Pharmacogn. Commn. 2018; 8(1): 15-24
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

In vitro and *in vivo* Antioxidant, Antihemolytic and Anti-inflammatory Activities of *Santolina chamaecyparissus* Extracts.

Djarmouni meriem*, Baghiani Abderrahmane, Adjadj Moufida, Arrar Lekhmici
Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, University Setif, Setif 19000, ALGERIA.

ABSTRACT

Introduction: *Santolina chamaecyparissus* L. is a small medicinal herb, cultivated in Europe, Asia and Africa due to the antihelmintic, antiseptic, antispasmodic, bactericidal, fungicidal, digestive and vulnerary properties. Despite this, *S. chamaecyparissus* aerial part extractions have not been examined for antioxidant, antihemolytic and anti-inflammatory properties. **Methods:** *S. chamaecyparissus* aerial parts were extracted with solvents of varying polarity: methanol (crude) extract (CrE) chloroform extract (CHE), ethyl acetate extract (EAE), and aqueous extract (AE). The content of total phenolics, and flavonoids in all the extracts were determined with spectrophotometric methods. Both enzymatic and non-enzymatic methods were used to evaluate the antioxidant activity of the extracts. Antioxidant activity of all the extracts were investigated using free radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl, DPPH$^{\bullet}$ and 2,2′-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) assay), capacity of the inhibition of linoleic acid peroxidation (β-carotene assay), chelation of metals (iron chelating assay) and inhibition of xanthine oxidase (XO) activity. To investigate antihemolytic activity of the extracts, the 2, 2,-azobis (2-amidinopropane) dihydrochloride (AAPH) assay was used to induce erythrocyte oxidative hemolysis. An *in vivo* approach was carried out on mice treated with the methanol extract at a dose of 100 mg/kg/day for 21 consecutive days, and one group was treated with vitamin C (vitamin C 50 mg/kg) as a standard drug. To determine the improvement of antioxidant potential, basic biochemical parameters were used in tissue (liver), plasma and whole blood. Phorbol 12-myristate 13-acetate (PMA)-induced ear edema was used to investigate the anti-inflammatory activity of methanolic extract in mice. **Results:** Among all the extracts analyzed, the EAE exhibited a higher phenolic and flavonoids content (373.83 ± 0.23 mg gallic acid/g, and 61.51±7.86 mg quercetin/g respectively), followed by CE and CHE. DPPH and ABTS scavenging assays showed that EAE exhibited the highest effect in the both assays, with an IC_{50} of 0.01 mg/ml and 0.0085 mg/ml respectively. All extracts moderately inhibited linoleic acid oxidation, with 57 % inhibition. The CE had a considerable chelating activity on ferrous iron (IC_{50}= 0.32 mg/ml). In the enzymatic method by xanthine oxidase, results demonstrated that EAE had the highest XO inhibitory effect on both XO activity and Cyt-c reduction with IC50= 0.052±0.0003 mg/ml and 0.057±0.0006 mg/ml, respectively followed by CHE and CE. In the cellular system, CrE and CHE showed a hemolysis effect with % hemolysis (62.81% ± 1.43), (62.71% ± 1.01). However, EAE provided protection against AAPH-induced hemolysis with 53.67% ± 0.97. The *in vivo* assay exhibited a significant decrease (79.56%) of the content of malondialdehyde (MDA) in the liver and increased glutathione (GSH) and catalase (71.95% and 59.16% respectively). The methanol extract clearly demonstrated anti-inflammatory effects by reduced ear edema induced by PMA with 61.51%. **Conclusion:** Our results indicate that the *S. chamaecyparissus* extracts (SCE) possesses potent antioxidant and anti-inflammatory properties and may be a valuable natural source that could be applicable to both the medical and food industries.

Key words: Xanthine oxidase, Total phenolic, Flavonoid compound, Erythrocyte oxidative, Ear edema.

Correspondence:
Djarmouni meriem,
Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, University Setif, Setif 19000, ALGERIA.
E-mail: magistairebiochimie@gmail.com
DOI: 10.5530/pc.2018.1.4

INTRODUCTION

Plants and their products are rich sources of a phytochemicals and have been found to possess a variety of biological activities, including antioxidant potential. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference.[1,2] Among dietary antioxidants, phenolic compounds are the most abundant natural antioxidants.[3,4] Phenolics can act as antioxidants in many ways. These antioxidants act as reducing agents, hydrogen donators, free radical's scavengers and singlet oxygen quenchers. They inhibit oxidising enzymes, chelation of transition metals, and transfer of hydrogen or single electron to radicals, and therefore act as cell saviours.[4,5]

Physiological concentrations of free radicals are required to mediate physiological processes such as inflammatory reactions. Indeed, during inflammation, cells of the immune system are recruited to the site of damage. This results in respiratory burst, an overproduction of reactive oxygen species that can propagate inflammation by stimulating a release of cytokines and causes oxidative damage to bimolecular constituents in the body. This eventually leads to chronic diseases such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases.[6]

Erythrocytes have been used as a model to investigate oxidative damage due to their high concentration of O_2 and their known sensitivity to endogenous reactive oxygen species (ROS), especially to peroxyl radicals (ROO$^{\bullet}$) that may attack membrane components, inducing changes in membrane rheology, conformation of membrane proteins, cellular morphology, protein cross-linking, and hemolysis.[7]

Santolina chamaecyparissus L. (Asteraceae) is a shrub with yellow inflorescences that is widely used in Mediterranean folk medicine. The plant is used in northern Africa as a remedy against intestinal worms and as a spasmolyticum, amongst other uses. Akerreta *et al.*[8] maintain that the plant is commonly used as a 'chamomile' tea for digestive disorders in Navarra and other Mediterranean areas. The inflorescences of *S.chamaecyparissus* are widely used in Mediterranean folk medicine for their analgesis, anti-inflammatory, antiseptic, antispasmodic, bactericidal, fungicidal, digestive and vulnerary properties, and is used in phytotherapy for different kinds of dermatitis.[9] The essential oil has anti-Candidadal properties,[10] and finds some limited uses in perfumery and cosmetics. The aim of our study was to evaluate the potential of *S. chamaecyparissus* aerial part extracts to act as an antioxidant, antihemolytic and anti-inflammatory agent. As far as we know, this is the first time that antioxidant activity of *S. chamaecyparissus* is evaluated by using this bioassay.

MATERIALS AND METHODS

Plant Materials

The aerial part of *Santolina chamaecyparissus* (SCE) was harvested from natural resources from Setif (Algeria) during the spring June 2012 in the stage of flowering. The taxonomic identity of the plant was confirmed by Pr. Laouar H. (Setif University), and a voucher specimen (No. S.c. 2009-1) was preserved at the local Herbarium of Botany, Department of Botany, University of Sétif.

The plant was air dried in the dark and powdered using an electrical grinder (Retsch SK100). All reagents and Solvents were from Fluka and Sigma Aldrich (Germany) and were of analytical grade.

Animal materials

Male *Swiss albino's* mice (20 - 30 g) were purchased from the Pasteur Institute of Algiers, Algeria and they were group-housed (6-8 mice per cage) with free access to food and water, and kept in a regulated environment at 25C° under 12 h light/12 h dark conditions. They had free access to the standard pellet diet and drinking water during the experiments. After 1 week of acclimatization to the home cage, the mice were randomly divided into three groups and i.p. injected for 21 days. The feeding scheme was as follows:

Group 1: Normal control group (NCG) which received 0, 9% physiological saline solution per day.

Group 2: positive control group (PCG), received 50 mg/kg of vitamin C per day.

Group 3: extract treatment groups which received 100 mg/kg of methanol extract MHGE per day.

Extraction and fractionation

The extractions were carried out using various polar and non-polar solvents according to the method of Markham.[11] One hundred grams of powdered aerial plant parts were macerated for 24 h with 85% aqueous-methanol. The macerates were then extracted twice using 50% of the same solvent for 4 h with continuous stirring. The extracts were then filtered and evaporated on rotary evaporator (BÜCHI) to give crude extract (CrE).

CrE was successively extracted with hexane, chloroform and ethyl acetate. Each organic layer was evaporated to dryness under reduced pressure to yield HxE, CHE, EAE and AqE, respectively. These fractions were stored at -20C° prior to use.

Determination of total phenolic compounds and flavonoid content in the extract

Total phenolic content was determined by the Folin–Ciocalteau method.[12] Briefly, 200µl of SCE was mixed with 1ml of of 1:10 diluted Folin–Ciocalteau reagent for 4 min and 800µl of 75 g/l Na_2CO_3 were then added. The absorbance was measured at 765 nm after 2 h of incubation at room temperature. Results were compared to a gallic acid standard curve and are expressed as gallic acid equivalents.

Total flavonoids content was estimated according to an established procedure.[13] To 1 ml of each sample, 1 ml of 2% $AlCl_3$ methanol solution was added. After 10 min at room temperature, the absorbance was measured at 430 nm. The total flavonoid content was calculated and compared to a quercetin calibration curve.

Assay of antioxidant activity *in vitro*
DPPH radical scavenging assay

The free-radical-scavenging activity of the different extracts was measured using DPPH according to the procedure described by our previously published method,[14] with slight modifications. The extract solution (50µl) was mixed with a solution of 0.004% DPPH in methanol (1250 μl). The mixture was shaken vigorously and allowed to stand for 30 min before the absorbance was measured at 517 nm. Radical-scavenging activity was calculated as the following percentage:

[(As-Ai)/As] ×100 (As = absorbance of DPPH alone, Ai = absorbance of DPPH in the presence of various extracts). A concentration of BHT that was identical to the experimental samples was used as reference.

ABTS radical scavenging assay

The ability of various extracts to scavenge the ABTS radical cation was determined by our previously published method.[15] A solution of ABTS radical cation ($ABTS^+$) was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulfate at room temperature in the dark for 12 h. The $ABTS^+$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. The extract solution at a range of concentrations (50µl) was mixed with ABTS solution (1mL) and the reaction mixture could stand for 30 min. The absorbance was recorded at 734 nm. Trolox was used as reference. The level of radical scavenging was calculated using the equation described above for DPPH.

β -Carotene bleaching assay

The antioxidant activity assay was carried out according to the β-carotene bleaching method of Aslan *et al.*[16] In the assay, 1mL β-carotene solution (0.5mg/ml chloroform) was pipetted into a round-bottom 250ml flask containing 25µl linoleic acid and 200mg Tween 40. The mixture was then evaporated at 40 C° by means of a rotary evaporator to remove the chloroform. The mixture was then diluted with 100ml distilled water saturated with oxygen, which was added slowly to the mixture with vigorous agitation to form an emulsion. Then, 2.5ml aliquots of the emulsion were transferred into different test tubes containing 350µl of samples at 2mg/ml. The tubes were gently mixed and incubated up to 24h in the dark at room temperature. After this incubation period, absorbance of the mixtures was measured at 490 nm after 0h, 1h, 2h, 4h, 6h, 12h and 24h of incubation. BHT was again used for comparative purposes. Lipid peroxidation (LPO) inhibition was calculated using the following equation: LPO inhibition = (As − Ai) /As × 100

Whereas is the absorbance of the assay control; Ai is the absorbance of the assay 24 h later.

Metal ion chelating assay

The chelating activity of a metal ion (Fe^{2+}) was measured using a modified version of the method of Decker and Welch.[17] The reaction mixture, containing 250 μl of the SCE solution, 50 μl of ferrous chloride ($FeCl_2$) solution (0.6 mM), 50µl of ferrozine solution (5 mM), and 450µl of methanol was shaken well and was incubated at room temperature for 10 min. The absorbance of the Fe^{2+}- ferrozine complex was measured at 562 nm against a blank. The chelating activity was calculated using the following equation:

Chelating rate = [(As−Ai)/As]×100, where As is the absorbance of the control and Ai is the absorbance in the presence of the extract. Ethylenediamine tetraacetic acid (EDTA) was used as the control.

Xanthine oxidase activity assay

Xanthine oxidase activity was evaluated by the spectrophotometric measurement of the formation of uric acid by xanthine at 295 nm. An aliquot of a 100 μM solution of xanthine in 50 mM phosphate buffer (pH 7.4), containing 0.1 mM EDTA with (1227 ηmole/min/mg of XO). Different concentrations of tested compounds were added to the samples before the enzyme was added and their effects on the generation of uric acid was used to calculate regression lines and IC_{50} values.[18] Allopurinol

was used as a positive standard.

Superoxide anion generation by xanthine-/xanthine oxidase assay

Anti-radical activity was determined spectrophotometrically according to the method of Robak and Gryglewski.[19] by monitoring the effect of SCE extracts on superoxide anion radicals produced by the xanthine/xanthine oxidase system. The reaction mixture contained xanthine (100 µM), horse heart cytochrome c (25 µM), in air-saturated sodium phosphate buffer (50 mM, pH 7.4), supplemented with 0.1mM EDTA and various concentrations of SCE extracts. The reactions were started by the addition of XO. Within 1min, reduced cytochrome c was spectrometrically determined at 550 nm against enzyme-free mixture.

AAPH-induced hemolysis assay *in vitro*

Blood was obtained from healthy mice by venipuncture and collected into tubes containing EDTA as anticoagulant. Samples were immediately centrifuged at 1500 rpm for 10 min at 4 C° and the plasma was then carefully discharged. Erythrocytes were washed three times with phosphate buffered saline (PBS; pH 7.4). To induce free radical chain oxidation in erythrocytes, aqueous peroxyl radicals were generated by thermal decomposition of AAPH (dissolved in PBS; final concentration 300 mM). To study the protective effects of the SCE extracts against AAPH-induced hemolysis, an erythrocyte suspension at 2% hematocrit was incubated with the CrE, CHE, EAE and MOHE extracts (0,1 mg/ml) followed by incubation with and without 300 mM AAPH at 37C° for 4.5 h. The extent of hemolysis was determined spectrophotometrically as described previously.[20] The results were expressed as percentage inhibition of erythrocyte hemolysis. The half-time of hemolysis corresponds in the time so that the initial optical density decreases by 50%.

In vivo antioxidant assays

Biochemical assay

Twenty-four h after the last drug administration, the mice were sacrificed. Blood samples were collected, a volume of whole blood was immediately transferred to another tube containing phosphate buffer (300 mOs, pH 7.4) to obtain a dilution of 1:25 to determine the total antioxidant of the red blood cells by hemolysis assay. The remaining blood was centrifuged at 1500g at 4C° for 5 min to afford the serums. The serum was analyzed for antioxidant of plasma capacity (CPA) with DPPH and reducing power assays. The livers were removed rapidly, washed and homogenized in ice-cold physiological saline to prepare the homogenate. Then homogenate was centrifuged at 4000g at 4C° for 10 min to remove cellular debris, and the supernatant was collected for determination of enzyme activity (CAT), lipid peroxidation (MDA), and GSH level.

Determination of the activity of antioxidant enzyme (CAT)

To measure the catalase (CAT) activity, the reaction mixture was composed of 0.1 M phosphate buffer pH 7.4, 19 mM H_2O_2 and liver homogenate. The reduction rate of H_2O_2 was followed for 240 nm at 2 min, at room temperature.[21] CAT activity was expressed as U/mg protein.

Determination of GSH content

GSH in liver tissues was determined according to the Ellman method,[22] which measures the reduction of 5-5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent) by sulfhydryl groups to 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color. The results were expressed in mg per g protein (mg/g protein).

Protein assay

All results were normalized by the protein concentrations, which were determined by Peterson's modification of the procedure of Lowry *et al.*[23]

Lipid peroxidation assay

Lipoperoxidation was evaluated by thiobarbituric acid reactive substances (TBARS) tests during an acid-heating reaction, as previously described.[24] Aliquots of samples were incubated with 20% trichloroacetic acid and 0.67% thiobarbituric acid. The mixture was heated (15 min) in boiling water. TBARS was determined by reading the absorbance of the pink-colored complex formed in a spectrophotometer at 532 nm.

Antioxidant capacity of plasma

Reducing power assay

The reducing ability of biological sample was determined by the reducing power assay of Chung *et al.*[25] Each sample (0.1ml), was mixed with 0.1 ml of 200 mM PBS (pH 6.6) and 0.1 ml of 1% $K_3Fe(CN)_6$, and the mixture was incubated at 50 C° for 20 min. After 0.25 ml of 1% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 2790g for 10 min. The upper layer (0.25 ml) was mixed with 0.25 ml of deionised water and 0.5 ml of $FeCl_3$, and the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicates increased reducing power of the sample.

DPPH assay

Serum was treated with solution of 0.004% DPPH solution. After incubation at room temperature for 30 min, the absorbance of the solution was determined at 517 nm. The results were compared with the DPPH solutions in the absence of the serum. The assay was carried out as described above.

Antioxidant capacity of red blood cells (hemolysis assay)

Mice's erythrocytes from citrated blood were isolated by centrifugation at 1500g for 5 min and washed three times with PBS, in order to induce free-radical chain oxidation in erythrocytes, peroxyl radicals were generated by tBH as a free radical generator.[20] An erythrocyte suspension at 2% hematocrit with phosphate buffer (300 mOs, pH 7.4) was incubated with or without tBH. The kinetics of erythrocytes resistance to hemolysis was determined at 37C° by continuous monitoring of changes in 620 nm absorbance. The time to reach 50% of total hemolysis was determined (half hemolysis Time; HT_{50}).

Anti-inflammatory activity

The PMA-induced mice ear oedema test has been used as an experimental model for screening the anti-inflammatory activity. According to a modified method of Garrido *et al.*,[26] 4µg per ear of PMA, in 20 µl of DMSO, was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (DMSO, 20 µl). MHGE was administered topically (100 mg/kg) 1 h before PMA application. The reference group was treated with diclofenac (10 mg/kg). Six h after PMA application, mice were killed by cervical dislocation and a 6mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight.

Statistical analysis

All experiments were done in triplicate and results were reported as mean ± SD. Data were analyzed by one-way ANOVA. Statistically significant effects were further analyzed, and means were compared using Tukey test.

Table 1: Yields, total polyphenols and flavonoids content of *S. chamaecyparissus* extracts.

Extracts	Yeild(%)	Total phenols content µg GAE/mg of extract	Flavonoids content µg QE/mg extract
CrE	9.71 ± 0.20	196.08±0.35	16.94±1.96
HXE			
CHE	1.487±0.22	143.08±0.47	8.71±0.98
EAE	1.352±0.34	373.83±0.23	61.51±7.86

RESULTS

Determination of total polyphenols and flavonoids content

The extraction yields calculated showed that methanol, chloroform, and ethyl acetate extracts of SCE registered a yield of 9.71%, 1.487%, and 1.352% respectively.

The total phenolic and flavonoid contents of the SCE extracts as determined by Folin–Ciocalteu and $AlCl_3$ method (Table 1). Among the three extracts, EAE contained the highest amount of phenolic and flavonoid compounds (373.83 ± 0.23 mg gallic acid/g, and 61.51±7.86 mg quercetin/g respectively) followed by CrE and CHE.

In vitro antioxidant activity of SCE

DPPH free-radical scavenging

The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants.[29]

The results showed that SCE extracts exhibited a dose-dependent activity in scavenging DPPH radicals presented. The IC_{50} values of SCE competing with the standard (BHT), were found to be in the order of BHT> EAE > CrE > CHE, (Figure 1). EAE exerted high DPPH radical scavenging activity (IC_{50}: 0.01±0.0001 mg/ml), which was very near to that of the reference compound BHT (IC_{50}: 0.032±0.0186 mg/ml) P>0.05.

$ABTS^{\cdot+}$ free-radical scavenging

The reduction capability of ABTS radical was determined by the decrease in absorbance induced by plant antioxidants. The concentration of each extract required to inhibit each radical by 50% (IC_{50}) is shown in Figure 2. The EAE showed the greatest potency (IC_{50}= 0.0085 ±9.40 10^{5-} mg/ml) among the three extracts, and exhibits the most robust radical-scavenging activity, which is lower by 6-folds than that of Trolox, followed by that of CHE (0.024 ±0.0003 mg/ml).

β-Carotene bleaching assay

As for β-carotene-linoleic acid assay, the addition of the SCE at 2 mg/ml prevented the bleaching of β-carotene to same degrees (57% and 58%). Compared to standard antioxidant, the SCE are 1.7 times less active than BHT (Figure 3).

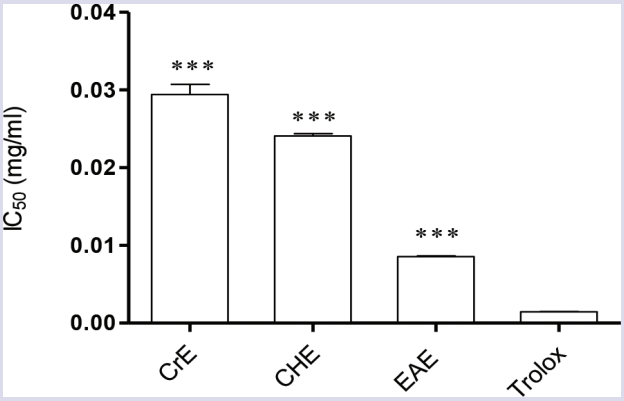

Figure 2: $ABTS^{\cdot+}$ radical scavenging activity of *S. chamaecyparissus* extracts (CrE: crude extract; CHE: chloroform extract; EAE: ethylacetate extract). Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3).

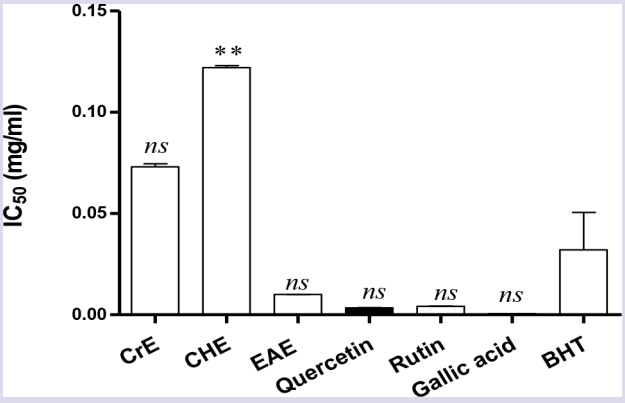

Figure 1: DPPH radical scavenging activity of *S. chamaecyparissus* extracts; Data are presented as Inhibitory concentration for 50% of DPPH activity (IC_{50}) values. CrE: crude extract; CHE: chloroform extract; EAE: ethylacetate extract. Each value represents the mean ± SD (n = 3).

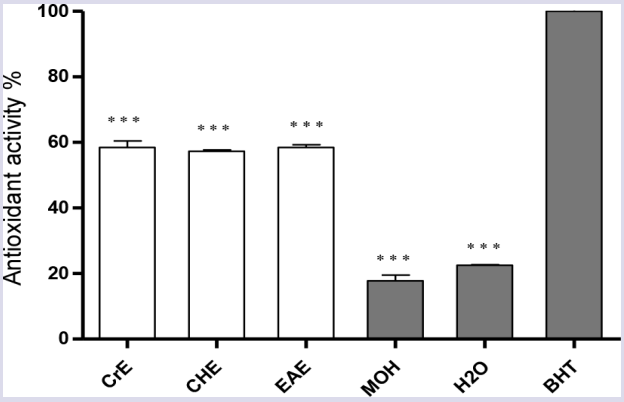

Figure 3: Percentage inhibition of the linoleic acid oxidation by *S. chamaecyparissus* extracts (CrE: crude extract; CHE: chloroform extract; EAE: ethylacetate extract) compared with BHT after 24h. ($p \leq 0.01$).

Chelating activity

Although iron is essential for oxygen transport, respiration, and enzyme activity, it is a reactive metal that catalyses oxidative damage in living tissues and cells. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease of the blue color of the complex.[29]

SCE was capable of chelating iron (II) and did so in a concentration dependent manner. CrE have an excellent chelating ability (IC_{50}= 0.32 ± 0.009 mg/ml), which was even lower than that of EDTA (IC_{50}= 5.8± 0.0831 μg/ml), with approximately 55 folds followed by CHE and EAE respectively (Figure 4).

Xanthine oxidase inhibitory and $O_2^{\cdot-}$ scavenging activity

The enzyme XOD plays a crucial role in the production of uric acid, catalyzing the oxidation of hypoxanthine and xanthine. During the reoxidation of XOD, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. Both, inhibition of XOD and the scavenging effect on the superoxide anion, were measured. SCE exhibited an inhibitory effect on xanthine oxidase in a concentration dependent manner. CHE and EAE, showed a high inhibition of XO P>0.05 with IC_{50} of (0.051± 0.0002 mg/ml) and (0.052±0.0003 mg/ml) respectively, followed by CrE (0.091±0.001 mg/ml). The inhibition of XO by CHE and EAE showed a less efficient than allopurinol (IC_{50} = 0.0082 ± 0.0005 mg/ml), as a specific inhibitor for XOR, by 6.2-folds and 6.3-folds, respectively (Figure 5).

The effect of SCE was studied for their ability to scavenge $O_2^{\cdot-}$ generated by the xanthine/xanthine oxidase system. The $O_2^{\cdot-}$ scavenging activity of the SCE are increased markedly with increasing concentrations. The most potent scavengers of superoxide anion radical were observed for EAE with IC_{50} of (0.057± 6.65 10^{5-} mg/ml), followed by CHE with IC_{50} of (0.074±0.001mg/ml), which was approximately 1.3-folds higher than that of EAE, however CrE showed the lowest effect in compared with previous extracts ($p \leq 0.001$) (Figure 6).

AAPH-induced hemolysis assay *in vitro*

Recent research has demonstrated an increasingly interest in the protective biochemical function of naturally occurring antioxidants in biological systems and on the mechanism of their action. The *in vitro* oxidative hemolysis of mice erythrocytes was used here as a model to study the free radical-induced damage of biological membranes and the protective effect of SCE. Figure 7 shows erythrocyte hemolysis induced by AAPH with the addition of CrE, CHE, EAE and MOHE extracts (0.1mg/ml). When erythrocytes were incubated in air at 37C° as a 2% suspension in phosphate buffered saline (PBS) and the water-soluble radial inhibitor

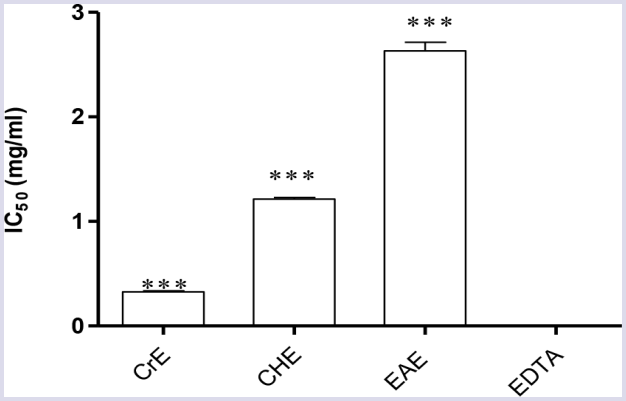

Figure 4: 50% of chelating activity of *S. chamaecyparissus* extracts (CrE: crude extract; CHE: chloroform extract; EAE: ethylacetate extract) and EDTA. Comparison was realized against EDTA; ***: $p \leq 0.001$.

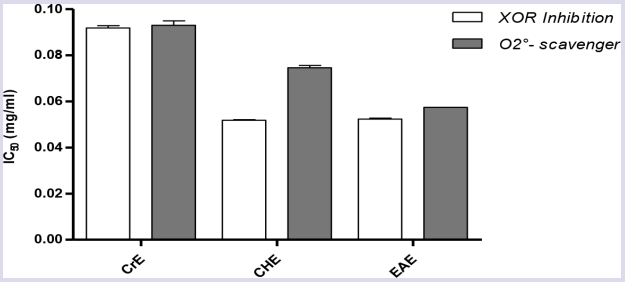

Figure 6: Evaluation of S. *chamaecyparissus* extracts as inhibitors of xanthine oxidase and as scavengers of superoxide produced by the action of XO enzyme. Each value is represented as mean ± SD (n = 3).

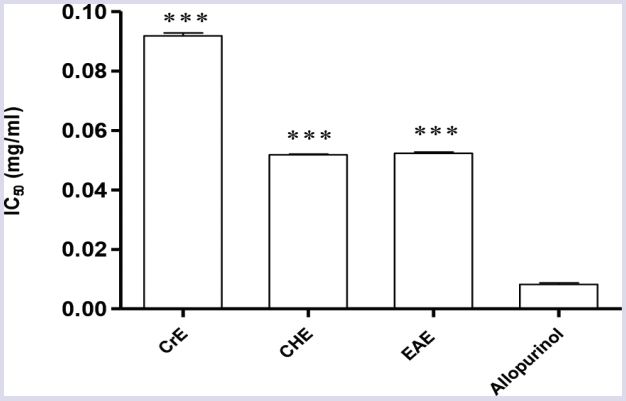

Figure 5: IC_{50}, Inhibitory concentration of *S. chamaecyparissus* extracts for 50% of XO activity. Comparison was realized against allopurinol. ***: $p \leq 0.001$.

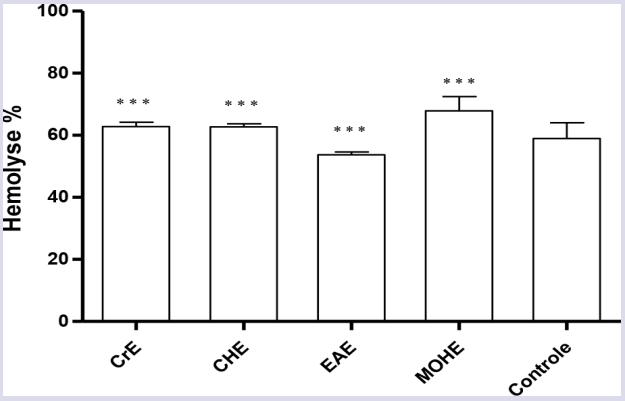

Figure 7: Effect of *S. chamaecyparissus* extracts (0.1 mg/ml) on AAPH (300 mM) induced oxidative stress in red blood cells. Values are means ± SEM; n=3.

AAPH (300 mM), was added to the aqueous suspension of erythrocytes, hemolysis induction was time dependent for the 4.5h period of the assay.

When the cells were incubated with 0,1 mg/ml extracts of SCE, we showed that CrE, CHE and MOHE extracts have hemolysis effect with % hemolysis (62.81% ± 1.43), (62.71% ± 1.01) and (67.88% ± 4.60) respectively, however this concentration of EAE provided protection against AAPH-induced oxidative hemolysis with (53.67% ± 0.97).

In vivo antioxidant activity of SCE

Enzymatic and non-enzymatic endogenous antioxidant system in liver tissue

We measured the activity of CAT and the content of GSH and MDA in livers of mice. In this assay, the methanol extract treatment significantly increased the CAT activity and GSH levels, but decreased the MDA levels in the liver compared with group control. MOHE group showed 59.16% increase in CAT activity relative to control group (0.382±0.068 *versus* 0.156 ± 0.033 U/mg protein) (Figure 8), and 71.95% increase in GSH levels (8.19±2.98 *versus* 3.34± 1.12 μmol/g tissus) (Figure 9), and 79.56% decrease in MDA levels (23.37±6.73 *versus* 64.78± 7.14 nmol /g tissus) (Figure 10).

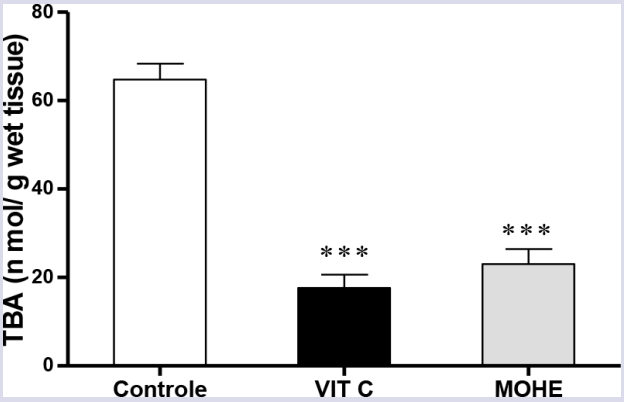

Figure 10: Lipid peroxidation by TBARS assay in liver for different groups group (Methanol extract, VIT C and control group). Values are means ± SEM (n = 6-8). Comparisons are made relative to the control group, *** p<0.001.

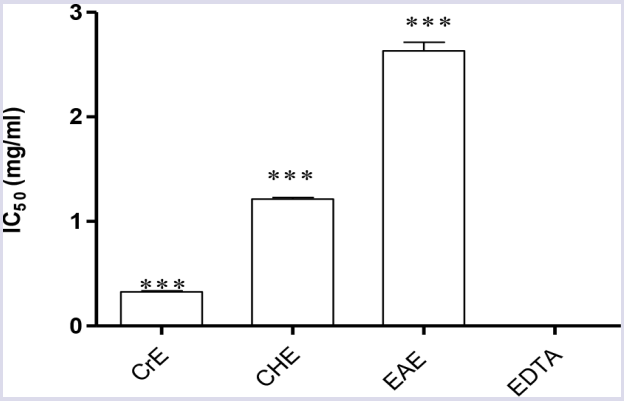

Figure 8: Activity of catalase enzyme in liver tissues for different group (Methanol extract, VIT C and control group). Values are means ± SEM (n = 6-8). Comparisons are made relative to the control group, *** p<0.001; p>0.05, ns: not significant.

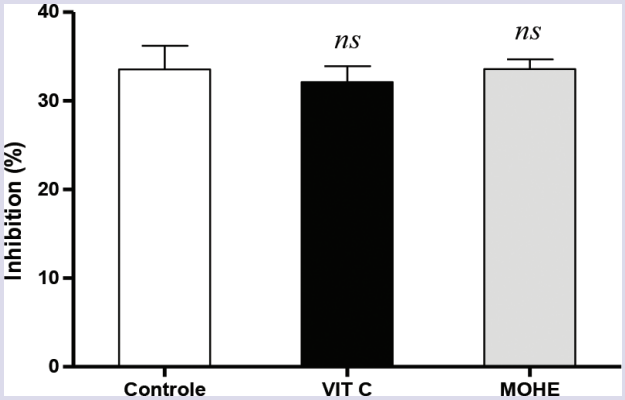

Figure 11: Plasma antioxidant capacity toward DPPH radical for different groups (Methanol extract, VIT C and control group). Values are means ± SEM (n = 6-8). Comparisons are made relative to the control group, p>0.05, ns: not significant.

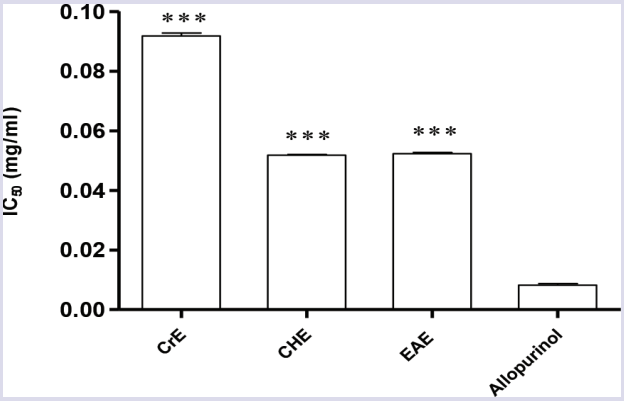

Figure 9: Thiol group content (GSH) in liver for different groups group (Methanol extract, VIT C and control group). Values are means ± SEM (n = 6-8). Comparisons are made relative to the control group, p>0.05, ns: not significant, ** p<0.01.

Plasma antioxidant capacity

The obtained results, showed that administration of MOHE (100 mg/ml) leads to increased plasma antioxidant capacity by DPPH assay (33.59±2.40% *versus* 32.11±4.03% for the control group) (Figure 11). This increase is statistically not significant compared with the control group, however the administration of MOHE (100 mg/ml) significantly increased ($p \leq 0.001$) the antioxidant capacity (1.237±0.298 *versus* 0.53±0.14) by the reducing power assay (Figure 12).

Total antioxidant of red blood cells (hemolysis test)

We measured the effect of treatment the mice with MHGE on whole blood resistance to free radical aggression calculated HT_{50} values, which revealed a half time for hemolysis in all treated groups compared to the control group (Figure 13). For the group MOHE treated with (100mg/kg), the increase in HT_{50} (HT_{50}=72.05±4.95 min) is statistically significant compared with the control group (HT_{50}=59.88±9.32 min) ($p < 0.01$). Also, administering of vitamin C (50 mg/kg), caused no significant delay of hemolysis (HT_{50} = 61.65 ±9.81min) ($p > 0.05$).

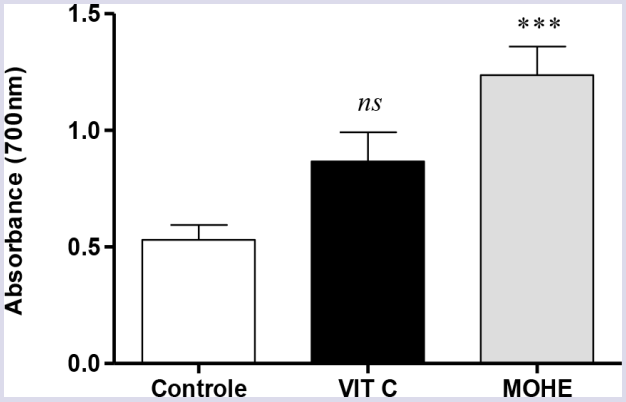

Figure 12: The reducing ability of plasma for different groups (Methanol extract, VIT C and control group). Values are means ± SEM (n=6-8). Comparisons are made relative to the control group, *** p<0.001; p>0.05, ns: not significant.

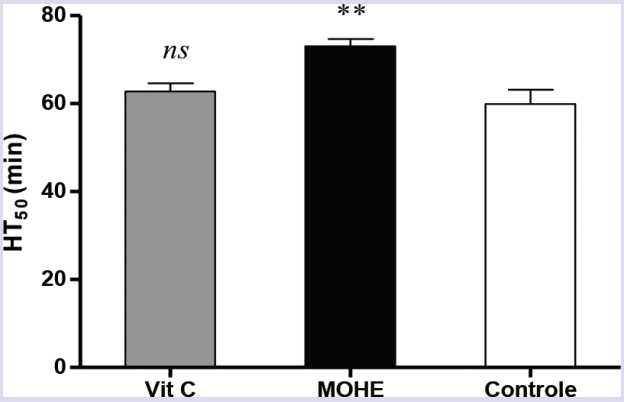

Figure 13: Half-hemolysis time (HT_{50}) for different studied groups (Methanol extract, VIT C and control group). The values are means ± SEM (n = 6-9). Comparisons are made relative to the control group, p>0.5 ns: not significant, ** p<0.01.

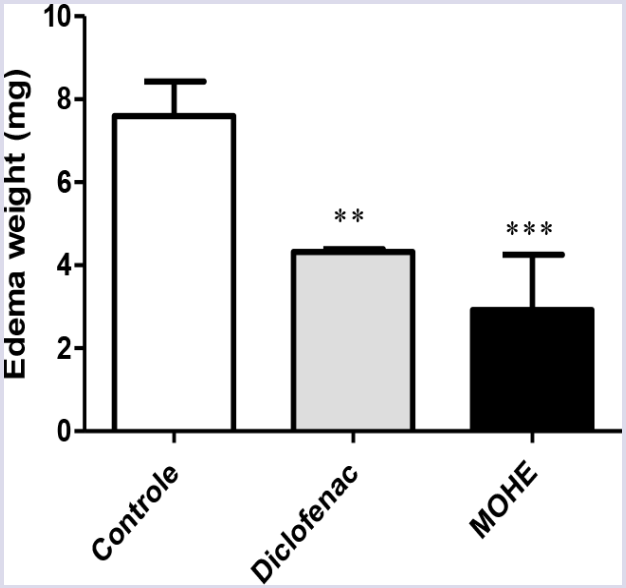

Figure 14: Inhibition of PMA induced ear edema by MOHE and Diclofenac applied topically before PMA application. Each point represents the mean ± SEM (n = 7-8). P < 0.01, P < 0.001 vs. control group.

Anti-inflammatory activity

Ear edema was induced by the application of PMA to the mouse ears. As shown in Figure 14, the application of PMA for 6 h significantly increased the weight of ear edema. Diclofenac, a common clinical non-steroidal anti-inflammatory drug, was used as a positive control for the inhibitory effect on the ear edema. The pre-treatment of 10 mg/kg of Diclofenac effectively reduced ear edema after 6 h PMA stimulation ($p < 0.01$). Similarly, pre-administration of MOHE (100 mg/kg) markedly inhibited the PMA-induced ear edema with 61.51% inhibition ($p < 0.001$).

DISCUSSION

Phenolic antioxidants are products of secondary metabolism in plants and their antioxidant activity is mainly due to their redox properties and chemical structure, which might play an important role in chelating transition metals and scavenging free radicals. Consequently, the antioxidant activities of plant extracts are often explained by their total phenolic and flavonoid contents. Also, the sterical structures of antioxidants or free radicals are known to play a more important role in their abilities to scavenge different types of free radicals.[27]

When making a comparison between the yields of extracts, it was found that the polar solvents gave better extraction yields, which is true in the study of Chigayo *et al.*[28] Since biologically active compounds occur naturally in very small concentrations, the choice of an extraction method and the corresponding suitable solvent is an important step in the drug discovery process.

Addition of water to methanol proved that the extraction efficiency can be increased significantly as the extraction yields obtained with 80% methanol (52.9% yield) and 60% methanol (46.9% yield) were much higher than the yield obtained from the use of pure methanol (36.6% yield).[28]

DPPH$^{\cdot}$ is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In the present study, there was increased scavenging activity of the DPPH radicals with increasing concentration of the plant extracts, which may indicate an increased ability to donate hydrogen ions resulting in a lighter solution which is proportional to the number of electrons gained. Therefore, it may be postulated that SCE has DPPH scavenging activity by reducing the radical to corresponding hydrazine because of its hydrogen ion-donating ability.[30]

The results revealed a good correlation between antiradical potent and phenolic content. Many authors have reported excellent linear correlations between antioxidant/antiradical activity tests and total phenolic content.[31,28,32] Generally certain structural features in flavonoids are responsible for their high antioxidant activity; the presence of 2, 3-unsaturation on the C-ring and the number and substitution of hydroxyl groups on the A and B-rings.[32]

The scavenging of the radical $ABTS^{+}$ by the extracts was found to be much lower than that of DPPH. Stereo selectivity of the radicals and the solubility of extracts in diverse solvent systems are some factors that have been reported to affect the capacity of extracts to react and quench different radicals. This further showed the capability of SCE to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.[30]

The β-carotene bleaching assay is based on the discoloration of β-carotene owing to its reaction with linoleic acid-generated free radicals in an emulsion system. In the presence of an antioxidant compound, this degradation process is prevented. It also reflects the ability to inhibit the lipid peroxidation *in vitro*.[29,33] Burda and Oleszek.[34] reported that the differences in solubility of flavonoids, both aglycones, glycosides, and methoxylated derivatives, in a micellar water–lipid system may influence the results obtained from this test, and the partition of the compounds between the two phases can influence the oxidation results.

There is no correlation between total phenolic content and chelating activity, the ability for extracts to chelate metal ions depends on the availability of other antioxidants responsible for metal chelation. Non-phenolic metal chelators include phosphoric acid, citric acid, ascorbic acid, carnosine, some amino acids, peptides and proteins such as transferrin and ovotransferrin.[35]

SCE have an inhibitory effect on XO activity, indicating that their scavenging effects on superoxide anion is due to dual effect of the extracts on XO activity and superoxide anion scavenging. This activity could be related to the presence of flavonoids in SCE and their structural differences. The insaturation in the C ring and the free hydroxyl group at C-7 enhanced the activity.[36] To our knowledge, there have been no studies or reports to date regarding inhibition of XO activity by SCE.

SCE were evaluated for their protection capacity on red blood cells (RBCs) against oxidative damage by AAPH, a peroxyl radical generator that attacks the erythrocytes to induce the chain oxidation of lipids and proteins, disturbs the membrane organization and eventually leads to hemolysis.[37] Hemolytic activity of most extracts of SCE may be explained by the presence of saponins in the erythrocyte suspension, since these compounds are also extracted by methanol and recognized by their hemolytic activity and foaming properties. In the EAE, the hemolysis is lagged, indicating that endogenous antioxidants in the erythrocytes, namely glutathione, tocopherol, ascorbate and enzymes such as catalase and superoxide dismutase, can efficiently quench radicals to protect them against free radical induced hemolysis, as described previously.[38]

CAT is a key component of the antioxidant defense system, which metabolizes hydrogen peroxide into oxygen and water and protects tissues against reactive hydroxyl species. GSH, a nonenzymatic antioxidant, decomposes H_2O_2 into molecular oxygen and water, and constitutes the first line of defense against free radicals.[39] Lipid peroxidation is also one of the most important mechanisms contributing to oxidative stress. Hence, the measurement of lipid peroxidation is an important indicator in the assessment of antioxidant potential. We calculated the amount of MDA, which is one of the most important by-products of lipid peroxidation.

Some research which evaluated the antioxidant activity of some plant extracts *in vivo*, Wang *et al.*[40] found that in Douchi extracts groups, SOD and CAT activities increased significantly in liver tissue. Nonetheless, glutathion peroxidase (GSH-Px) activities had no change in liver tissue. SOD and GSH-Px activities increased significantly in kidney tissue; however, CAT activities exhibited no change in kidney tissue, they proposed that the change of enzyme activities is related to the components or metabolites of extracts, which could affect enzymatic activities or enzyme contents. However, da Silva *et al.*[41] showed that *Passiflora edulis*' aqueous extract showed an increase in GSH level in kidneys, but didn't affect significant change in the GSH level in liver compared to control, and enhanced the activity of glutathione reductase (GR) and decreased the activity of (GPx) and SOD relative to control group. In contrast, this extract was able to reduce liver TBARS. However, it did not modify the serum lipid peroxidation. The beneficial effects of plant extracts in the reduction of lipid peroxidation by the decrease of TBARS levels in liver and serum tissues is also observed in several other studies.[42,43] The enhanced activities of antioxidant enzyme (CAT) and GSH level with reduction of MDA level may provide an effective defense of MHGE from the damaging effects of free radicals in vital tissues. It is believed that the enhanced activities of antioxidant enzymes were partially due to the increased mRNA expression of these enzymes.[44]

In a previous study,[41] serum antioxidant potential was not affected by *Passiflora edulis*' extract aqueous in experimental group with (FRAP and ORAC assays) suggesting that the experimental period was too short to promote such modifications. In humans, the consumption of black tea did not change the plasmatic antioxidant status (by ORAC and FRAP assay) even after the chronic experimental period.[45] Published data about antioxidant response after short-term treatment with phenolic compounds are conflicting. Some studies have reported no change in the antioxidant response after consumption of *Pinnus maritima* extract,[46] anthocyanins from *Myrciaria jaboticaba,*[47] controlled diets high in fruits and vegetables.[48] However, other studies with berry administration have shown an increase in *in vivo* antioxidant activity.[47,49]

In the present study, the incubation of erythrocyte together with t-BH led to remarkable antihemolytic effect. The protective effects of plants against hemolysis may be due to: i. their kind of phenolic content, because there is no significant if correlation between antihymolitic effect of extracts and their phenolic compound content, ii. And/or the difference in the degree of the penetrations of the flavonoid molecules in intact erythrocytes.[50] The behavior of these extracts against the radical attack is always difficult to explain because the RBCs are a complex matrix in which the substances pharmacologically evaluated could be involved in many reactions in the cell membrane.[51]

The application of PMA for 6 h significantly increased the weight of ear edema. PMA and other phorbol esters has been reported that cyclo-oxygenase inhibitors and 5-lipoxygenase inhibitors are highly effective against inflammation caused by PMA. Also, polyphenols and flavonoids such as caffeic acid, quercetin, luteolin, have been recognized as potent inhibitors of cyclo-oxygenase in other studies.[52,53] These reports, together the results of Khouya *et al.*[6] suggest a possible relationship between the protective effects of aqueous extracts in an acute inflammatory animal model and the rich content of polyphenol in these extracts.

CONCLUSION

The antioxidant and anti-inflammatory properties of these plants could justify its importance in the fight against oxidative stress diseases and its corollaries. The presented study could explain the effectiveness of plants in traditional medicine in Algeria. In biological activities, the plant extracts presented some potential antioxidant and anti-inflammatory effects. Future studies will focus on isolating the bioactive compounds from these plants.

CONFLICT OF INTEREST

The authors declare none.

REFERRENCES

1. Ramya R, Kalaiselvi M, Narmadha R, Gomathi D, Bhuvaneshwari V, Amsaveni R, *et al.* Secondary metabolite credentials and *in vitro* free radical scavenging activity of *Alpinia calcarata*. J Ac Med. 2015;5(2):33-7. http://dx.doi.org/10.1016/j.jacme.2015.02.005.
2. Verma AR, Vijayakumar M, Rao CV, Mathela CS. *In vitro* and *in vivo* antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*. Food Chem Toxicol. 2010;48(2):704-9. http://dx.doi.org/10.1016//j.fct.2009.11.052.
3. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, *et al.* Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources: A review. Int J Mol Sci. 2017;18(1):96. doi:10.3390/ijms18010096.
4. Šamec D, Durgo K, Grúz J, Kremer D, Kosalec I, Piljac-Žegarac J, *et al.* Genetic and phytochemical variability of six *Teucrium arduini* L. populations and

their antioxidant/prooxidant behaviour examined by biochemical, macromolecule- and cell-based approaches. Food Chem. 2015;186:298-305. http://dx.doi.org/10.1016/j.foodchem.2014.07.135.

5. Simin N, Orcic D, Cetojevic-Simin D, Mimica-Dukic N, Anackov G, Beara I, *et al*. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (Allium flavum L. subsp. flavum, Alliaceae). Food Sci Technol. 2013;54(1):139-46. http://dx.doi.org/10.1016/j.lwt.2013.05.023.
6. Khouya T, Ramchoun M, Hmidani A, Amrani S, Harnafi H, Benlyas M, *et al*. Anti-inflammatory, anticoagulant and antioxidant effects of aqueous extracts from Moroccan thyme varieties. Asian Pac J Trop Biomed. 2015;5(8):636-44. http://dx.doi.org/10.1016/j.apjtb .2015.05.011.
7. Chisté RC, Freitas M, Mercadante AZ, Fernandes E. Carotenoids inhibit lipid peroxidation and hemoglobin oxidation, but not the depletion of glutathione induced by ROS in human erythrocytes. Life Sci. 2014;99(1):52-60. http://dx.doi.org/10.1016/j.lfs.2014.01.059.
8. Akerreta S, Cavero SY, López V, Calvo MI. Analyzing factors that influence the folk use and phytonomy of 18 medicinal plants in Navarra. J Ethnobiol Ethnomedicine. 2007;3(1):16. doi:10.1186/1746-4269-3-16.
9. Da Silva JAT. Mining the essential oils of the Anthemidea. Afr J Biotechnol. 2004;3(12):706-20. http://www.academicjournals.org/AJB
10. Suresh B, Srirama S, Dhanaraja SA, Elangoa K, Chinnaswamy K. Anticandidal activity of *Santolina chamaecyparissus* volatile oil. J Ethnopharmacol. 1997; 55(2):151-9. https://doi.org/101016/ S0378-8741(96)01490-0.
11. Markham KR. Techniques of flavonoid identification. London: Academic Press. 1982;1-113.
12. Li HB, Cheng KW, Wong CC, Fan KW, Chen FD, Jiang YS. 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.
13. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, *et al*. Oxigen species scavenging activity of phenolic extract from howthorn fresh plant organs and pharmaceutical preparation. Arzneimittel-forsch. 1996;46(11):1086-94.
14. Cuendet M, Hostettmann K, Dyatmiko W, Potterat O. Iridoid glucosides with free radical scavenging propreties from Fagraea blumei. Helv Chim Acta. 1997;80(4):1144-52. DOI: 10.1002/hlca.19970800411.
15. Re R, Pellegrini N, Proteggente A, Annala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolourisation assay. Free Radical Bio Med. 1999;26(9):1231-37. https://doi.org/10.1016/S0891-5849(98)00315-3.
16. Aslan A, Güllüce M, Sökmen M, Adigüzel A, Sahin F, Özkan H. Antioxidant and antimicrobial properties of lichens Cladonia foliacea, Dermatocarpon miniatum, Everinia divaricata, Everinia prunastri and Neofuscella pulla. Pharm Biol. 2006;44(4):247-52. http://dx.doi.org/10.1080/13880200600713808.
17. Decker EA, Welch B. Role of Ferritin as lipid oxidation catalyst in muscle food. J Agr Food Chem.1990;38(3):674-7. http://dx.doi.org/10.1021/jf00093a019.
18. Avis PG, Bergel F, Bray RC. Cellular constituents. The chemistry of xanthine oxidase. Part III. Estimations of the cofactors and the catalytic activities of enzyme fractions from cow's milk. J Chem Soc.1956;1219-26. DOI:10.1039/JR9550001100.
19. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem pharmacol. 1988;37(5):837-41. doi:10.1016/0006-2952(88)90169-4.
20. Girard AS, Madani F, Boukortt M, Cherkaoui-Malki J. Belleville J. Fructose-enriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats. Nutrition. 2006;22(7):758-66. http://dx.doi.org/10.1016/j.nut.2006.05.006.
21. Claiborne A. Catalase activity. In: Handbook of Methods for Oxygene Radical Research. Greenwald RA. Ed. CRC Press: Boca Raton, FL. 1985;283-4.
22. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. https://doi.org/10.1016/0003-9861(59)90090-6.
23. Lowry OH, Rosegrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193(1):265-75. http://www.jbc.org/content/193/1/265.citation.
24. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric reaction. Anal Biochem. 1979;95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3.
25. Chung YC, Chen SJ, Hsu CK, Chang CT, Chou ST. Studies on the antioxidative activity of Graptopetalum paraguayense E. Walther. Food Chem. 2005;91(3):419-24. https://doi.org/10.1016/j.foodchem.2004.06.022
26. Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G, *et al*. *In vivo* and *in vitro* anti-inflammatory activity of Mangifera indica L. extract (VIMANG). Pharmacol Res. 2004; 50(2):143-9. DOI:10.1016/j.phrs.2003.12.003.
27. Ndoye-Foe FMC, Tchinang TFK, Nyegue AM, Abdou JP, Yaya AJG, Tchinda AT, *et al*. Chemical composition, *in vitro* antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. Complem Altern Med. 2016;16(1):117. DOI 10.1186/s12906-016-1096-yy.
28. Chigayo K, Mojapelo PEL, Mnyakeni-Moleele S, Misihairabgwi JM. Phytochemical and antioxidant properties of different solvent extracts of Kirkia wilmsii Tubers. Asian Pac J Trop Biomed. 2016;6(12):1037-43. http://dx.doi.org/10.1016/j.apjtb.2016.10.004.
29. Chaouche TM, Haddouchi F, Ksouri R, Atik-Bekkara F. Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. J Chin Med Assoc. 2014;77(6):302-7. http://dx.doi.org/10.1016/j.jcma.2014.01.009.
30. Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z. *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia. Beni-Suef University Journal of Basic and Applied Sciences. 2017;6(1):10-4. http://dx.doi.org/10.1016/j.bjbas.2016.12.003.
31. Wong-Paz JE, Contreras-Esquivel JC, Rodríguez-Herrera R, Carrillo-Inungaray ML, Lopez LI, Nev´arez-Moorillon GV, *et al*. Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. Asian Pac J Trop Med. 2015;8(2):104-11. doi: 10.1016/S1995-7645(14)60299-6.
32. Noreen H, Semmar N, Farman M, McCullagh JSO. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pac J Trop Med. 2017;10(8):792-801. http://dx.doi.org/10.1016/j.apjtm.2017.07.024.
33. Baghiani A, Boumerfeg S, Adjadj M, Ameni D, Djarmouni M, Khelifi-Touhami F, *et al*. Antioxidants, free radicals scavenging and xanthine oxidase inhibitory potentials of Ajuga iva L. extracts. Free Radic Antioxid J. 2011;1(4):21-30. doi: 10.5530/ax.2011.4.5.
34. Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. J Agr Food Chem. 2001;49:2774-79. DOI: 10.1021/jf001413m.
35. Lee JH, Renita M, Fioritto RJ, StMartin SK, Schwartz SJ, Vodovotz Y. Isoflavone characterization and antioxidant activity of Ohio soybeans. J Agric Food Chem. 2004;52(9):2647-51. DOI:10.1021/jf035426m.
36. Baghiani A, Ameni D, Boumerfeg S, Adjadj M, Djarmouni M, Charef N, *et al*. Studies of Antioxidants and Xanthine Oxidase Inhibitory Potentials of Root and Aerial Parts of Medicinal Plant Capparis Spinosa L. Amer Jour of Med and Med Scien. 2012;2(1):25-32. doi: 10.5923/j.ajmms.20120201.06.
37. Kumar KH, Rachitha P, Krupashree K, Jayashree GV, Abhishek V, Khanum F. LC-ESI-MS/MS analysis of total oligomeric flavonoid fraction of Cyperus rotundus and its antioxidant, macromolecule damage protective and antihemolytic effects. Pathophy. 2015;22(4):165-73. DOI: http://dx.doi.org/10.1016/j.pathophys.2015.07.001.
38. Magalhαes AS, Silva BM, Pereira JA, Andrade PB, Valentαo P, Carvalho M. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. Food Chem Toxicol. 2009;47(6):1372-7. doi:10.1016/j.fct.2009.03.017.
39. He J, Huang B, Ban X, Tian J, Zhu L, Wang Y. *In vitro* and *in vivo* antioxidant activity of the ethanolic extract from Meconopsis quintuplinervia. J Ethnopharmacol. 2012;141(1):104-10. http://dx.doi.org/doi:10.1016/j.jep.2012.02.006.
40. Wang D, Wang L, Zhu F, Zhu J, Chen XD, Zou L, *et al*. *In vitro* and *in vivo* studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese salt-fermented soybean food). Food Chem. 2008;107(4):1421-28. https://doi.org/10.1016/j. foodchem. 2007.09.072.
41. Da-Silva JK, Cazarin CBB, Colomeu TC, Batista ÂG, Meletti LMM, Paschoal JAR, *et al*. Antioxidant activity of aqueous extract of passion fruit (Passiflora edulis) leaves: *In vitro* and *in vivo* study. Food Res Int. 2013;53:882-90. http://dx.doi.org/10.1016/j.foodres.2012.12.043.
42. Khennouf S, Amira S, Arrar L, Baghiani A. Effect of some phenolic compounds and quercus tannins on lipid peroxidation. World Appl Sci J. 2010;8(9):1144-49.
43. Da-Silva PAC, Dionsísio AP, Wurlitzer NJ, Alves RE, Brito ES, Silva AMO, *et al*. Effect of antioxidant potential of tropical fruit juices on antioxidant enzyme profiles and lipid peroxidation in rats. Food Chem. 2014;157:179-85. DOI: 10.1016/j.foodchem.2014.01.090.
44. Liu J, Jia L, Kan J, Jin C. *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food Chem Toxicol. 2013;51:310-16. http://dx.doi.org/10.1016/j.fct.2012.10.014.
45. Widlansky ME, Duffy SJ, Hamburg NM, Gokce N, Warden BA, Wiseman S. Effects of black tea consumption on plasma catechins and markers of oxidative stress and inflammation in patients with coronary artery disease. Free Radical Bio Med. 2005;38(4):499-506. https://doi.org/10.1016/j.freeradbiomed.2004.11.013.
46. Silliman K, Parry J, Kirk LL, Prior RL. Pycnogenol does not impact the antioxidant or vitamin C status of healthy young adults. J Am Diet Assoc. 2003;103(1):67-72. doi: 10.1053/jada.2003.50004.
47. Leite AV, Malta LG, Riccio MF, Eberlin MN, Pastore GM, Marostica MR. Antioxidant potential of rat plasma by administration of freeze-dried Jaboticaba peel (*Myrciaria jaboticaba Vell Berg*). J Agr Food Chem. 2011;59(6):2277-83. doi: 10.1021/jf103181x.
48. Paterson E, Gordon MH, Niwat C, George TW, Parr L, Waroonphan S. Supplementation with fruit and vegetable soups and beverages increases plasma carotenoid concentrations but does not alter markers of oxidative stress or cardiovascular risk factors. J Nutr. 2006;136(11):2849-55.
49. Hassimotto NMA, Pinto MD, Lajolo FM. Antioxidant status in humans after consumption of blackberry (*Rubus fruticosus* L.) juices with and without defatted milk. J Agr Food Chem. 2008;56(24):11727-33. doi: 10.1021/jf8026149.
50. Chaudhuri S, Banerjee A, Basu K, Sengupta B, Sengupta PK. Interaction of

flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. Int J Biol Macromolec. 2007;41(1):42-8. DOI: 10.1016/j.ijbiomac.2006.12.003.

51. Djeridane A, Yousfi M, Nadjemi B, Vidal N, Lesgards JF, Stocker P. Screening of some *Algerian medicinal plants* for the phenolic compounds and their antioxidant activity. Eur Food Res Technol. 2007;224(6):801-9. DOI: 10.1007/s00217-006-0361-6
52. Armutcu F, Akyol S, Ustunsoy S, Turan FF. Therapeutic potential of caffeic acid phenethyl ester and its anti-inflammatory and immunomodulatory effects: Review. Exp Ther Med. 2015;9(5):1582-8.
53. Carlsen IG, Frøkiær J, Nørregaard R. Quercetin attenuates cyclooxygenase-2 expression in response to acute ureteral obstruction. Am J Physiol Renal Physiol. 2015;308(11):1297-305. http://dx.doi.org/ 10.1152/ajprenal.00514.2014.