LC-MS/MS ANALYSIS, *IN VITRO*, *IN VIVO* AND *IN SILICO* ANTI-INFLAMMATORY EVALUATION OF *ANABASIS ARTICULATA* (FORSSK.) MOQ. EXTRACTS

YASMINA MAKHLOUF,[*] AMEL BOUAZIZ,[*] ZOHRA DOUAA BENYAHLOU,[**] WALID BOUSSEBAA,[***] NABIL BENAZI,[****] SALEM YAHIAOUI,[**] SEDDIK KHENNOUF[*] and DAHAMNA SALIHA

[*]*Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1, Setif, Algeria*
[**]*Abdelhamid Ibn Badis University of Mostaganem, Laboratory of Technology and Solid Properties (LTPS), Mostaganem, Algeria*
[***]*Scientific and TechnicalResearch in Physico-Chemical Analysis Center – CRAPC, Tipaza, Algeria*
[****]*Institut Pasteur Algeria, Antenna M'sila, M'sila, Algeria*
✉ *Corresponding author: A. Bouaziz, a.bouaziz@univ-setif.dz*

Received July 22, 2024

Anabasis articulata (Chenopodiaceae), commonly called Ajrem, is a medicinal plant of Algerian flora of arid and semi-arid regions, extensively used in complementary medicine to treat diabetes, eczema, fever, and kidney diseases. The current investigation was intended to evaluate the anti-inflammatory potential of *A. articulata* ethanolic extract (EEAA) and its fractions that were separated using decreasing polarity solvents (hexane, chloroform, ethyl acetate, and butanol) to obtain an ethanolic extract (EEAA), a chloroform extract (ChFA), an ethyl acetate extract (EAFA), an n-butanol extract (nBFA), and an aqueous extract (AqFA). These fractions were analyzed using LC-MS-MS, whereas polyphenols, flavonoids, and tannins were evaluated using colorimetric methods. For the acute toxicity study, one oral dose of 2 and 5 g/kg was administered to mice. The *in vitro* anti-inflammatory properties were determined by using the egg albumin denaturation test, whereas the *in vivo* anti-inflammatory effect was assessed using carrageenan, croton oil, and xylene-induced edema tests. The anti-inflammatory properties of these natural compounds were assessed *in silico* via molecular docking simulations applying the cyclooxygenase COX_2 inhibitory impact. Seven metabolites were identified: anthrone, beta-carotene, butylated hydroxyanisole, butylatedhydroxytoluene, gallic acid, myricetin, and rutin. ChFA contained the greatest quantity of polyphenols and flavonoids (497.98±0.377 mg GAE/g and 79.89±0.789 mg QE/g). While nBFA showed the highest amount of total tannins (162.89±2.103 mg TAE/g). The evaluation of the *in vitro* anti-inflammatory properties revealed that all fractions of *A. articulata* had a potent anti-inflammatory effect. No death, no toxicological symptoms, and no appreciable body weight changes between the treated and control groups were observed. Oral administration of EEAA (200 mg/kg) significantly reduced the edema induced by carrageenan, croton oil, and xylene. The molecular docking showed that beta carotene, myricetin, and rutin exhibited the most promising inhibition against COX_2. Significant anti-inflammatory effects were demonstrated by *A. articulata* extract *in vitro*, *in vivo*, and *in silico*. The administration of *A. ariculata* ethanolic extract can be regarded as non-toxic. These findings are consistent with the plant's traditional applications, which include therapy of anti-inflammatory illnesses.

Keywords: *Anabasis articulata* (Forssk) Moq., anti-inflammatory activity, molecular docking, carrageenan, croton oil, xylene

INTRODUCTION

Oxidative stress is defined as an imbalanced production of reactive oxygen species relative to antioxidant defenses.[1] Free radical overproduction and a deficiency in antioxidants are the causes of this disorder.[2] However, oxidative stress develops when a cell is unable to produce the antioxidants to repair this harm. One of the main factors sustaining inflammation is oxidative stress. Therefore, it plays a part in the appearance of disorders linked to chronic inflammation.[3]

Unfortunately, using synthetic anti-inflammatory medications frequently has unfavorable side effects, such as gastrointestinal,[4] renal disorders,[5] cardiovascular complications.[6] Also, one of the major causes is the non-selective inhibition of both of COX_1 and COX_2.[7]

Consequently, there is a pressing need to find and employ more effective and natural medicines.[8] The stability, quality, and shelf durability of products have been found to be improved by antioxidants, both natural and synthetic. However, there may be toxicity risks. Thus, the use of synthetic antioxidant molecules is currently under consideration.[9] Due to their relatively mild side effects, herbal plants are effective at treating a wide range of illnesses. Due to their powerful antioxidant action, the demand for natural medications, primarily derived from plant sources, has grown over the past few decades.[10]

A. articulata or Ajrem, as it is known locally, belongs to the Chenopodiaceae family and is a herbal remedy that is widely used in traditional Algerian therapy to treat diabetes, high temperature, headaches, and skin diseases, including dermatitis.[11] As a solitary herb or in combination with other therapeutic herbs, it is taken orally after being decocted in water.[12] According to Abdallaha *et al*.,[13] the methanolic crude extract of the aerial portion has shown the strongest anti-inflammatory efficacy in rats. Abdulsahib[14] investigated the beneficial impact of *A. articulata* stem extract on decreasing intraocular pressure in a glaucoma rat models. Current phytochemical research on *A. articulata* resulted in the identification of four acknowledged saponins: 3-O-gluco-pyranosyl of stigmasterol, ßeta-sitosterol, sitostanol, 3-O-(ßeta-D-the gluco-pyranosyl] oleanolic acid, 3-O-[ßeta-D-gluco-pyranosyl -28-O-ßeta-D- xylopyranosyl] oleanolic acid, in addition to proceric acid.[15] According to Gamal *et al*.,[16] seven triterpenes were separated by various chromatographic techniques from methylene chloride, ethyl acetate, and n-butanol fractions of the aerial portions of *A. articulata* (Forssk) Moq.

The outcomes of molecular docking can be used to evaluate and supplement the findings of biological experimentation. In biological experiments, the impact of a molecule on the functioning of a system is experimentally assessed. Examples of these tests include binding assays and activity tests on cells or organisms.[17] ,mmResearchers can direct the design of novel compounds, evaluate their therapeutic potential, and enhance their biological features by employing this integrated method. By giving predictive information and removing the need for expensive and time-consuming studies, this increases and refines the drug discovery process.[18]

Few studies have looked at the composition, antioxidant, anti-inflammatory, and toxicity of the ethanolic extract of *A. articulata* leaves and flowers mixture, despite reports from researchers that the plant has therapeutic qualities. Therefore, the present study is an attempt to explore this extract for its composition of secondary metabolites (total phenolics, total flavonoids, and total tannins), for its antioxidant activity, and for the acute toxicity to explore natural antioxidant agents. The purpose of this investigation is also to determine safety, *in vitro* antidiabetic activity, and effective dose requirements for anti-inflammatory effects *in vitro* and *in vivo*. These studies have been preceded by *in vitro* studies and docking exploration to elucidate the process of action.

EXPERIMENTAL

Plant material

A. articulata was harvested in October–November 2020 during the flowering period in El Mergueb natural reserve in M'sila province (North Algeria), at 35° 42′ N latitude and 4° 32′ E longitude. Professor Oujhih, a renowned taxonomist at the University of Batna, identified this plant. The laboratory received a voucher specimen with the identification number SNV 0045-2020. An electric mixer was used to crush and powder the *A. articulata* flowers and leaves after they had been air-dried.

Animal models

Albino mice and rats (weighing 18–30 and 120–200 g, respectively) were purchased from Pasteur Institute in Algiers, and fed with unrestricted access to food and water. They were adapted during ten days to optimal laboratory conditions (12 h light/dark cycle and 25 ±0.2 °C).

Preparation of the crude extract

According to the Markham[19] method, the extracts were made by homogenizing powdered flowers and leaves, mixing them with 1 liter of ethanol and water (70:30 v/v), and agitating the mixture for five days. The first filtrate was obtained by filtering the resulting solution. This process was then repeated with the residue, utilizing a 50:50 v/v mixture and agitating it for two days to obtain the final filtrate. This final filtrate was mixed with the initial one. Crude ethanolic

extract (EEAA) was obtained after evaporating the solvent set at 40°C.

Crude ethanolic extract fractionation

Fractions were obtained according to the method of Markham.[19] Several hexane washes were performed on the concentrated hydroethanolic solution until a transparent layer of hexane was formed. The bottom layer was subsequently extracted with chloroform, ethyl acetate, and n-butanol, respectively, to get four fractions after the solvent of each fraction was evaporated: chloroform (ChFA), ethyl acetate (EAFA), n-butanol (nBFA) and aqueous fraction (AqFA).

Liquid chromatography mass spectrometry analysis (LC-MS/MS)

Chromatographic analysis was performed using a Shimadzu 8040 UPLC-ESI-MS/MS system equipped with an Ultra-Fast Mass Spectrometry (UFMS) technology and a binary pump Nexera XR LC-20AD. The ESI source conditions were optimized as follows: CID gas, 230 kPa; conversion dynode, -6.00 kV; interface temperature, 350 °C; DL temperature, 250 °C; nebulizing gas flow, 3.00 L/min; heat block, 400 °C; drying gas flow, 15.00 L/min.

The ion trap mass spectrometer was operated in both positive and negative ion modes using Multiple Reaction Monitoring (MRM).

According to Ben Amor *et al*.,[20] the chromatographic conditions separation were achieved using the Ultra-Force C18 Column (I.D. 2.5 mm 100 mm, 1.8 m particle size; Restek), maintained at 35 °C. The mobile phase consisted of a binary gradient of solvent A (water, 5 mM ammonium formate, and 0.1% formic acid) and solvent B (methanol, 5 mM ammonium formate, and 0.1% formic acid). The gradient program was as follows: 0-10 min, 95% A; 10-25 min, 5% A; 15-35 min, 5% A; 35-55 min, 95% A. The flow rate was set to 0.4 mL/min.

A solid phase extraction (SPE) was prepared. So, 20 mg aliquot of the extract was dissolved in 10 mL of ultrapure water and purified using a vacuum-driven isolute C18 SPE cartridge (pre-conditioned). The retained polyphenols were eluted with 3 mL of methanol and filtered through a 0.22 µm nylon filter. The resulting filtrate was directly injected into the LC-MS/MS system.

The polyphenol standards used were: acetylsalicylic acid, benzoic acid, cennamic acid, coumaric acid, folic acid, gallic acid, maleic acid, vanillic acid, ascorbic acid, beta carotene, caffeic acid, catechol, chrysine, coumarin 4-hydroxy, epicatechin, hesperitin, hydroxy anisol butyl, hydroxy toluene butyl, merycetin, quercitin, rutin, pyrogallol, para coumaric acid, keampferol, and chlorogenic acid.

Preliminary phytochemical analysis

Determination of extraction yield

The weight ratio of the extract to the treated plant weight is known as the yield of the plant extract. The yield, reported in percentages, was estimated using the following equation:

$$\text{Yield, \%} = W_{extract} / W_{plant} \times 100 \quad (1)$$

where $W_{extract}$ = the extract's weight, W_{plant} = the plant's weight.

Determination of total phenolic content

Spectrophotometric estimation of the phenolic component amount in *A. articulata* fractions was conducted using the Folin-Ciocalteu reagent technique, as reported by Li *et al*.[21] In order to perform the technique, 0.5 mL of the diluted Folin-Ciocalteu reagent were added to 0.1 mL of either the extract or gallic acid. Four minutes later, 0.4 mL of sodium bicarbonate (7.5%) were added. During a 90 minutes incubation period in the dark, at ambient temperature, the absorbance was measured at 765 nm. The total polyphenols were quantified using the regression equation of the gallic acid calibration curve at various concentrations. The findings are reported in milligrams of gallic acid per gram (mg EAG/g of extract).

Determination of flavonoids content

Quantitative determination of flavonoids was performed using the aluminum trichloride method.[22] 1 mL of the $AlCl_3$ solution (2%) was combined with the same volume of the fraction solution, each at varying concentrations produced in the suitable solvent. The absorbance was measured at 430 nm after a 10 minutes incubation period. The amount of flavonoids was estimated using the regression equation of the quercetin calibration curve at various concentrations. The findings were reported in milligrams of quercetin per gram (mg EQ/g of extract).

Determination of total tannin content

Prasanth *et al*.[23]described an approach for determining the total quantity of tannins in extracts utilizing the Folin-Ciocalteu reagent and tannic acid as standard. 2.5 milliliters of water-mixed 10% Folin-Ciocalteu's reagent, 0.5 milliliters of each extract, and 2.5 milliliters of Na_2CO_3 (7.5%) were added to the reaction mixture. For 45 minutes, in complete darkness, the samples were incubated at 45 °C. Then, using various tannic acid concentrations (25–300 µg/mL), the absorbance was measured at 765 nm, and the calibration curve was created. The total tannin was quantified using the regression equation of the tannic acid calibration curve at various concentrations. The findings are reported in milligrams of tannic acid per gram (mg TAE/g E of extract).

In vitro *anti-inflammatory estimation*

The protocol described by Bouaziz *et al.*[24] was used to conduct the protein denaturation experiment. To get a dilution solution of 1:100, the volume of egg white was adjusted using buffer solution Tris-HCl (20 Mm, pH = 6.8). After gently stirring the mixture for ten minutes, the solution was filtered. After transferring identical quantities to tubes, either aspirin or extract was added, and the tubes were incubated for 15 minutes at 74 °C. The percentage of protein denaturation inhibition was estimated based on the absorbance measured at 650 nm.

Protein denaturation inhibition, % = [(Ab $_{control}$ − Ab $_{treated}$)/ Ab $_{treated}$] × 100 (2)

***In vivo* investigations**

Acute toxicity study

The toxicity of EEAA at a single dose or acute toxicity was assessed on mice according to the Organization of Economic Co-operation and Development Guidelines 423.[25] The animals were randomly divided into three groups, containing five male mice each. The ethanolic extract of *A. articulata* was supplied orally to two treatment groups at a single dose of 2 and 5 g/kg body weight, whereas the control group received just distilled water. Day 0 was designated as the first dose day, while day 14 was designated as the sacrifice day. Individual lots of mice were monitored for 4 hours after the treatment to detect any behavioral and physiological changes in comparison to the control. Gross behavioral and toxic effects were observed at short intervals for 24 h, then every day for the following 14 days. To detect late effects, all of the mice were sacrificed on day 14. Then, mortality, body weight, macroscopic analysis, and relative organ weight were then calculated as parameters:

-Mortality: during the 14 days period, all the animals were detected daily for clinical signs and mortality patterns;

-Body weight: the body weight of each mouse was calculated using a sensitive balance during the study period, once on day 0, day 7, and day14 of the experiment;

-Relative organ weight: on day 14 of the study period, all the animals were euthanized by exsanguination under anesthesia of chloroform. Diverse organs, namely the heart, liver, kidneys, and spleen were cautiously dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

Relative organ weight = (absolute organ weight / body weight of mouse on sacrifice day) × 100 (3)

Anti-inflammatory activity

Three procedures were used to examine the anti-inflammatory activity of EEAA: Carrageenan induced paw edema in rats, croton oil, and xylene induced ear edema in mice.

Carrageenan-induced paw edema in rats

The approach previously reported by Tsai and Lin[26] was used to test our extract's anti-inflammatory properties against carrageenan-induced acute paw edema in rats. Indomethacin (10 mg/kg, p.o.), distilled water (10 mL/kg, p.o.), and EEAA (50, 100, and 200 mg/kg, p.o.) were administered to five groups of five rats each. When 0.1 mL of 1% carrageenan in normal saline was subplantarly injected into the rats' left hind paw one hour after administration, the animals experienced acute inflammation. A digital caliper was used to determine each paw's thickness. The measurements were taken before the carrageenan injection V_{t0} and one, two, three, four, five, and six hours after the inflammation was induced V_t. Edema volume was determined as the difference between V_t (1, 2, 3, 4, 5 and 6 h) and V_{t0}. To calculate the percentages of inhibition, the following equation was applied:

Carrageenan induced paw oedema inhibition (%) = $\left[\frac{[(Vt - Vt0)\ control - (Vt - Vt0)\ treated\ group]}{[(Vt - Vt0)\ control]}\right] \times 100$ (4)

Croton oil-induced ear edema in mice

With a few minor adjustments, the technique outlined by Dulcetti Jr. *et al*.[27] was used to create the mouse ear edema via topical injection of croton oil. A 5% acetone solution (v/v) was used to dissolve the croton oil, and 10 µL of the solution was applied to the right ear's anterior and posterior surfaces using a micropipette. Acetone 80 and distilled water 20 v/v were administered as the vehicle to the left ear (control). One hour before to the application of croton oil, groups of mice (n = 5) were given oral doses of EEAA (100 and 200 mg/Kg), vehicle (distilled water), or indomethacin (10 mg/Kg). Six hours later, each ear's thickness was measured using a digital caliper, and the anti-inflammatory activity was calculated using the method below to indicate the percentage of edema inhibition:

Croton oil induced ear edema (%) = [$Diff_{(neg)}$–$Diff_{(trt)}$]/ $Diff_{(neg)}$ ×100 (5)

where $Diff_{(trt)}$ represents the difference in ear edema thickness in the treatment group and $Diff_{(neg)}$ indicates the difference in ear edema thickness in the negative group.

Xylene-induced ear edema in mice

The xylene-induced ear edema test was conducted using the method of Manouze *et al*.,[28] with minor adjustments. Briefly, each group (five animals per group) was given by gavage one dose (100 or 200 mg/kg) of EEAA, indomethacin (10 mg/kg) or vehicle (10 mL/kg) one hour before. The right ear's inner and outer surfaces were treated topically with 0.02 mL of

xylene to cause ear edema. A control was the left ear. One hour after xylene application, the thickness of the ear was measured with a digital caliper before. The percentage of inhibition of ear edema was calculated using the following formula:

$$\text{Xylene-induced ear edema inhibition (\%)} = [\text{Diff}_{(neg)} - \text{Diff}_{(trt)}]/\ \text{Diff}_{(neg)} \times 100 \quad (6)$$

where $\text{Diff}_{(trt)}$ represents the difference in ear edema thickness in the treatment group and $\text{Diff}_{(neg)}$ indicates the difference in ear edema thickness in the negative group.

Molecular docking study

The study of molecular docking of the organic molecules that have been extracted from the plant was performed to complement and confirm the results in the experimental section for the *in vitro* and *in vivo* activities using AutoDock Vina program. The protein used to simulate this activity, named COX_2, has been obtained from the RCSB database with the code [3LN0].[29] The active site of the protein used is delimited by a box of dimensions: a volume of 80 × 80 × 80 $Å^3$ and a center x, y, z: 42, 32 and 32, respectively.

To ensure an effective simulation, the ligands (7 molecules: gallic acid, anthron, beta-carotene, butylated hydroxytoluene (BHT), butylhydroxyanisole (BHA), myricetin and rutin were initially prepared by optimizing them using the Gaussian program[30] to achieve a stable geometry with minimum energy. Next, the receptors (proteins) for the two activities were prepared using Discovery Studio. This involved removing water molecules and heteroatoms, and adding polar hydrogen atoms and Kollman charges.[31] Afterwards, molecular docking simulations were conducted using the AutoDock Vina program.[32]

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 *in-vitro* experiments, the statistics were presented as mean ± standard deviation (SD) with (n = 3), and as mean ± standard error of the mean (SEM) for *in-vivo* experiments with (n = 5) when the p-value was less than 0.05.

RESULTS AND DISCUSSION

Nowadays, it is commonly acknowledged that medicinal plants constitute a substantial source of bioactive substances. *A. articulata* is well known for having a few medicinal benefits. In order to value this plant, as there is little research on it in the literature, it is explored in this study as a potential source of active chemicals.

LC-MS-MS analysis

Liquid chromatography-mass spectrometry analysis (LC-MS-MS) was used to qualitatively identify the secondary metabolites found in the n-butanol, ethyl acetate, and chloroform fractions of *A. articulata*. The ion trap mass spectrometer was used in multiple reaction monitoring (MRM) mode, utilizing both positive and negative ions. Table 1 displays the phenolic chemicals that LC-MS-MS determined for the *A. articulata* fractions. The pics of molecules identified in fractions were illustrated in Figure 1. Anthrone, beta-carotene, butyledhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), gallic acid, myricetin, and rutin were the biomolecules detected.

Table 1
LC-MS-MS-determined phenolic compounds of *A. articulate* fractions

Compound name	Charge +/-	Precursor m/z	Product ion m/z	ChFA	EAFA	nBFA
Anthrone	+	195.1500	177.200 165.250	ND	D	ND
Beta carotene	+	537.200	282.500 199.25	D	D	D
Butyledhydroxyanisole BHA	+	181.100	99.15 81.05	ND	D	D
Butylatedhydroxytoluene BHT	+	221	161.3 203.25	D	D	D
Gallic acid	-	168.800	125.100	D	D	D
Myricetin	+	46.15	336.2	D	D	D
Rutin	+	611.200	73.200 282.200	D	D	D

D: detected, ND: not detected, ChFA: chloroform fraction, EAFA: ethyl acetate fraction, nBFA: n-butanol fraction

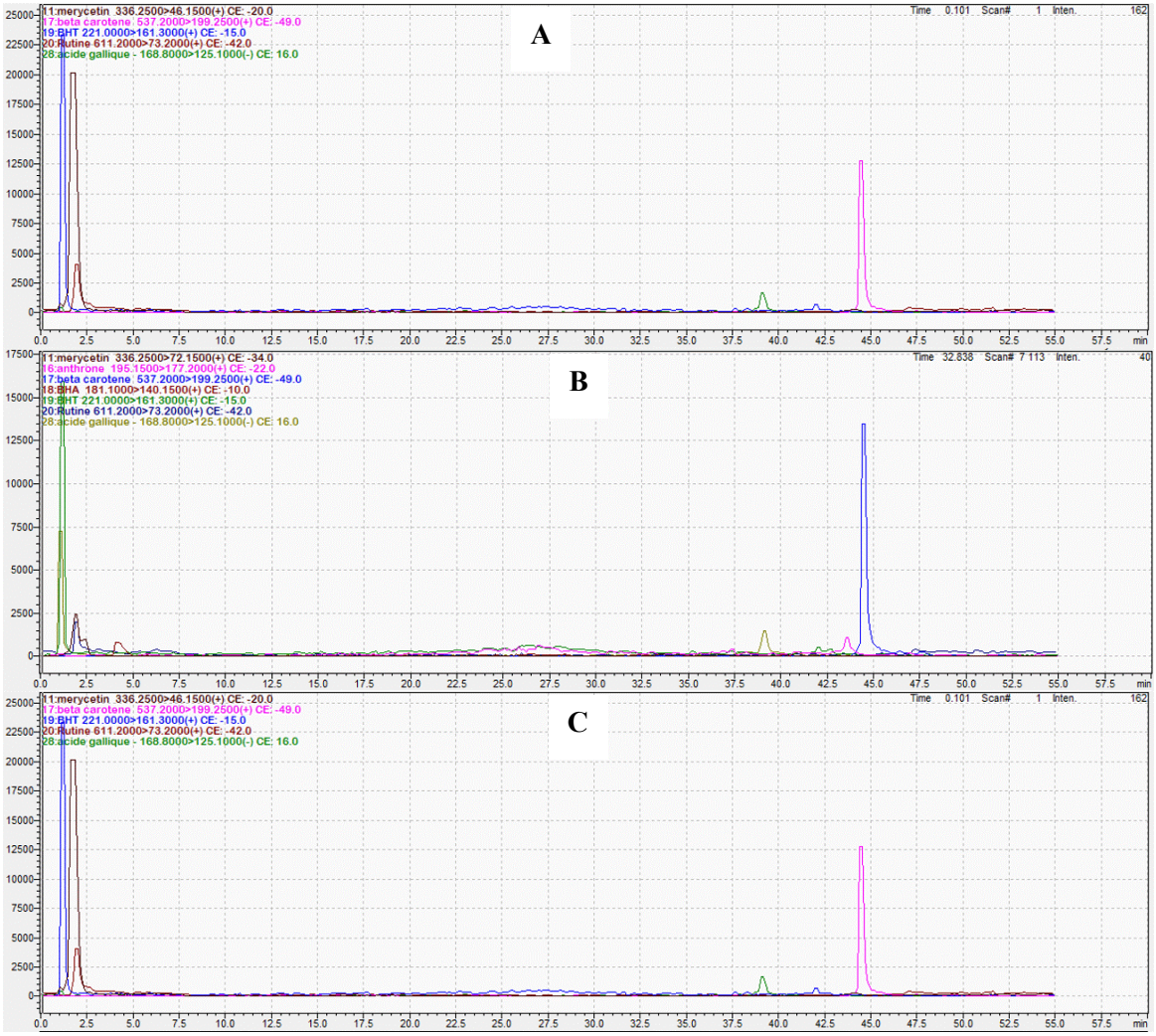

Figure 1: LC/MS-MS chromatograms obtained in the multiple reaction monitoring (MRM) mode of polyphenols detected in *A. articulata* fractions – A: chloroform fraction, B: ethyl acetate fraction, C: n-butanol fraction

Metwally *et al.*[15] isolated β-sitosterol from the unsaponifiable fraction of *A. articulata*, caffeine from the methylene chloride fraction, and 4-acetoxy phenol from the ethyl acetate fraction using chromatography analysis. These isolates were structurally elucidated using IR, EI-MS, 1H NMR, and ^{13}C NMR methods.[33] However, the chromatographic analysis of the MeOH extracts of *Anabasis aretioides* Coss. &Moq. revealed the identification of 25 phenolic compounds,[34] and two of them: gallic acid and rutin, were detected in the present study in the three studied fractions.

Preliminary phytochemical analysis

Extract yield

A. articulata was extracted using fractionation of the crude ethanolic extract. The extraction yields were calculated, and the results are presented in Table 2.

The yields of the various fraction extracts ranged from 7.6 to 42.2% for the chloroform fraction, ethyl acetate fraction, ethanolic extract, n-butanol fraction, and aqueous fraction, respectively. When the yields of the different extracts were compared, the chloroform fraction had the lowest extraction yield (7.6%), while the aqueous fraction had the highest yield (42.2%). According to Benhammou *et al.*,[35] the root of *A. articulata* methanolic extract, ethyl acetate fraction, and butanolic fraction yielded lower values than our results. Solvent extractability is primarily determined by the component's solubility in the solvent.[36]

Total phenolic, total flavonoids and total tannins contents

Total phenolic component content in fraction extracts ranged from 178.23 mg to 497.98 mg/g, as estimated by the calibration curve regression equation (y = 0.0081x - 0.0317) and expressed in gallic acid equivalents (GAE). The extracts' flavonoid content (mg/g), as estimated by the regression equation of the calibration curve (y = 0.038x - 0.0336) and expressed in quercetin equivalents, ranged from 7.50 to 79.89 mg/g. The extracts' tannin concentration (mg/g) ranged from 58.58 to 162.89 mg/g DW, as estimated by the regression equation of the calibration curve (y = 0.0083x + 0.136) and expressed in tannic acid equivalents.

The results given in Table 2 showed that the chloroform fraction indicated the highest content of total phenolics and flavonoids (497.98±0.377 and 79.89±0.789 mg/g, respectively). The n-butanol fraction has the highest content of tannins (162.89±2.103 mg/g). The amount of total phenolics and flavonoid compounds in different species of the Chenopodiaceae family, such as *Atriplex halimus* and *A. articulata*, reported by Belyagoubi-Benhammou *et al*.,[37] and in *Agatophora alopecuroide*, *A. articulata*, *Hammada elegans*, *Salsola baryosma*, *Salsola vermiculata*, reported by Djeridane *et al.*[38] were lower than those found for *A. articulata* extracts in this study. There are various explanations for the variations in the phenolic content. Many intrinsic factors, such as plant variety (genetic), plant parts, environmental circumstances (*e.g.* soil, climate irrigation, temperature range, exposure to diseases and pests), cultural techniques, harvest season, drying methods, handling and storage factors can also impact the phenolic content during the plant development cycle.[39] In terms of total tannins content, all extracts demonstrates a higher TTC than that published by Berrani *et al.*[34] for methanolic extracts of aerial parts and roots of *A. aretioides* Coss. & Moq. When compared to the common anti-inflammatory medication – aspirin, all fractions demonstrated good suppression of protein denaturation. According to these findings, *A. articulata* extracts have strong anti-inflammatory properties that are dose dependent.

***In vivo* investigations**

Acute toxicity investigation

Effect of plant extract on mortality

Mice given single doses of the ethanolic extract of *A. articulata* at 2 and 5 g/Kg b.w. demonstrated no mortality after 14 days of observation. Furthermore, there were no agitation, no lethargy and no toxic influences displayed by the animals. No lethal effects were noted throughout the observation period. No toxicity signs (no seizures and no respiratory difficulties) were observed in the animals throughout the 14 days study period. Therefore, the extract may be safe at these doses and the oral LD50 is considered greater than 2 g/kg in rats and mice.

Effect of plant extract on body weight gain of mice

The changes in the body weight of control and mice treated with the EEAA extract during the 14 days of treatment are presented in Figure 3. The body weight gain showed no significant difference (P>0.05) between the control and treated groups. However, the body weight of mice after 24 h, 48 h, 72 h, and 7 days of administration of EEAA 2 g/kg or 5 g/kg exhibited a decrease, but statistically not significant (P>0.05) as compared to the negative control.

Table 2
Yield, total phenolics content, total flavonoids content and total tannins content of *A. articulata* fractions

Extract	Yield (%)	T.P.C. (mg G.A.E/g)	T.F.C. (mg Q.E/g)	T.T.C. (mg T.A.E/g)
EEAA	15.5	396.33± 0.556	21.69± 0.154	112.49± 0.184
ChFA	7.6	497.98±0.377	79.89±0.789	140.19±2.135
EAFA	10.6	325.14±0.498	25.10±0.795	117.01±1.740
Nbfa	29.1	404.15±0.958	44.48±0.933	162.89±2.103
AqFA	42.2	178.23±1.049	7.50±0.277	58.58±1.607

Values expressed as means ±SD; T.P.C: total phenolic content; T.F.C: total flavonoids content; T.T.C: total tannins content; G.A.E: gallic acid equivalent; Q.E: quercetin equivalent; T.A.E: tannic acid equivalent; EEAA: ethanolic extract; ChFA: chloroform fraction; EAFA: ethyl acetate fraction; nBFA: n-butanol fraction; AqFA: aqueous fraction

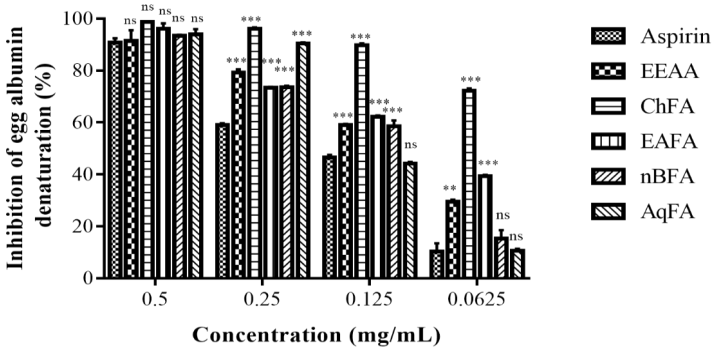

Figure 2: Egg albumin denaturation of EEAA (values are means ±SD (n=3); ns: no significant difference, **P<0.01, ***P<0.001 compared with aspirin)

Effect of plant extract on relative organ weight

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 of the extract (Fig. 4). The liver, kidneys, heart, and spleen showed no alteration in the relative weight compared to the organs of the control group.

Zero fatalities were reported over the first 14 days of the gavage acute toxicity investigation. Even at the maximum dosage of 5000 mg/kg b.w., the animals showed no physical symptoms of toxicity, as demonstrated by regular breathing and the lack of paralysis, tremors, convulsions, diarrhea, and salivation. According to OECD categorization,[25] this implies that EEAA was non-toxic and safe for mice to use during the observation period. Since no harmful effects were seen even after high-dose administration, it may be concluded that chemicals from the ethanolic extract of *A. articulata* flowers and leaves do not present acute toxicity. This finding is in line with that found by Chichi *et al*.,[42] who found that *Atriplex halimus* L. (Chenopodiaceae) at doses of 2 g/kg did not cause any behavioral abnormalities or mortality during the assay.

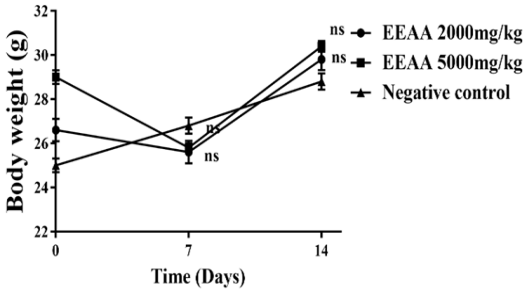

Figure 3: Body weight changes in mice treated with EEAA extract compared to negative control on days 0, 7 and 14 in the acute toxicity experiment of *A. articulata* extract (results expressed as mean±SEM (n=5 for each extract), ns – no significant difference)

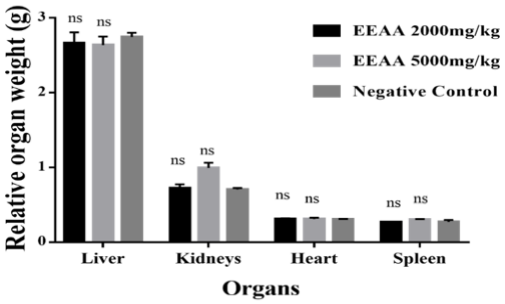

Figure 4: Relative organ weights of mice receiving EEAA after 14 days of the acute toxicity experiment compared to negative control (results are expressed as mean±SEM (n = 5), ns – no significant difference; *P<0.05)

Following the administration of EEAA, there was no discernible difference (P>0.05) in the body weight gain between the negative control and treated groups. The weight gain of the mice was unaffected by the extract during the research, indicating that changes in body weight, compared to the negative control group, would have revealed any potential toxicity of the extract. This may be partly explained by the fact that EEAA had no detrimental effects on appetite or food consumption.[43]

The present study, as illustrated in Figure 4, did not reveal any significant changes (P>0.05) in organ weight after administration of EEAA. Relative organ weights are important indicators of target organ injury and physiological disturbances.[41]

In vivo *anti-inflammatory properties*

Three procedures were used to examine the anti-inflammatory activity of EEAA: carrageenan induced paw edema in rats, croton oil, and xylene induced ear edema in mice.

Carrageenan-induced paw edema

Tests were conducted with two doses of 100 and 200 mg/kg. The acquired findings were compared with the drug; indomethacin (10 mg/kg). Table 3 illustrated the paw thickness of inflammation during 6 h of administration of standard and two doses of EEAA, respectively. In carrageenan treated animals, after a progressive increase, the paw edema peaked at 6.138±0.023 mm after 6 hours.

Table 3
Effect of EEAA on carrageenan-induced paw edema in rats over time

Treatment (mg/kg)	Paw thickness (mm)					
	0 h	1 h	2 h	3 h	4 h	6 h
Indomethacin, 20 mg/kg	4.496 ±0.078	5.62 ±0.08	5.254 ±0.073	5.058 ±0.024	4.858 ±0.046	4.62 ±0.073
Untreated group	4.816 ±0.099^{ns}	5.516 ±0.065^{ns}	5.69 ±0.066**	5.792 ±0.054***	5.918 ±0.038***	6.138 ±0.023***
EEAA 100 mg/kg	4.184 ±0.035^{ns}	5.268 ±0.143*	5.156 ±0.02^{ns}	4.954 ±0.023^{ns}	4.796 ±0.038^{ns}	4.56 ±0.037^{ns}
EEAA 200 mg/kg	4.246 ±0.081^{ns}	5.042 ±0.057**	5.054 ±0.028^{ns}	4.93 ±0.04^{ns}	4.784 ±0.043^{ns}	4.504 ±0.069^{ns}

Results are presented as mean ± SEM (n=5); ns: no significant difference, *P<0.05, **P<0.01, ***P<0.001 compared with indomethacin

However, the inhibition of paw edema in rats receiving EEAA showed anti-inflammatory activity at oral doses of 100 and 200 mg/kg of 67.515 ± 0.383% and 77.926 ± 1.733%, respectively, compared to the indomethacin treated group with a percentage of inhibition of 89.984 ± 1.323%. Abdallaha *et al*.[13] and Ben Menni *et al*.[44] reported similar findings for *A. articulata* using methanolic fraction for the first and using the sterols for the second. On the other hand, the *Chenopodium ambrosioides* (Chenopodiaceae) extract described by Ouadja *et al*.[45] could not reduce carrageenan-induced paw edema in rats.

Carrageenan can cause a range of symptoms in humans that are comparable to acute inflammation, including fast edema, increased vascular permeability, localized telangiectasia, and plasma extravasation.[46] A well-known and often used model of inflammation in rats is carrageenan-mediated paw edema, which results in a biphasic phase in the inflammatory pathway.[47] This inflammation is characterized by the successive release of many inflammatory chemicals. The initial phase (1-3 hours) contains serotonin, histamine, and bradykinin, whereas the latter phase (3-6 hours) contains prostaglandins. Inflammatory mediators and reactive oxygen species are both elevated at the final phase of inflammation. NSAIDs often do not suppress the first phase of inflammation.[48] Oral EEAA treatment reduced inflammation in the final phase of carrageenan-induced paw edema, while a dosage of 200 mg/kg reduced inflammation during the early phase.

Croton oil-induced ear edema in mice

Croton oil is extracted from *Croton tiglium* L. seeds,[49] and it causes topical irritation and edema when applied topically.[50] This test measures a variation in ear plug volume after the injection of an irritant agent in order to determine the inflammatory response. The findings of this investigation are shown in Figure 5.

Edema is effectively reduced by 65.340±1.154% and 76.193±1.135% at doses of 100 and 200 mg/kg of EEAA, respectively. Indomethacin, employed as a positive control has an estimated anti-inflammatory effect of 80.68±1.258%. Croton oil-induced dermatitis exemplifies an acute inflammatory reaction at its peak. This test is commonly used for discovering prospective anti-inflammatory medications in the management of skin conditions.[51] The 12-O-tetra-decanoyl-13-phorbol acetate (TPA) molecule is what gives croton oil its phlogogenic properties.

TPA causes inflammation that manifests as a significant increase in blood vessel permeability, neutrophils infiltration, edema, and the generation of mediators of inflammation.[52] According to Pérez *et al*.,[53] TPA-induced mouse ear edema was effectively prevented by the essential oil of *Chenopodium album* L. (Chenopodiaceae).

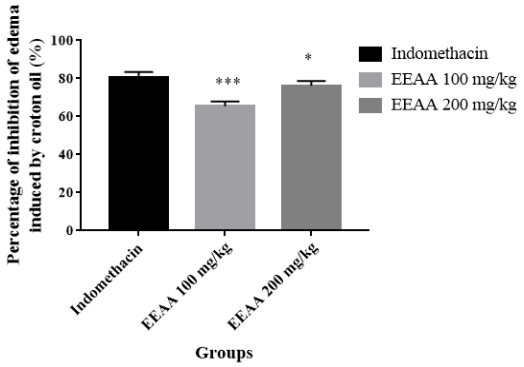

Figure 5: Percentage of inflammation reduction of EEAA in mice with croton oil-induced ear edema (values are presented as means ±SEM (n=5), *P<0.05, ***P<0.001 compared with indomethacin)

Xylene-induced ear edema

Figure 6 displays the results of this experiment. The edema is successfully reduced by EEAA at doses of 100 and 200 mg/kg, at 70.25% and 81.74%, respectively. The concurrent anti-inflammatory impact of indomethacin, the positive control, was assessed to be 82.98%.

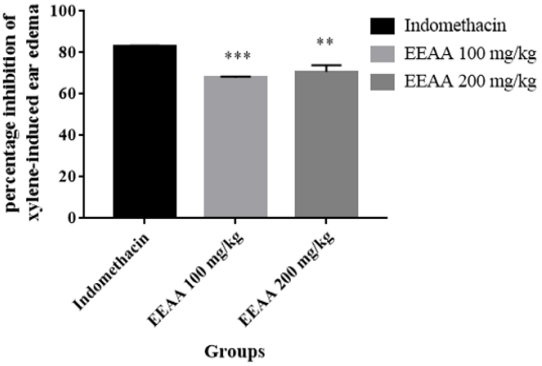

Figure 6: Percentage of inhibition inflammation of EEAA in mice with xylene-induced ear edema (values presented as means ±SEM (n=5), **P<0.01, ***P<0.001 considered significant when compared with indomethacin)

The oedematization caused by xylene in the ears of the mice resembles oedematization that occurs during the early phases of acute inflammation.[54] Following topical application of xylene, signs of acute inflammation include significant vasodilation, skin edema, and inflammatory cell infiltration.[55] The increase in ear edema caused by xylene was considerably and dose-dependently reduced by the plant extract.

EEAA has anti-inflammatory properties that may be attributed to the presence of steroids, alkaloids, and flavonoids present in various fractions. The phytochemical studies of *A. articulata* revealed a variety of secondary metabolites, including phenolics,[33] alkaloids[56] and saponins, such as 3-O-gluco-pyranosyl of stigmasterol, ßeta-sitosterol, sitostanol, 3-O-(ßeta-D-the gluco-pyranosyl] oleanolic acid, 3-O-[ßeta-D-gluco-pyranosyl -28-O-ßeta-D- xylopyranosyl] oleanolic acid, in addition to procericacid.[15]

Analysis of molecular docking

Docking of molecules is a recent bioinformatics technique that predicts the likely experimental orientation as well as the binding affinity required to establish a stable complex structure between a ligand and a target.[57] Molecular docking studies were carried out using AutoDock Vina program to determine whether the seven compounds detected provided an anti-inflammatory impact compared to the standard (indomethacin).

The result allowed us to determine the binding energy between the protein and various positions of the ligand. A negative value of the binding energy suggests a likelihood of binding between the ligand and the receptor. The inhibition constant (Ki) was then calculated applying the formula: $Ki = \exp(\Delta G/RT)$, where ΔG, R, and T represent the binding energy, the gas constant (1.9872036×10^{-3} kcal.Mol^{-1}), and the surrounding temperature (298.15 K), in that order.[58] A smaller inhibition constant indicates a more effective drug derived from the title molecule.

Table 4 gathers the results obtained from molecular docking regarding the binding energy and inhibition constant for the 7 bio-compounds that showed interaction with the protein. It was noticed that the two ligands named Rutin and Myricetin have the best affinity, with values of -10.4 kcal.Mol^{-1} and -9.9 kcal.Mol^{-1}, respectively, as well as a low inhibition constant of 0.023 µM and 0.055 µM, respectively. Additionally, we performed molecular docking using a

commercialized drug named indometacine. According to Table 4, beta-carotene, myricetin, and rutin have shown favorable results compared to the drug (indomethacin).

Table 4
Results for affinity and inhibition constant of ligands with the same protein

Biomolecules	Anti-inflammatory activity	
	Protein: COX-2 (3LN0)	
	ΔG (kcal.Mol^{-1})	Ki (μM)
Gallic acid	-6.5	17.19
Anthron	-8.6	0.49
Beta carotene	-8.8	0.35
Butylatedhydroxytoluene (BHT)	-7.4	3.76
Butylhydroxyanisole (BHA)	-6.9	8.75
Myricetin	-9.9	0.055
Rutin	-10.4	0.023
Indometacine (standard)	-8.7	0.42

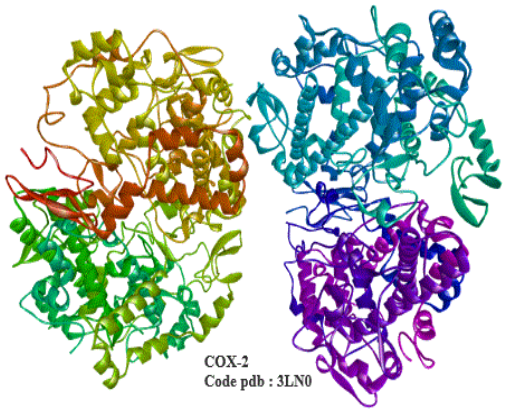

Figure 7: 3D structure of the inflammatory protein COX-2 [PDB ID: 3LN0]

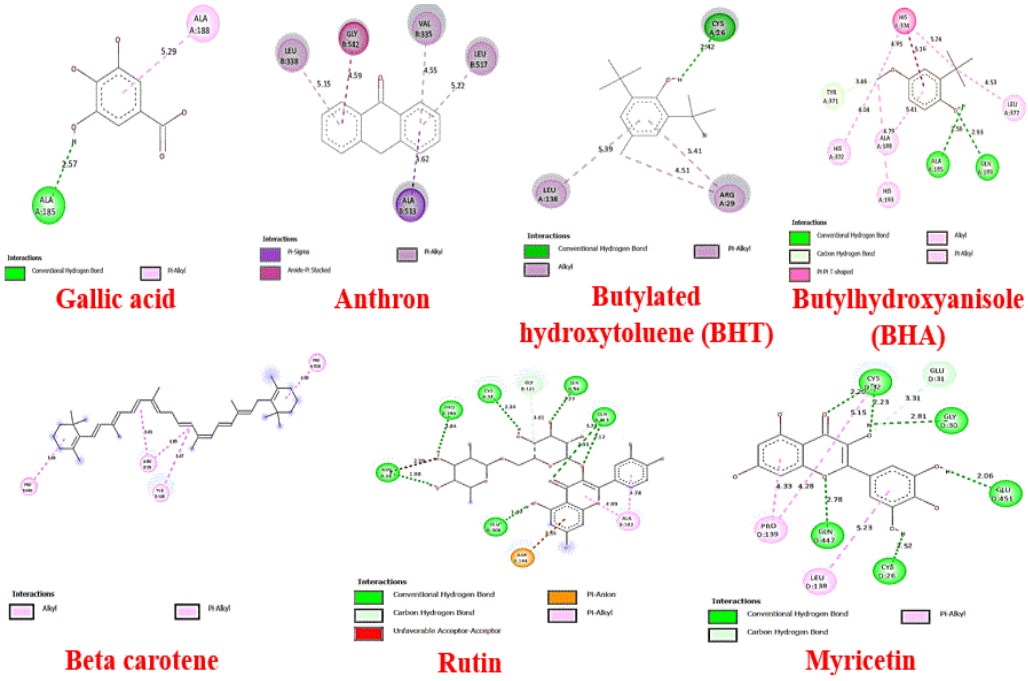

Figure 8: 2D detailed binding sites of each ligand into the receptor [PDB ID: 3LN0]

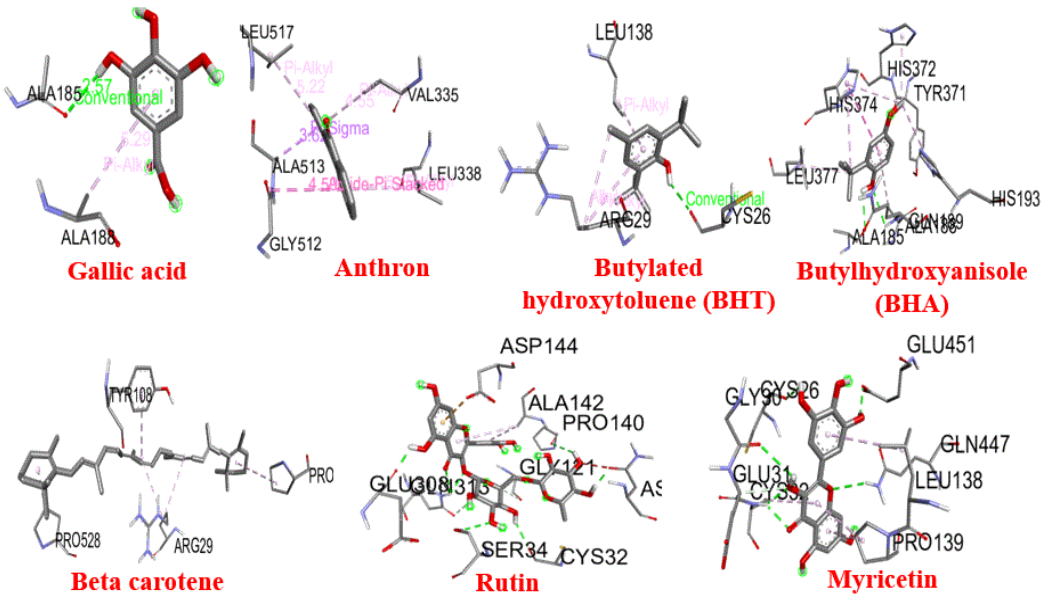

Figure 9: 3D detailed binding sites of each ligand into the receptor [PDB ID: 3LN0]

Figures 8 and 9 show many hydrogen bonding connections between each ligand and the protein. Additionally, non-covalent interactions like hydrophobic and electrostatic connections were discovered. These non-covalent interactions help the ligand-protein complexes stay stable and particular, which eventually increases their effectiveness. Based on the results obtained, each of these ligands has the potential to be an effective principle in the fight against this illness, as we can expect. Additionally, the combined efforts of these 7 ligands may produce even better results. Finally, *in silico* findings support the results of the investigation study presenting action that reduces inflammation, which demonstrated an excellent correlation with the experimental findings.

CONCLUSION

As far as we are aware, this is the first article to discuss the safety, and anti-inflammatory *in vivo* and *in vitro* and *in silico* properties of ethanolic extract from leaves and flowers mixture of *A. articulata* (Forssk.) Moq. that grows in Algeria. The findings of the inquiry into anti-inflammatory effects support the traditional usage of *A. articulata*, and the *in silico* study confirms these results. The acute toxicity research indicated that the ethanolic extract is tolerable up to a dose of 5 g/kg for mouse body weight when taken orally after a single administration. Molecules that reduce inflammation could be extracted from EEAA.

ACKNOWLEDGEMENTS: Authors thank the Algerian Ministry of Higher Education and Scientific Research (MESRS) for supporting this research study.

REFERENCES

[1] C. Cabello-Verrugio, F. Simon, C. Trollet and J. F. Santibañez, *Oxid. Med. Cell. Longev.*, **2017**, 4310469 (2016), https://doi.org/10.1155/2017/4310469
[2] M. D. Shastri, S. D. Shukla, W. C. Chong, K. Dua, G. M. Peterson *et al.*, *Oxid. Med. Cell. Longev.*, **2018**, 7695364 (2018), https://doi.org/10.1155/2018/7695364
[3] J. Lugrin, N. Rosenblatt-Velin, R. Parapanov and L. Liaudet, *Biol. Chem.*, **395**, 203 (2014), https://doi.org/10.1515/hsz-2013-0241
[4] J. B. Raskin, *Am. J. Med.*, **106**, 3S (1999), https://doi.org/10.1016/S0002-9343(99)00112-6
[5] G. Gambaro and M. A. Perazella, *J. Int. Med.*, **253**, 643 (2003), https://doi.org/10.1046/j.1365-2796.2003.01146.x
[6] B. H. McCarberg and B. Cryer, *Am. J. Ther.*, **22**, e167 (2015), https://doi.org/10.1097/MJT.0000000000000123
[7] V. Limongelli, M. Bonomi, L. Marinelli, F. L. Gervasio, A. Cavalli *et al.*, *PNAS*, **107**, 5411 (2010), https://doi.org/10.1073/pnas.0913377107
[8] M. S. Bensaad, S. Dassamiour, L. Hambaba, M. A. Kahoul and M. Benhoula, *TJPR*, **20**, 1647 (2021), https://doi.org/10.4314/tjpr.v20i8.14
[9] V. Yeşilyurt, B. Halfon, M. Öztürk and G. Topçu, *Food Chem.*, **108**, 31 (2008), https://doi.org/10.1016/j.foodchem.2007.09.055

[10] M. Jeevitha, P. V. Ravi, V. Subramaniyam, M. Pichumani and S. K. Sripathi, *Future J. Pharm. Sci.*, **7**, 228 (2021), https://doi.org/10.1186/s43094-021-00375-4
[11] V. Hammiche and K. Maiza, *J. Ethnopharmacol.*, **105**, 358 (2006), https://doi.org/10.1016/j.jep.2005.11.028
[12] N. Kambouche, B. Merah, A. Derdour, S. Bellahouel, J. Bouayed *et al.*, *AJB*, **8**, 5589 (2009)
[13] H. M. Abdallaha, A. B. Abdel-Naimc, O. M. Ashourd, I. A. Shehataa and E. A. Abdel-Sattarb, *Z. Naturforsch. C.*, **69C**, 1 (2014), https://doi.org/10.5560/ZNC.2012-0168
[14] W. K. Abdulsahib, *Iraqi J. Pharm. Sci.*, **30**, 1 (2022), https://doi.org/10.31351/vol30issSuppl.pp1-8
[15] N. S. Metwally, A. M. Mohamed and F. S. El-Sharabasy, *J. Appl. Pharm. Sci.*, **2**, 54 (2012), https://doi.org/10.7324/JAPS.2012.2403
[16] G. Gamal, K. A. Abo-El-Seoud and G. Attia, *Nat. Prod. Res.*, **36**, 4076 (2022), https://doi.org/10.1080/14786419.2021.1961769
[17] R. Mathammal, N. Jayamani and N. Geetha, *Int. J. Spectrosc.*, **2013**, 1 (2013), https://doi.org/10.1155/2013/171735
[18] Z. D. Benyahlou, F. T. Baara, S. Yahiaoui, Y. Megrouss, N. Boukabcha *et al.*, *J. Mol. Struct.*, **1277**, 134781 (2023), https://doi.org/10.1016/j.molstruc.2022.134781
[19] K. R. Markham, "Techniques of flavonoid Identification", London Academic Press, 1982, Chapters 1 and 2, p. 113
[20] S. Ben Amor, S. Mekious, L. Allal Benfekih, M. H. Abdellattif, W. Boussebaa *et al.*, *Life*, **12**, 927 (2022), https://doi.org/10.3390/life12070927
[21] H. Li, K. Cheng, C. Wong, K. Fan, F. Chen *et al.*, *Food Chem.*, **102**, 771 (2007), https://doi.org/10.1016/j.foodchem.2006.06.022
[22] T. Bahorun, B. Gressier, F. Trotin, C. Brunet, T. Dine *et al.*, *Arzneimittel-Forschung*, **46**, 1086 (1996)
[23] D. S. N. B. K. Prasanth, A. S. Rao and R. P. Yejella, *Int. J. Pharmacogn. Pharm.*, **4**, 109 (2017), https://doi.org/10.13040/IJPSR.0975-8232.IJP.4(4).109-117
[24] A. Bouaziz, S. Djidel, A. Bentaher and S. Khennouf, *JDDT*, **10 (2-s)**, 22 (2020), http://dx.doi.org/10.22270/jddt.v10i2-s.4022
[25] *OECD Guidelines for the Testing of Chemicals, Acute Oral Toxicity-Fixed Dose Procedure*, **4**, 1 (2001)
[26] C.-C. Tsai and C.-C. Lin, *J. Ethnopharmacol.*, **64**, 85 (1998), https://doi.org/10.1016/S0378-8741(98)00108-1
[27] O. Dulcetti Jr, V. C. Andreucci, I. B. da S. Cunha, C. E. P. Araujo, F. de Oliveira *et al.*, *Acta Farm. Bonaerense*, **23**, 285 (2004)
[28] H. Manouze, O. Bouchatta, A. C. Gadhi, M. Bennis, Z. Sokar *et al.*, *Front. Pharmacol.*, **8**, 598 (2017), https://doi.org/10.3389/fphar.2017.00598
[29] J. L. Wang, D. Limburg, M. J. Graneto, J. Springer, J. R. B. Hamper *et al.*, *BMCL*, **20**, 7159 (2010), https://doi.org/10.1016/j.bmcl.2010.07.054
[30] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb *et al.*, *J. Comput. Chem.*, **29**, 839 (2008)
[31] "Life Sciences and Material Sciences. BIOVIA – Dassault Systèmes" (n.d.)
[32] O. Trott and A. J. Olson, *J. Comput. Chem.*, **31**, 455 (2010), https://doi.org/10.1002/jcc.21334
[33] G. Gamal, K. Abouelsaoud, E. Elekhnawy and G. Attia, *JAMPR*, **3**, 29 (2022), https://doi.org/10.21608/jampr.2022.131451.1027
[34] A. Berrani, I. Marmouzi, M. Kharbach, A. Bouyahya, M. El Hamdani *et al.*, *JBCPP*, **30**, 251 (2019), https://doi.org/10.1515/jbcpp-2018-0154
[35] N. Benhammou, N. Ghambaza, S. Benabdelkader, F. Atik-Bekkara and F. K. Panovska, *Int. Food Res. J.*, **20**, 2057 (2013)
[36] T. Dhanani, S. Shah, N. A. Gajbhiye and S. Kumar, *Arab. J. Chem.*, **10**, S1193 (2017), https://doi.org/10.1016/j.arabjc.2013.02.015
[37] N. Belyagoubi-Benhammou, L. Belyagoubi and F. A. Bekkara, *J. Med. Plant Res.*, **8**, 1198 (2014), https://doi.org/10.5897/JMPR2014.5554
[38] A. Djeridane, A. Hamdi, W. Bensania, K. Cheifa, I. Lakhdari *et al.*, *Diabetes Metab. Syndr.*, **9**, 324 (2015), https://doi.org/10.1016/j.dsx.2013.10.007
[39] M. Moghaddam, S. N. Khaleghi Miran and L. Mehdizadeh, *Int. J. Hortic. Sci. Technol.*, **5**, 93 (2018), https://doi.org/10.22059/ijhst.2018.253067.219
[40] M. Kpemissi, Y. T. Kantati, V. P. Veerapur, K. Eklu-Gadegbeku and Z. Hassan, *IBRO Neurosci. Rep.*, **14**, 21 (2023), https://doi.org/10.1016/j.ibneur.2022.12.001
[41] G. Nouioura, M. Tourabi, A. Tahraoui, K. El-yagoubi, S. Maache *et al.*, *SPJ*, **31**, 101701 (2023), https://doi.org/10.1016/j.jsps.2023.101701
[42] I. Chikhi, H. Allali, D. M. El Amine, M. Houria and T. Boufeldja, *Asian Pac. J. Trop. Dis.*, **4**, 181 (2014)
[43] S. Sireeratawong, N. Lertprasertsuke, U. Srisawat, A. Thuppia, A. Ngamjariyawat *et al.*, *SJST*, **30**, 611 (2008)
[44] D. Ben Menni, N. Belyagoubi-Benhammou, A. Benmahieddine, H. Ben Menni, A. Gismondi *et al.*, *Waste Biomass Valor.*, **13**, 3283 (2022), https://doi.org/10.1007/s12649-022-01717-w
[45] B. Ouadja, G. Katawa, G. A. Toudji, L. Layland, E. H. Gbekley *et al.*, *JABS*, **162**, 16764 (2021), https://doi.org/10.35759/JABs.162.7
[46] X. Dai, M. Ding, W. Zhang, Z. Xuan, J. Liang *et al.*, *Med. Sci. Monit.*, **25**, 7958 (2019), https://doi.org/10.12659/MSM.916977
[47] S. Ben Khedir, M. Mzid, S. Bardaa, D. Moalla, Z. Sahnoun *et al.*, *eCAM*, **2016**, 6108203 (2016), https://doi.org/10.1155/2016/6108203

[48] J. Necas and L. Bartosikova, *Vet Med*., **58**, 187 (2013), https://doi.org/10.17221/6758-VETMED
[49] A. Salatino, M. L. F. Salatino and G. Negri, *J. Braz. Chem. Soc*., **18**, 11 (2007), https://doi.org/10.1590/S0103-50532007000100002
[50] M. Al Amin, I. A. Chowdhury, K. Mahbub, M. Sattar, M. Shahriar *et al*., *Bangla Pharma J*., **15**, 171 (2012), https://doi.org/10.3329/bpj.v15i2.12586
[51] J. Mo, P. Panichayupakaranant, N. Kaewnopparat, A. Nitiruangjaras and W. Reanmongkol, *J. Ethnopharmacol*., **148**, 901 (2013), https://doi.org/10.1016/j.jep.2013.05.040
[52] A. Karbab, K. Mokhnache, S. Ouhida, N. Charef, F. Djabi *et al*., *J. Ethnopharmacol*., **258**, 112936 (2020), https://doi.org/10.1016/j.jep.2020.112936
[53] G. S. Pérez, S. M. Zavala, G. L. Arias and L. M. Ramos, *J. Essent. Oil Res*., **23**, 38 (2011), https://doi.org/10.1080/10412905.2011.9700480
[54] M. Torres-Rêgo, A. A. Furtado, M. A. O. Bitencourt, M. C. J. D. S. Lima, R. C. L. C. D. Andrade *et al*., *BMC Complement. Altern. Med*., **16**, 275 (2016), https://doi.org/10.1186/s12906-016-1259-x
[55] H.-D. Kim, H.-R. Cho, S. Moon, H.-D. Shin, K.-J. Yang *et al*., *Arch. Pharm. Res*., **30**, 323 (2007)
[56] N. Belyagoubi-Benhammou, L. Belyagoubi, A. Gismondi, G. Di Marco, A. Canini *et al*., *Med. Chem. Res*., **28**, 754 (2019), https://doi.org/10.1007/s00044-019-02332-6
[57] S. Kumar and S. Kumar, Molecular Docking: A Structure-Based Approach for Drug Repurposing, IsDD, 2019, p. 161
[58] C. Yung-Chi and W. H. Prusoff, *Biochem. Pharmacol*., **22**, 3099 (1973), https://doi.org/10.1016/0006-2952(73)90196-2