ANTI-INFLAMATORY AND ANTI-LIPID PEROXODATION POTENTIALS OF *SALVIA CHUDAEI* BATT. et TRAB. AND *LAVANDULA ANTINEAE* MAIRE: ENDEMIC PLANTS FROM ALGERIAN SAHARA

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The present study was undertaken to investigate the anti-inflammatory and anti-lipid peroxidation activities of two endemic plants of the central Sahara; Salvia chudaei Batt. et Trab and Lavandula antineae Maire (Lamiaceae), as well as their chemical composition of hydromethanolic extracts. In the test of carrageenan-induced oedema in mice, L. antineae extract, at the dose of 200 mg/kg, revealed good anti-inflammatory activity (P<0.05) showing 80.74% reduction in the paw thickness comparable to that produced by the standard drug aspirin an83.44% at 4h, while S. chudaei extract was found to have moderate activity (50.74%). When the plant extracts were applied topically at a dosage of 1 and 2 mg per ear, the reduction of ear oedema inflammation was very remarkable compared to the mice of the control group. The plant extracts also showed anti-lipid peroxidation activity with no significant difference compared to ascorbic acid (77.63 ± 1.58%). In addition, the chemical characterization of two extracts by HPLC-DAD revealed the presence of various phenolic acids and flavonoids. These findings showed that Lavandula antineae and Salvia chudaei displays remarkable anti-inflammatory and anti-lipid peroxidation activities and hence can be potential natural sources in health and medicine.

Keywords*: Lavandula antineae; Salvia chudaei; anti-inflammatory, anti-lipid peroxidation activities; HPLC-DAD analysis.*

INTRODUCTION

Inflammation is an immunological defense process generated by the body in response to infection, injury, and irritation. However, uncontrolled and persistent inflammation contributes to the progression of many diseases, including arthritis, atherosclerosis, asthma, Alzheimer's disease (AD), and malignancies[1].

The mechanism of inflammation wound is allocated, in part, to the liberation of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. The resulting oxidative stress may lead to aging, inflammation, cancer, stroke, myocardial infarction and other chronic diseases[2&3].

Medicinal plants are relevant in the world as sources of drugs or herbal extracts for various therapeutic purposes. These natural sources are composed of a variety of biologically active compounds, mainly phenolic, and these phytochemicals have been

known to exhibit different biological properties. Thus, it is crucial to develop effective natural antioxidants and anti-inflammatory drugs with fewer adverse effects[3].

Among the medicinal plants used in Algeria which is believed to have beneficial effects are *Lavandula antineae* Maire and *Salvia chudaei* Batt. et Trab. These species belong to the family of Lamiaceae, syn: Labiatae, they are endemic plants in the central Sahara. *Lavandula antineae* (Tamahaq name: tehenok) is a small semi-evergreen perennial distributed in Algeria, Nigeria, Chad and Sudan.

Its aerial parts are used in Algerian folk medicine for the treatment of chills, bruises, oedema and rheumatism. *Salvia chudaei* (Tamahaq name: aouit) which is a perennial shrub very ramous grow only in Hoggar, Tefedest (Algeria) and Tibesti (Chad). Indigenous people employ this species for the treatment of dysmenorrhea, abdominal pains, spasms, sunstroke and gonorrhea[5&6].

Our previous studies showed that the organic extracts of these species have strong antioxidant properties with remarkable antimicrobial activity. The extracts have also been found to possess an excellent cytotoxic effect on *Artemia salina*[7&8].

In order to continue our search for the bioactive products present in these two species, we investigated for the first time their anti-inflammatory potential using carrageenan-induced paw oedema and croton oil-induced ear oedema models in mice, and their anti-lipid peroxidation activity by ferric thiocyanate method. The phenolic constitution of the plant extracts was also analyzed by the HPLC-DAD technique.

MATERIAL AND METHODS

Chemicals and Equipment
Chemicals

All organic solvents used in this work are from Sigma Aldrich, Germany, DMSO, carrageenan , croton oil, α-tocopherol , Vitamin C and BHT (Honeywell, Fluka, Germany), absolute ethanol, and linoleic acid (Sigma Aldrich, Germany), standards used for HPLC analyzise: gallic acid, caffeic acid, syringic acid, rosmarinic acid, coumaric acid, chlorogenic acid, naringenin-7-glucoside, kaempferol, catechin, rutin, luteolin-7-glycoside, quercetin, luteolin, naringenin and apigenin-7-glucoside. (Honeywell, Fluka, Germany).

Swiss albino mice (20 to 25 g) were purchased from Pasteur Institute (Algiers).

Equipment

Sensitive balance (Kern and Sohn Gmbh, Germany), Rotary evaporator (Büchi, R-200, France), UV-1800 spectrophotometer (Shimadzu, Japan), Centrifuge and *Digital Vernier Caliper* Germany, (EBA 8, Hettich, Germany), HPLC UV-DAD, Agilent 1260 (Canada)

Plant Material

Arial parts of *Salvia chudaei* and *Lavandula antineae* were collected from Hoggar, Southern Algeria (Coordinates; UTM: GF96; Latitude: 23°10'0"; Longitude: 5°49'60"), at the flowering stage. Plant identification was carried out by botanists at, National Institute for Forest Research, Tamanrasset, Algeria. The aerial plant parts were cleaned and air-dried at room temperature in the shade, and then powdered.

Extraction Procedure

Powdered plant material (20 g) was extracted for 48 hrs with 200 ml of methanol-water (7:3) at room temperature. The solvent was then removed by filtration and the fresh solvent was then added to the residue, this process was repeated three times. The combined filtrates were then concentrated under reduced pressure at 40 °C using vacuum rotary evaporator. The obtained dried extracts were kept in the dark at +4 °C prior to use.

Evaluation of anti-inflammatory activity
Experimental animals

Swiss albino mice (20 to 25 g) were purchased from Pasteur Institute (Algiers). The animals were housed for one week for acclimatization in standard cages under controlled conditions maintained at 25 ± 1 °C with a 12hrs:12hrs dark-light cycle, free access to water and a standard pellet diet. The animal experiments were done as suggested by the ethical guidelines for the care of laboratory animals.

Acute toxicity

The plant extracts were prepared in physiological solution (NaCl 0.9%) containing 3% DMSO at different doses: 100, 500, 1000 mg/kg body weight. These extracts were administrated orally to test groups (n=6). All animals were observed for 72 hrs for signs of toxicity and mortality cases.

Carrageenan-induced paw oedema

The method of Winter et al., (1962) [9], was used. Paw oedema was induced by injecting 0.05 mL of 1% of carrageenan suspended in physiological solution into the subplantar tissues of the left hind paw. The thickness of paw was measured by *Digital Vernier Caliper* before and after the injection of carrageenan every hr over a 4-hr period.

One hr before the injection of carrageenan, the animals received orally the following treatments:

- Control group receiving physiological water
- Group treated with aspirin: 100 mg/kg
- Two groups treated with *S. chudaei* extract (100 and 200 mg/kg, respectively)
- Two groups treated with *L. antineae* extract (100 and 200 mg/kg, respectively)

The anti-inflammatory activity was calculated by the following equation:

% oedema inhibition= [(Tt– T0) control– (Tt – T0) treated]/(Tt – T0) control

Tt = thickness of paw after 4 hrs
T0 = thickness of paw before the injection of carrageenan

Croton oil-induced ear oedema

The evaluation of anti-inflammatory activity using this model was carried out according to the protocol described by Sosa et al., 2005 [10]. Skin inflammation was induced on the inner surface of the right ear of the mouse by the application of 10 μl of an acetone solution containing 5% croton oil. The untreated left ear served as a control. 5 μL of different doses of the test substance solution were applied locally to the right ear 30 minutes before the deposition of the croton oil solution. Different groups of mice (n = 6) were treated as follows:

- Control group received only the croton oil solution.

- Group receiving aspirin (0.5 mg / ear) + croton oil solution.
- Two groups receiving S. chudaei extract (1 and 2 mg/ear) + the croton oil solution.
- Two groups receiving *L. antineae* extract (1 and 2 mg/ear) + the croton oil solution.

Four hrs after the induction of inflammation, the mice were sacrificed. The left and the right ear were then sectioned at the level of their implantation and discs with a thickness of 6 mm were taken and weighed immediately.

The topical anti-inflammatory activity was calculated according to the following formula:

% oedema inhibition = [(Wr-Wl) control mouse - (Wr-Wl) treated mouse) / (Wr-Wl) control mouse

Wr = Weight of the right ear.
Wl = Weight of the left ear

Evaluation of anti-lipid peroxidation activity

The anti-lipid peroxidation activity analysis using ferric thiocyanate (FTC) was performed according to the procedure reported by Kikuzaki and Nakatani (1993) [11]. A mixture containing 4 mg of the sample in 4 ml of absolute ethanol, 4.1 ml of 2.52 % linoleic acid in absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at 40 °C in the dark. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid, the absorbance of the red color was measured at 500 nm every 24hrs until one day after the absorbance of control reached its maximum. Ascorbic acid, BHT and α-tocopherol were used as standards while the mixture without the plant extract was used as the control.

The lipid peroxidation inhibition was calculated as follows:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where A_C is the absorbance of the control reaction and A_S is the absorbance of sample extracts. The values obtained for the control samples were taken for 100% lipid peroxidation. The experiment was carried out in triplicate

HPLC-DAD analysis

High-performance liquid chromatography (HPLC) of samples was performed with an Agilent 1260 Infinity equipped with UV-VIS detector DAD. The analysis was carried out in reverse phase with a C18 column (5 μm, 250 × 4.6 mm). The temperature was maintained at 22 ± 0.8° C and the volume injection was 5 μl. The solvents used are HPLC grade and the flow rate is set at 1 mL/min. The chromatographic conditions consist of a gradient A: acetonitrile / B: acetic acid 0.2% with the following gradient: 0 min.: 95% A + 5% B; 30 min.: 5% A + 95% B. Detection was performed at 270 nm, 320 nm and 370 nm. The identification of compounds was done by comparing the retention times and the UV spectra obtained by those of the standards.

Statistical Analysis

The data were expressed as means ± S.D. Differences were evaluated by a one-way analysis of variance (ANOVA) test completed by the Tukey test. Differences were considered significant at P< 0.05.

RESELTS AND DISCUSSION

Results
Plant toxicity in mice

The lethal dose (LD_{50}) of *S. chudaei* and *L. antineae* is not known in the literature. Thus, the search for eventual toxicity is necessary to carry out anti-inflammatory tests. In this study, we found no symptoms of toxicity and no mortality in mice given orally the extracts of *S. chudaei* and *L. antineae* at doses of 100, 500 and 1000 mg/kg. The LD_{50} is therefore greater than 1 g / kg. These results reveal that the use of doses below 1000 mg/kg presents no risk to experimental animals.

Anti-inflammatory activity
Paw oedema Model

In this model, carrageenan is widely used as a phlogistic agent and the evaluation of inflammation can be performed by measuring the thickness of oedema. The results are represented in figure 1.

In the control group, the injection of 50 μL of the 1% carrageenan solution into the left hind paw causes visible inflammation within one hr after this injection (the thickness of the oedema is 3.21 ± 0.34 mm), oedema increases gradually and reaches 4.00 ± 0.46 mm after four hrs. However, oral administration of aspirin (reference molecule) causes a significant reduction in oedema progressively from the first hr with a percentage inhibition of 83.44% (Table 1). The pretreatment of mice with plant extracts significantly inhibits the development of oedema in a dose-dependent manner (Fig.1). The results in table 2 revealed no significant difference between the effect of aspirin (at 100 mg/kg) and that of *L. antineae* extract (at 200 mg/kg) which induced inhibition of 80.74%, while *S. chudaei* extract was found to have moderate activity (50.74%).

Table 1: Inhibition of paw oedema in different groups of mice in the presence of pretreatment with hydromethanic extracts and reference product (Aspirin).

Treatments	inhibition of oedema %	
	Dose of 100 mg/kg	Dose of 200 mg/kg
S. chudaei extract	22.41±3.56 (c)	50.74±5.12 (b)
L. antineae extract	62.54±3.53 (b)	80.74 ± 6.53 (a)
Aspirin	83.44 ±5.06 (a)	−

[Different letters indicate significantly different activities (P<0.05)]**.**
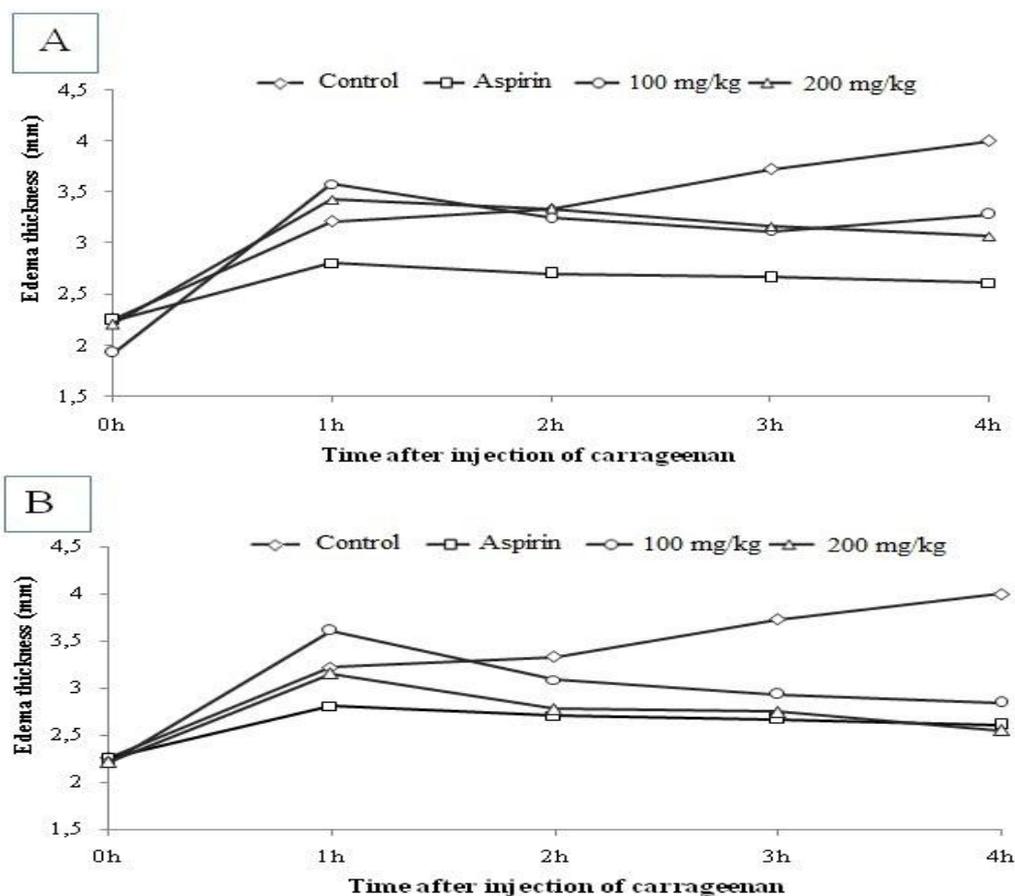
Fig.1: Evolution curve of paw oedema (mm) in the presence of different oral pretreatment: physiological saline (control group); aspirin prepared at 100 mg/kg (Reference group); *S. chudaei* extracts prepared at 100 and 200 mg/kg (A); *L. antineae* extracts prepared at 100 and 200 mg/kg (B).

Ear oedema model

As shown in table 2, four hrs after topical application of croton oil to the right ear, the mice in the control group developed oedema weighing 5.33 mg. In the mice of the group treated locally with 0.5 mg of aspirin, we noticed a very significant reduction (P< 0.05) in the weight of the ear (1.40 mg) compared to that of the control mice, which corresponds to inhibition of inflammation of 73.73%.

The plant extracts showed a dose-dependent anti-inflammatory activity. The treatment of the mice with 2 mg of *L. antineae* extract and *S. chudaei* extract induced a very remarkable decrease in inflammation compared to the mice of the control group. The weight of the ear is 1.44 mg and 1.93 mg, which means inhibition of 72.98% and 63.72%, respectively.

Table 2: Effect of *L. antineae* and *S. chudaei* hydromethanolic extracts on ear oedema.

Treatments	Dose (mg/ear)	Difference in weight (left and right ear) in mg	Inhibition of oedema %
Control	−	5.33±0.30	−
S. chudaei extract	1.0 2.0	4.66±0.63 1.93±0.40	12.44 (d) 63.72 (b)
L. antineae extract	1.0 2.0	3.76±0.81 1.35±0.42	29.45 (c) 74.67(a)
Aspirin	0.5	1.40±0.68	73.73 (a)

[Different letters indicate significantly different activities (P<0.05)].

Evaluation of anti-lipid peroxidation activity

To measure the amount of peroxide formed at the primary stage of linoleic acid peroxidation, the FTC method was used. The peroxide reacts with ferrous chloride to make a reddish ferric chloride pigment. Lipid peroxidation was monitored in daily intervals, over a period of nine days (Fig.2). According to the results, the tested samples exhibited a good effect in inhibiting linoleic acid oxidation compared to control which is indicated by their low absorbance values. *S. chudaei* and *L. antineae* extracts showed a good anti-lipid peroxidation activity (75.95 ± 0.31% and 77.44 ± 0.36%, respectively) with no significant

difference compared to ascorbic acid (77.63 ± 1.58%). These extracts were also found to be more active than BHT (63.8±0.84%) and α-tocopherol (58.50 ± 3.71%).

Chemical composition

The HPLC analysis of plant extracts showed that *S. chudaei* and *L. antineae* are plants rich in phenolic acids and flavonoids. The comparison of the retention times and the UV spectra of the peaks obtained with those of different standards injected in the same conditions, allowed us to identify 5 compounds in *S. chudaei* and 8 in *L. antineae* (Table 3).
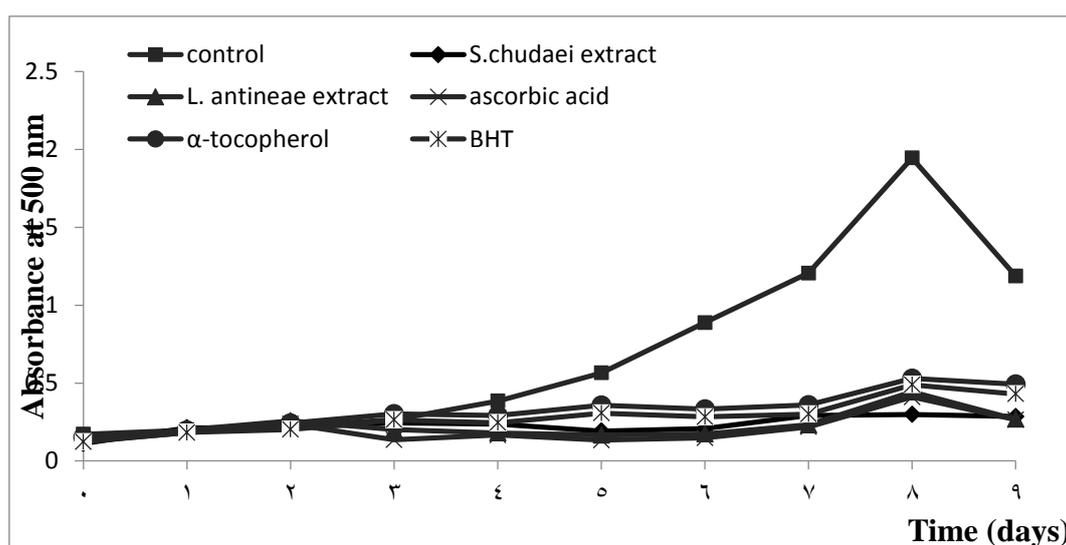

Fig.2: Inhibitory effect of *S. chudaei* and *L. antineae* extracts on the primary oxidation of linoleic acid system measured by FTC method. Each value is expressed as mean ± SD (n = 3).

Table 3: The phenolic compounds identified by HPLC-DAD in hydromethanolic crude extracts of *S. chudaei* and *L. antineae*.

Standards	RT (min.)	λUV max (nm)	Area % in *S. chudaei* extract	Area % in *L. antineae* extract
Naringenin-7-glycoside	2.250	286 ;332	0.42% [a]	0.15%
Syringic acid	2.969	218 ;274	4.61%	−
Catechin	7.262	220 ;277	−	0.20%
Rutin	9.414	254 ;356	−	3.02%
Ferrulic acid	9.902	215 ;287 ;312	−	20%
Hesperidin	10.955	242 ;281 ;363	−	1.32%
Rosmarinic acid	11.598	290 ;328	2.11%	8.26%
Quercitin	13.041	254 ;365	0.22%	−
Luteolin	13.207	256 ;266 ;370	−	1.83%
Naringenin	14.836	213 ;283 ;330	0.10%	0.30%

(−) not detected

Discussion

In this study, two models of *in vivo* inflammation were used: carrageenan-induced paw oedema and croton oil-induced ear oedema.

A carrageenan-induced paw oedema is an excellent tool for the quantification of inflammation. The development of oedema in the paw of the mouse after injection of carrageenan is a biphasic event. The initial phase observed during the first hr is attributed to the release of histamine, serotonin and bradykinins. As a result, vascular permeability is increased, which facilitates the infiltration of neutrophils and the accumulation of plasma in the interstitial space that leads to oedema. Platelets-activating factors and arachidonic acid metabolites also play a role. The second phase of oedema is associated with the production of cyclooxygenase (COX) which increases the synthesis of prostaglandins (PGs) essentially PGE2, the production of oxygen free radicals and neutrophils infiltration. This explains the late effect of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin which has a weak effect in the first hr. The inhibition curve of NSAIDs shows a stabilization from 3 hrs which corresponds to the stabilization of these mediators[12-14].

The treatment of mice with hydromethanolic extracts of *L. antineae* and *S. chudaei* leads to a significant reduction in the thickness of oedema in comparison with control, with kinetics similar to that of aspirin (Figure 1). Therefore, it can be supposed that the inflammation inhibitory effect of these extracts could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis.

The results obtained by the methanolic extract of *S. chudaei* are in agreement with those obtained by Semaoui et al., 2021 who found also that the infusions prepared from this plant at doses of 250, 500 and 1000 mg/kg body weight exhibited moderate activity (45, 48 and 58%, respectively)[15].

In the ear oedema model, the phylogenic effect of croton oil is mainly due to the active compound 12-O-tetradecanoylphorbol-13-acetate (TPA) that it contains. TPA induces an inflammatory reaction characterized by a significant production of pro-inflammatory mediators, an increase in vascular permeability and oedema. This reaction is induced by the activation of protein kinase C (PKC), which promotes an increase in phospholipase A2 (PLA2). This enzyme catalyzes the hydrolysis of phospholipids membrane to arachidonic acid, the enzymatic oxidation of this acid by cyclooxygenases, or lipooxygenases leads to eicosanoids, prostaglandins, leukotrienes and cytokines [16, 17].

The results obtained through this test show that the tested extracts, in particular, that of *L. antineae*, have a potent anti-inflammatory activity, this suggests that these extracts could act on the phospholipase A2, the cyclooxygenase and/or the lipooxygenase which are the mediators involved in the inflammatory reaction induced by TPA.

The remarkable anti-inflammatory activity could be attributed to the presence of phenolic acids and flavonoids present in these plants in different proportions. The chromatographic analysis carried out in the present work and also in the study of Semaoui et al., 2021 [15] on *S. chudaei* allowed the identification of some polyphenolic components which have already been investigated by other researchers for their anti-inflammatory activity inflammatory.

The observed anti-inflammatory activity may be attributed to the presence of phenolic acids and flavonoids present in these plants in different proportions. Some polyphenolic components which are identified in these extracts have already been examined by other researchers for anti-inflammatory activity. For example, it has been demonstrated that luteolin is capable to inhibit the cyclooxygenase-2 and 15-lipooxygenase activity and reduce PGE2 levels and the release and expression of cyclooxygenase-2. Quercitin and naringenin inhibit the activity of phospholipase PLA2 and 12-lipooxygenase. Quercitin, apiginine and catechin are cyclooxygenase inhibitors. Also, apiginine, quercetin, Isorhamnetin, Epicatechin and ferulic acid inhibit COX-2 expression by suppressing the activation of transcription factors such as NF-kB and AP-1[18& 19, 3& 4]. In addition, the rosmarinic acid detected in both species is an essential component of the anti-inflammatory activity because it can decrease the production of interleukin (IL)-6 and prostaglandin E2 (PGE2), by inhibiting the activity of cyclooxygenase[20].

Lipid peroxidation is caused by the generation of hydroxyl and superoxide radicals, leading to the formation of peroxy radicals that eventually propagate the chain reaction in lipids. Therefore, the presence of natural antioxidant molecules such as phenolic compounds could prevent lipid peroxidation by naturalizing radical peroxides through different modes of action including free radical scavenging, electron transfer, radical addition and radical recombination[21, 22].

In this study, the potent inhibition effect of plant extracts observed on lipid peroxidation can be ascribed to their different active compounds. Previous studies have confirmed the anti-lipid peroxidation properties of various phenolic acids and flavonoids present and identified in our extracts such as ferulic acid, naringenin, luteolin and quercetin[23-26].

In accordance with other previous studies and findings, the potential anti-lipid peroxidation activity observed may at least in part be incharge of the anti-inflammatory activity of the plant extracts[2, 27, 28].

Conclusion

To our knowledge, this study is considered as a first source of information on the anti-inflammatory and anti-lipid peroxidation activity of the extracts of *L. antineae* and *S. chudaei*. It gives a scientific justification for the traditional use of *Lavandula antineae* as an anti-rheumatic remedy. Bioactive compounds found in these plants have the potential for application as natural anti-inflammatories and anti-lipid peroxidative agents in different pharmaceutical products. Further study will be aimed at isolating and identifying the substances responsible for the anti-inflammatory activity of plant extracts, which may be further exploited in herbal formulations.

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نشرة العلوم الصيدليــــة

جامعة أُسيوط

المضاد الكمون للالتهاب و أكسدة الليبيدات لكل من SALVIA CHUDAEI BATT. et TRAB. AND LAVANDULA ANTINEAE MAIRE، نباتين مستوطنين من صحراء الجزائر

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أجريت هذه الدراسة للتحقيق في الأنشطة المضادة للالتهابات ومضادات أكسدة الدهون لنباتين مستوطنين في الصحراء الوسطى ؛ Salvia chudaei Batt. et Trab and Lavandula antinee Maire (Lamiaceae) (المستخلصات المائية–الكحولية)، وكذلك تركيبها الكيميائي. في اختبار الوذمــة التــي يسببها الكاراجينان في الفئران ، أظهر مستخلص L. antineae ، بجرعة ٢٠٠ ملجم / كجــم ، نشــاطًا جيدًا مضادًا للالتهابات(P<٠.٠٥) أظهر انخفاضًا بنسبة ٨٠.٧٤٪ في سماكة القدم مقارنة بتلك الناتجة عن المركب المرجعي. عقار الاسبرين ٨٣.٤٤٪ عند ٤ ساعات بينما خلاصة S. chudaei ذات نشــاط معتدل (٥٠.٧٤٪). عندما تم تطبيق المستخلصات النباتية موضعيًا بجرعة ١ و ٢ مجم لكل أذن ، كان الحد من التهاب وذمة الأذن ملحوظًا جدًا مقارنة بفئران المجموعة الضابطة. كما أظهرت المستخلصات النباتية فعالية ضد أكسدة الدهون مع عدم وجود فرق معنوي مقارنة بحمض الأســكوربيك (٧٧.٦٣ ± ١.٥٨٪). بالإضافة إلى ذلك ، أظهر التوصيف الكيميائي لمستخلصين بواســطة HPLC-DAD وجــود العديد من الأحماض الفينولية والفلافونويد. أظهرت هــذه النتــائج أن Lavandula antineae و Salvia chudaei يعرضان أنشطة رائعة مضادة للالتهابات ومضادة للدهون بيروكسيد وبالتالي يمكن أن تكــون مصادر طبيعية محتملة في الصحة والطب.