C. Phytochemical analysis and bioactivity evaluation of Moroccan Thymus atlanticus (Ball) fractions. Sci Afr. 2021;11:e00716. https://doi.org/10.1016/j.sciaf.2021.e00716