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ACUTE, SUB-ACUTE AND ANTIOXIDANT ACTIVITIES OF *ARTHROPHYTUM SCOPARIUM* AERIAL PARTS

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ABSTRACT: This study aims to evaluate the *in-vitro* and *in-vivo* antioxidant activity of different extracts prepared from the aerial parts of *Arthrophytum scoparium*. Polyphenols flavonoids and tannins contents were determined. The acute toxicity of ASE was carried out based on OECD during two weeks to ascertain the LD_{50}. In sub-acute toxicity, a 100, 200 and 400 mg/kg/day extract was orally administrated for 21 days consecutively. The results showed that decoction extract (DEC) was the richest fractions in Polyphenols, flavonoids and tannins content (23.29 mg/GAE, 99.77 mg/QE and 113.33 mg/TAE), respectively. *Arthrophytum scoparium* hydro-methanolic extract (ASE) had the strongest antioxidant activity using DPPH and ABTS scavenging assays with an IC_{50} of 0.045 and 0.0017 mg/ml, respectively. Moreover, the β-carotene/linoleic acid bleaching assay showed that ethyl acetate extract (EAE) had the highest inhibiting activity (83.29%). Furthermore, all *A. scoparium* fractions exhibited a good ferric reducing power. The administrated doses did not undergo changes in general behavior, toxicity or mortality of the tested mice. The LD_{50} was found to be superior to 5 g/kg. Daily administration of ASE leads to an increase in GSH level and decreasing the lipid peroxidation in the liver. *In-vivo*, ASE showed a strong scavenging effect on DPPH and reducing power. In conclusion, *A. scoparium* extracts exhibited considerable antioxidant effects *in-vitro* and *in-vivo*. These findings support the traditional use of this plant as an anti-inflammatory in therapeutics.

INTRODUCTION: Free radicals are continuously produced by the normal body using oxygen, which is an indispensable element for life. The normal cells use oxygen to generate energy; free radicals are produced by the mitochondria [1]. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are resulting from the cellular redox oxygen process [2, 3].

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Oxidative stress is described as a disproportion between the production of free radicals and reactive metabolites (oxidants), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to the damage of important biomolecules and cells, with potential impact on the whole organism [4].

In human, oxidative stress is thought to play a key role in the progression of many diseases ranging from neurodegenerative disorders to cancer [5]. The human body has multiple mechanisms especially enzymatic and nonenzymatic antioxidant systems to protect the cellular molecules against (ROS) induced damage. Secondary metabolites such as polyphenols constitute a big family of naturally

occurring substances in the plant kingdom. These substances have considerable interest in the field of food industry, pharmacy, and medicine due to their wide range of biological effects [6]. *Arthrophytum scoparium* belongs to the Chenopodiaceae family and is locally known as rimth. This plant has been reported as a plant rich in alkaloids [7, 8, 9].

This study aims to evaluate the antioxidant activity of *A. scoparium* aerial part extracts using deferent methods such as lipid peroxidation, reducing power and radical scavenging capacity. To valorize the traditional use of this plant from one hand and another hand to look for a new treatment which may be used as an alternative to the known pharmaceutical medicine already used in treating inflammation.

MATERIALS AND METHODS:

Plant Material: The vegetal material was collected in April 2016 from Ouled djellal, Biskra, Algeria. *A. scoparium* aerial parts were washed, cut, dried for two weeks in free air in dark room, and then powdered using the traditional mill. The powder was conserved in a glass bottle at ambient temperature until use. The plant specimen was identified by Pr. Oujhih B. Institute of Nutrition And Agronomy, University of Batna (Algeria) a voucher specimen was kept under the number 227/ISVSA/ DA/UHLB1/17.

Chemicals: Linoleic acid, β-carotene, butylated hydroxytoluene (BHT) was purchased from Fluka Chemical Co. (Buchs, Switzerland). Salicylate-sodium, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediamine tetra-acetic acid (EDTA), gallic acid, quercitin, 2.2´-Azino-bis (3-ethylbenzene-thiazoline 6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, potassium persulphate, potassium ferricyanide (K_3FeCN_6), trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrozine, ferrous and ferric chloride, furosemide, DTNB (5-5'-dithiobis2-nitrobenzoïc acid), H_2O_2, *n*-butanol, methanol. These chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) or Merck.

Animal Material: Healthy female adult Wistar rats (Nulliparous and non-pregnant) weighing 200-300g were used. They purchased from Pasteur Institute, Algiers, Algeria. The animals were acclimatized for one week, prior to experiments. They were fed with standard diet and water and kept in a standard with natural light and dark cycle (OECD, 2008), Ethics approval number- 2017/005.

Preparation of Plant Extracts: The phenolic compounds extraction was conducted as reported by Madoui *et al*. [10] Aerial parts of *A. scoparium* (100 gm) were extracted with one liter of methanol-water (85:15 v/v) and kept under agitation overnight at 4 °C the resulting solution was filtered to obtain the first filtrate. This procedure was repeated on the residue using water-methanol (50:50 v/v) under agitation for 4 h to obtain the last filtrate. The first and last filtrates were combined. The solvent was evaporated under reduced pressure to get CrE. The hydro-methanolic solution was washed with hexane several times until a clear upper layer of hexane was obtained. The lower layer was then extracted successively with chloroform and ethyl acetate to obtain 3 fractions; chloroform extract (ChE), ethyl acetate extract (EAE) and aqueous extract (AQE). Each fraction was stored at -20 ºC until use.

Antioxidant Activity *in-vitro*:

Estimation of Total Phenolic Content: Total phenolic compounds in *A. scoparium* extracts, was determined by the Folin-Ciocalteu reagent, using the mentioned by Boussoualim *et al*., [11] with slight modifications. 0.1 ml of samples and standard was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 fold). After incubation for 4 min, 0.4 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added. After 1 h and 30 min of incubation in the dark at room temperature, the measurement of the absorbance was affected at 760 nm using a spectrophotometer and the results are expressed as mg gallic acid equivalent (GAE)/g of extract.

Estimation of Flavonoid Content: Total flavonoids content in each extract was estimated using the aluminum chloride colorimetric method as described by Guemmaz *et al.* [12] Briefly; 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride in methanol. After incubation at room temperature for 10 min, the absorbance was measured at 430 nm, and the flavonoids content was expressed in mg quercetin equivalent per gram of dry weight (QE).

Estimation of Tannin Content: The capacity to precipitate hemoglobin was determined using bovine fresh blood according to Bouaziz *et al.* [13] Briefly, equal volumes of each extract and hemolysed bovine blood (absorbance = 1.6) were mixed. After 20 min, the mixture was centrifuged at 4000 rpm for 10 min, and the absorbance of the supernatant was measured at 756 nm. The results were expressed as mg equivalent to tannic acid per gram dried weight (mg TAE/g DW).

DPPH Radical Scavenging Activity: Quantitative measurement of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging properties was carried out using the method described by Aouachria *et al*., [14], with slight modifications. 1250 µl of DPPH (0.4 mM in methanol) was mixed with 50 µl of extract. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The absorbance was taken at 517 nm. DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (A_C - A_S / A_C) \times 100$$

A_C: is the absorbance without extract; A_S: is the absorbance in the presence of the sample.

ABTS Radical Scavenging Activity Assay: The spectrophotometric measurement of ABTS + (2 2'-azino- bis3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity was determined according to Bouaziz *et al.* [13] In this method, the radical cation was produced by reacting 2 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 16 h. Before usage, the ABTS + solution was diluted with sodium phosphate buffer (0.1 M, pH 7.4) to get an absorbance of 0.750 ± 0.025 at 734 nm. Then, 2 ml of fresh ABTS+ solution was added to 20 µl of extract solution in methanol at different concentrations. The percentage of inhibition at 734 nm was calculated for each concentration relative to a blank absorbance after 7 min. The extent of decolorization is calculated as the percentage reduction of the absorbance.

$$\text{ABTS + Scavenging effect (\%)} = (Ac - As) / Ac \times 100$$

Where AC: is the absorbance of the control (ABTS); AS: is the absorbance in the presence of the sample/ standard.

β-Carotene/Linoleic Acid Assay: The capacity of plant extracts to anticipate bleaching of β -carotene was evaluated as described by Guemmaz *et al.* [12] The solution mixture of β-carotene-linoleic acid was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform and 25 µl linoleic acid and 200 mg tween 40. Chloroform was completely evaporated, and then, 100 ml distilled water saturated with oxygen were added. Into test tubes, 2500 µl of the reaction mixture was disbursed and 350 µl of the various extracts with a same concentration (2 mg/ml), were added and the emulsion was incubated at room temperature for 48 h. The same procedure was repeated with synthetic antioxidant BHT as a positive control, and blanks (MeOH and H_2O). The measurement of absorbance of the reaction mixture was effected after; 0, 1, 2, 4, 6, 24 and 48 h at 490 nm. The antioxidant activity (AA) was measured in terms of successful bleaching of β -carotene by using the following equation:

$$AA\% = A \text{ sample} / A \text{ BHT} \times 100$$

A sample: Absorbance in the presence of the extract, A BHT: Absorbance in the presence of positive control BHT.

Reducing Power of Plant Extracts: The ability to reduce ferric ions was measured according to the method described by Bencheikh *et al.* [15] Briefly, 0.1 ml of each plant extract with different concentrations was mixed with an equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubation for 20 min at 50 ºC, 0.25 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Then 0.25 ml of the supernatant was added to 0.25 ml distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance was measured to determine the amount of ferric ferrocyanide (Prussian blue) formed at 700 nm against a blank.

Acute Toxicity and Determination of Lethal Dose (LD_{50}): The acute toxicity study of *A. scoparium* extracts was performed according to OECD (guidelines 425). LD_{50} of *A. scoparium* extracts was determined on rats. The animals were divided into two groups of five animals each. The crude extract was administrated orally in a single dose of 2000 and 5000 mg/kg. One female under fasting for 4 h. Sequentially, at intervals of 48 h, the same dose was administered to four females.

Five treated animals in total were received the vehicle (distilled water) and kept under the same conditions for the purpose to make a negative control group. The animals were observed if any sign of toxicity appears for 14 days (animal response and general physical condition). Results of this assay permit to determine the doses which will be used in the *in- vivo* tests and that which caused no lethality at all.

Sub-Acute Toxicity and Antioxidant Activity *in-vivo*: Animals Treatment: The Swiss albino's female mice of 25-30 g were randomly divided into 5 groups of five with similar average body weight. Neutral control (received distilled water). Positive control (received ascorbic acid at a dose of 100 mg/kg) and three other groups treated with a crude extract of *A. scoparium* aerial parts (100, 200 and 400 mg/kg, respectively) orally once a day [16]. After 21 days of treatment, blood samples were collected in tubes EDTA and centrifuged at 3000 rpm at 4 ºC for 10 min. Plasma was reserved for analyzing the antioxidant activity using DPPH scavenging assay and the reducing power. Livers were removed, washed and homogenized in ice-cold KCl (1.15%) with a ratio of 1/10, and then centrifuged at 5000 rpm for 15 min at 4°C. The resulting supernatant was used for the determination of catalase activity, malondialdehyde (MDA) and glutathione contents in organs.

Effect of Extracts on Plasma Antioxidant Capacity Using DPPH Radical: The capacity of plasma to scavenge the DPPH radical was evaluated according to the method mentioned previously *in-vitro* [14]. The appropriate volume of DPPH (0.004% in methanol) was added to the plasma. After 30 min of incubation in the darkness, the absorbance measured at 517 nm.

Effect of Extracts on Plasma Reducing Power: The reducing power test was carried out as described previously *in-vitro* [15]. Sodium phosphate buffer (0.2 M; pH 6.6) and potassium ferricyanide were added to the plasma. The mixture was incubated for 20 min at 50 °C. After that, trichloroacetic were added, the mixture was then centrifuged for 10 min at 3000 rpm. Distilled water and ferric chloride (0.1%) were mixed with the aliquot upper layer, and the absorbance was measured at 700 nm.

Assessment of Reduced Glutathione Concentration: The procedure to measure the reduced glutathione (GSH) level was estimated following Bentahar *et al.*, [17] with slight modifications. The mixture of the homogenate tissue and TBA (20%) with equal volume was centrifugation for 10 min at 2000 rpm. The supernatant (200 µl) was added to 1.8 ml of the Ellman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) (0.1 mM), which was prepared in phosphate buffer (0.3 M). Estimation of GSH is based on the reaction of DTNB with GSH and yield a yellow colored chromophore with a maximum absorbance at 412 nm. The amount of GSH present in the tissue was calculated using its extinction coefficient to be 1.36×10^4 M^{-1} cm^{-1} at 412 nm.

Assessment of Lipid Peroxidation: The lipid peroxidation in liver homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method mentioned by Zerrargui *et al.*, [18] which is based on spectrophotometric measurement of the pink color generated by the reaction of complex TBA-MDA. Tissue homogenate (0.5 ml) was mixed with TCA (0.5 ml, 20% w/v) and TBA (1 ml, 0.67% w/v) and then heated at 95 °C on a water bath for 15 min. After incubation, the tubes were cooled to room temperature, and final volume was made to 5 ml in each tube. 4 ml of *n*-butanol was added to each sample and centrifuged at 3000 rpm for 15 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample.

Assessment of Catalase Activity: Catalase activity was measured by the method of Aouachria *et al.* [14] The elimination of hydrogen peroxide in the presence of catalase was followed by the decrease in absorption of peroxide solution in the ultraviolet (UV) region. Briefly, 0.1 ml of supernatant was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm in an interval of time; the specific activity was expressed as units per gram of tissue according to this formula:

$$U/g\ tissue = (2.3033/ T) \times (\log A_1/A_2) /g\ tissue$$

A_1: Absorbance at T0, A_2: Absorbance at T1 and T: Interval of time.

Statistic Analysis Results were expressed as mean ± standard deviation. The differences between groups were determined by analysis of variance one-way ANOVA followed by Dunnett's test. Statistical analysis for *in-vitro* results was undertaken using student test. All results were analyzed using GraphPad Prism version 5.00. Differences were considered significant at $P<0.05$.

RESULTS:

Antioxidant Activity *in-vitro*:

Polyphenol, Flavonoid and Tannin Contents in Plant Extracts: In the present study, the quantification of total phenols was performed using Folin-Ciocalteu reagent.

The results showed that DEC was the richest fractions on phenolic, flavonoids and tannins, as shown in **Table 1**.

TABLE 1: TOTAL POLYPHENOL, FLAVONOID, AND TANNIN CONTENT IN *A. SCOPARIUM* EXTRACTS

Extract	Total phenolic content mg/GAE	Total flavonoid content mg/QE	Total tannins content mg/TAE
CrE	18.00 ± 3.54	83.11 ± 3.84	93.63 ± 2.65
ChE	21.30 ± 1.46	95.94 ± 2.81	68.33 ± 1.95
EAE	3.83 ± 1.361	7.88 ± 2.42	51.77 ± 0.48
AQE	20.741 ± 3.39	19.61 ± 1.18	24.97 ± 1.83
DEC	23.29 ± 1.01	99.77 ± 3.75	113.33 ± 0.08

Results are expressed as means ± standard (n = 3). CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction, GAE: gallic acid equivalent, QE: quercitin equivalent, TAE: tannic acid equivalent

DPPH Radical Scavenging Activity: DPPH is the Most frequently used methods to evaluate the radical scavenging activity of antioxidants substances, because of its quickness, reliability, and reproducibility. The IC_{50} value is the parameter used to measure radical scavenging activity. The CrE exhibited a higher scavenging effect (IC_{50} = 0.015 ± 0.005 mg/ml) that remains higher than that of standard with an IC_{50} of 0.087 ± 0.001 mg/ml **Table 2**.

ABTS Radical Scavenging Activity Assay: The ability of plant fractions to serve as electron or hydrogen donator in free radical reactions indicates their scavenging activity against cationic ABTS radical. The ABTS radical is reduced by antioxidants, but it is relatively constant [15]. The results shown in **Table 2** revealed that all plant fractions scavenge the ABTS radical. CrE exhibited the strongest antioxidant effect with IC_{50} = 0.001 ± 5.54^{E}-05 mg/ml, which is comparable to BHT as standard.

β-Carotene/Linoleic Acid Assay: The ability of extracts to inhibit the lipid peroxidation evaluated by β-carotene bleaching test showed that the peroxidation of lipids was effectively inhibited by *A. scoparium* extracts as mentioned in **Table 2**. The EAE showed an interesting antioxidant activity (83.29 ± 2.94%) when compared to the synthetic antioxidant standard BHT (96.93 ± 1.82%) at the same concentration (2 mg/ml).

TABLE 2: ANTIOXIDANT ACTIVITIES; SCAVENGING OF DPPH, ABTS, AND β-CAROTENE BLEACHING AND REDUCING POWER OF *A. SCOPARIUM* EXTRACTS

Extracts	DPPH IC_{50} (mg/ml)	ABTS IC_{50} (mg/ml)	β-carotene AA%	Reducing power EC_{50} (mg/ml)
CrE	0.015 ± 0.005 ***	0.001 ± 5.54^{E}-05***	58.28 ± 1.25***	0.43 ± 0.036**
ChE	0.041 ± 0.007 ***	0.007 ± 0.0002***	64.87 ± 1.53***	1.88 ± 0.104***
EAE	0.052 ± 0.002 ***	0.071 ± 0.005***	83.29 ± 2.94***	0.72 ± 0.11***
AQE	0.073 ± 0.004**	0.006 ± 7.9^{E}-05***	80.16 ± 3.73***	1.36 ± 0.084***
DEC	0.067 ± 0.002 ***	0.012 ±0.0005***	57.61 ± 1.99***	0.063 ± 0.021 ns
BHT	0.087 ± 0.001	0.18 ± 0.028	96.93 ± 1.82	0.18 ± 0.020

Data were presented as means ± SD (n = 3). BHT: butylated hydroxytoluene used as standards. Ns: no significant difference <0.05; ***, P<0.001;**, P<0.01.

Reducing Power: The presence of reducers causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The measurement of the formation of Perl's Prussian blue could be

representing an indicator of the Fe^{2+} concentration at 700 nm. In this assay, all extracts showed a good capacity of electron donation in a concentration-dependent manner **Table 2**.

Acute Toxicity: The present study was performed according to OECD guideline 425. The observations revealed that the ASE did not provoke any behavioral changes during the study period. Moreover, none mortality was registered throughout 14 days and, thus, the LD_{50} is higher than 5000 mg/kg BW for animals. The body weight increased gradually throughout the study period. Statistical analysis of body weight did not reveal any significant differences between treated and control groups **Fig. 1**.

Sub-Acute Toxicity and Antioxidant Activity *in vivo*: Observations and Mortality Patterns: The administration of 100, 200, and 400 mg/kg ASE doses to three groups of females did not indicate any behavioral changes, visual symptoms of toxicity or mortality in animals during the treatment period (21 days).

Body and Relative Organ Weights: Non significant weight loss was recorded in the treated group with ASE at different doses and control groups. During the three weeks, an improvement in body weight was noted in all groups **Table 3**. The administration of Vit. C (100 mg/kg) and ASE (100, 200 and 400 mg/kg) provoke no significant changes in relative weight of the organs (liver, kidneys, spleen, and stomach) in the treated groups compared to the control mice **Table 4**.

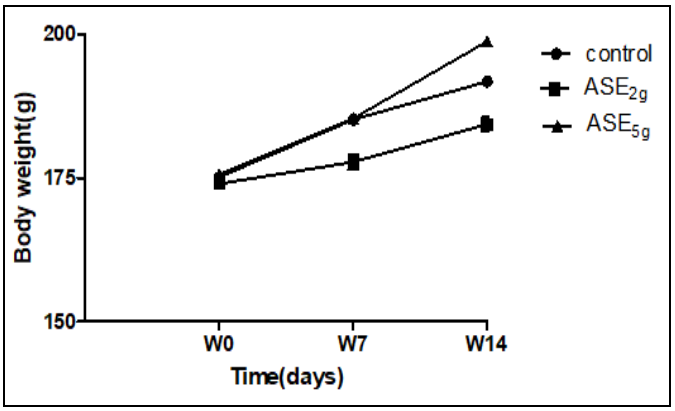

FIG. 1: BODY WEIGHT OF RATS TREATED WITH ASE. Values expressed as mean ± SD, n = 5

TABLE 3: BODY WEIGHT OF MICE TREATED WITH ASE AND CONTROL GROUPS

Groups	Control	Vit. C 100 mg/kg	ASE 100 mg/kg BW	ASE 200 mg/kg BW	ASE 400 mg/kg BW
W0	23.64 ±0.88	24.2 ± 0.83	26.0 ± 0.18	28.4 ± 0.89	25.25 ± 1.59
W7	26 .0 ± 0.82	26.0 ± 0.8	27.4 ± 0.54	29.4 ± 0.14	25.64 ± 0.13
W14	27.83 ± 0.8	26.6 ± 1.03	27.6 ± 0.14	29.8 ± 0.83	27.88± 0.38
W21	30.44± 0.97	26.8 ±1.08	28 ± 0.71	30.8 ± 0.11	28.27 ±0.70

Values expressed as mean ± SD, n = 5.

TABLE 4: RELATIVE ORGANS WEIGHT OF MICE TREATED WITH ASE, ViT. C AND CONTROL GROUP

Groups	Control	Vit. C 100mg/kg BW	ASE 100mg/kg BW	ASE 200mg/kg BW	ASE 400mg/kg BW
Liver	47.73 ±6.05	43.58 ± 9.44 ^{ns}	49.81 ± 12.89^{ns}	52.85 ± 8.81 ^{ns}	49.64 ± 10.32 ^{ns}
Kindey	8.78 ± 1.23	11.49 ± 1.03 ^{ns}	10.74 ± 1.91 ^{ns}	13.30 ± 1.56 ^{ns}	11.26 ± 2.17 ^{ns}
Spleen	4.73 ± 0.61	5.66 ± 0.58 ^{ns}	5.24 ± 1.14 ^{ns}	6.11 ± 1.33 ^{ns}	5.92 ± 0.82 ^{ns}
Stomach	8.43 ± 3.45	10.78 ± 1.40 ^{ns}	9.40 ± 2.75 ^{ns}	10.96 ± 2.89 ^{ns}	9.34 ± 2.34 ^{ns}

Vales expressed as mean ± SD (n = 5). Ns: non significant.

Effect of Extracts on Plasma Antioxidant Capacity Using DPPH Radical: The DPPH scavenging assay of plasma revelated that the ability of scavenging was significantly improved following the administration of ASE and Vit. C. The results showed that treatment with ASE: 100, 200 and 400 mg/kg improve the scavenging capacity in a dose-dependent manner (65.81 ± 2.31, 67 ± 0.63 and 90.22 ± 0.54%, respectively) **Table 5**.

Effect of Extracts on Plasma Reducing Power: Plasma of animals treated with the different concentrations of plant extract has an important reducing capacity.

The results indicated that there is a significant difference ($P<0.001$) between with negative, positive and treated groups as shown in **Table 5**.

Assessment of CAT Activity, GSH, and MDA: The changes in the activity of CAT, the content of GSH and MDA in livers of treated animals were investigated. The CAT activity in both livers and kidneys homogenates has undertaken a significant

changes during treatment with ASE (100 and 200 mg/kg). Also, no change was noticed in positive control and the group treated with 400 mg/kg. The analysis of these results indicated that the administration of ASE (100, 200 and 400 mg/kg) and Vit. C (100 mg/kg) leads to a significant increase in GSH level in livers. According to the results **Table 6**, a significant decrease of MDA level was observed in mice treated with Vit. C and ASE (100, 200 and 400 mg/kg).

TABLE 5: *IN-VIVO* EFFECTS OF DIFFERENT CONCENTRATIONS OF *A. SCOPARIUM* EXTRACT ON PLASMA ANTIOXIDANT ACTIVITIES; SCAVENGING DPPH AND REDUCING POWER

Groups	Control	Vit. C 100mg/kg BW	ASE 100mg/kg BW	ASE 200mg/kg BW	ASE 400mg/kg BW
DPPH%	46.66 ± 2.00	66.73 ± 0.25	65.81 ± 2.31***	67 ± 0.63***	90.22 ± 0.54***
Reducing power Abs at 700 nm	0.50 ± 0.015	0.42 ± 0.016	0.57 ± 0.02***	0.59 ± 0.02***	0.75 ± 0.021***

Data were expressed as mean ± SEM; (n = 5). ***: P<0.001.

TABLE 6: *IN-VIVO* EFFECTS OF ASE ON LIVER CATALASE ACTIVITY, GSH AND MDA CONTENTS

Liver	Control	Vit. C 100mg/kg BW	ASE 100mg/kg BW	ASE 200mg/kg BW	ASE 400mg/kg BW
CAT (IU/mg protein)	12.40 ± 0.64	13.26 ± 0.08	7.17 ± 0.45***	9.06 ± 0.88***	11.81 ± 0.74 ns
GSH (IU/mg protein)	44.08 ± 5.06	189.7 ± 5.54	72.07 ± 3.51 ns	127.85 ± 6.58***	139.1 ± 3.04***
MDA (nmole/mg protein)	103.3 ± 3.01	79.24 ± 1.36	78.66 ± 3.12***	74.84 ± 1.27***	64.09 ± 1.07 ns

Data were expressed as mean ± SEM; (n = 5). Ns: no significant. ***:P<0.001.

DISCUSSION: Based on the represented data, it can be concluded that the DEC extract possesses a high content of phenolic and flavonoid compounds. The results reported for other extracts tested (*Atriplex halimus* and *Anabasis articulata*) shown that the content ranged from 3.77 ± 0.06 to 43.14 ± 0.63 mg GAE/g DM [19], where these contents were lower than those reported for *A. scoparium* extracts. The differences in total phenolic contents could be due to genotypic and environmental variations (climate, location, temperature…), within species plant part tested, harvesting time and extraction procedure [20].

The free radical scavenging capacities of the different extracts are shown in **Table 2**. In this study, the methanolic extract exhibited high scavenging potency, which might be due to the presence of specific molecules. According to the results reported by Belyagoubi-Benhammou *et al*., [21] the ethyl acetate fraction of *A. halimus* process an interesting radical scavenging effect against DPPH (IC_{50} = 2.04 mg/ml). These capacities could be attributed to the abundance of flavonols (kaempferol, quercrtin) which constitute the main class of *Atriplex species* [22]. Because of the difference in amounts of total phenolic and flavonoids contents, antioxidant activities are more variable between species of plants (inter-species) than within the same species (intra-species) [23]. The results shown in **Table 2** revealed that CrE exhibits the strongest antioxidant activity in ABTS assay (IC_{50} = 0.0017 ± 5.45E-5 mg/ml) superior to the results found for *Atriplex hamilus* and *Haloxylon scoparium* fractions. According to the literature, It was found that no significant difference in antioxidant activities between ethyl acetate fraction of *Atriplex hamilus* (AHAE), dichloromethane fraction of *Atriplex hamilus* (AHDCM), butanolic fraction of *Atriplex hamilus* (AHB) [22].

All extracts of *A. scoparium* highly inhibit the oxidation of β-carotene compared to BHT, while this effect is due to the inhibition of linoleic acid peroxidation formed during the peroxidation of linoleic acid (scavenging effect). The best β-carotene oxidizing inhibitor in 24 h was EAE (83.29 ± 2.94%), followed by AQE (80.16 ± 3.73%) and ChE (64.87 ± 1.53%). EAE was the most active than other fractions. It was reported that any sample inhibits or retards the bleaching β-carotene can be described as a scavenger of free radicals and as a primary antioxidant [24]. The highest ferric reducing power activity was noticed by DEC (0.063 ± 0.021 mg/ml), **Table 2**. This finding reveals that the favorable antioxidant activity observed in the DEC is most possibly due to its high total contents of phenolic and flavonoid compounds. The results obtained by Benhammou *et al*., showed that the ethyl acetate fraction of *A. halimus* exhibited an interesting antioxidant activity to reduce iron [20].

The presence of phenolic compounds in these fractions may be explained there reducing power. Relations between Fe^{3+} reducing activity and total phenol content have been reported in the literature [10].

According to the OECD guideline, in the acute toxicity, the single administration of the ASE at the doses of 2 and 5 g/kg did not cause any behavior changes or mortality during the period of treatment. These observations suggest that the LD_{50} is greater than 5 g/ kg. To evaluate the sub-acute toxicity and the in vivo antioxidant activity of *A. scoparium* extracts, healthy mice were used and treated for 21 successive days. Since the weight loss was not recorded in all of the groups treated; it can be concluded that the intake of ASE did not affect the body weight of mice. Statistically, no-significant differences in the relative organs weights in both control and treated groups were registered.

To evaluate the *in-vivo* anti-oxidant potential, the DPPH scavenging and reducing power as well as the CAT activity, GSH and MDA levels in liver homogenate was estimated. The results indicate that the ASE improve free radical scavenging activity and reducing capacity regardless of the importance of these activities of the ASE determined *in-vitro* and these effects may be primarily attributed to the polyphenolic contents. According to the literature, many types of research demonstrate the significant correlation between the measured plasma antioxidant capacity and the total phenol content of plant extracts [25, 26, 27]. Whereas, *in-vivo* these activities are limited by several factors such as low bioavailability and metabolic conversion of the absorbed polyphenols (plasma concentration rarely exceeds 1µM) [28].

In-vivo, it was observed that the treatment of animals with ASE at doses of 100 and 200 mg/kg lead to a decrease in the activity of catalase in liver **Table 6**. The mechanisms of activation of antioxidant enzymes using polyphenols are not fully understood, as well as many studies indicated the association between phenolic compounds and antioxidant enzyme expression [29, 30, 31]. It was found that both doses of ASE (200 and 400 mg/kg) had a strong effect on GSH level nearby that of vitamin C. GSH antioxidant system plays a central role in cellular defense against reactive free radicals, especially in redox status balance. Moreover, it can serve as a marker of a decrease in antioxidant protection, susceptibility, early and reversible tissue damage [32]. The treatment with ASE significantly reduced ($P<0.001$) the level of MDA in liver **Table 6**. The differences in the inhibition of lipid peroxidation between plant extracts may be attributed to their different phenolic contents and their compositions [33].

CONCLUSION: Biological systems are under the continuous influence of oxidative stress because of ROS excessive generation. The present study demonstrates that *A. scoparium* extracts contain an important amount of phenolics and flavonoids compounds, which, possess *in-vitro* and *in-vivo* antioxidant activities, and were capable of inhibiting lipid peroxidation.

These results explained the radical scavenging, lipid peroxidation inhibition and antioxidant activity of *A. scoparium* and gave a scientific clearance supporting the traditional use of this plant.

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