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Research Article

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The Total Phenolic Compounds and Antioxidant Activity of Atriplex Nummularia Leaves' Extract

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ABSTRACT

In the present study, four fractions were prepared from leaves extract of Atriplex nummularia. This study aimed to determine TPP (total polyphenols), FLV (flavonoids) and CT (condensed tannins) on one hand and on the other hand, to evaluate the antiradical activity of the hydroalcoholic and aqueous fractions by four different methods including: DPPH test (2,2-diphenyl-1-picrylhydrazil), FRAP test (antioxidant activity by the iron reduction method), BCB test (β-carotene bleaching), and TAC test (antioxidant capacity) total) by the phosphomolybdate method. High levels of total polyphenols and condensed tannins were found in F/DieEA (23,437 ± 0.251mgGEA/gE), and F/DcmEA (17,251 ± 0.354mgCE/gE), respectively. Therefore, flavonoid levels F/DieEA and F/DcmEA were almost identical (9.515 ± 0.277 mgQE/gE), (9.238 ± 0.119 mgQE/gE). The F/DcmEA showed an interesting and significant antioxidant activity in all tests as the other F/DieEA, F/n-BUOH, F/aqueous represented the activity of trapping the free radical DPPH of C50 = (3.073 ± 0.088mg/ml), a reducing power of iron (433.55 ± 24.36mgQE/gE), and a total antioxidant capacity of the Mo (VI) reduction test of (20.28 ± 1.96mgEAG/gE), and the determination of IC50 of β-carotene bleaching (0.92 ± 0.0175mg/ml).

Key words: *Atriplex nummularia, polyphenols, flavonoids, tannins, antioxidant activity.*

INTRODUCTION

The oxidation of organic matter by molecular oxygen is a fundamental part of the aerobic life of human metabolism. Free radicals occur when the reduction of oxygen is incomplete in cytochromes [1] or they occur by biological dysfunction. These Free Radicals (FR) and Reactive Oxygen Derivatives (ROD) including lipid peroxidation (LP) are causative agents of several types of diseases such as emphysema, central nervous system lesion, autoimmune diseases, anemia, cerebral ischemia [1], cancers [2], inflammatory and degenerative diseases [3]. They are also harmful in a reversible or irreversible way with regard to all biochemical classes, such as nucleic acids, proteins and amino acids, lipids and lipoproteins, carbohydrates and macromolecular connective tissue [4]. Antioxidants such as phenolic compounds; tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone, (TBHQ), propyl gallate (PG), lignans, flavonoids and phenolic acids), ubiquinone (coenzyme Q), carotenoids, ascorbic acids and amino acids can eliminate free radicals [5]. In the vicinity of primary metabolites of plants such as (nucleic acids lipids, proteins, carbohydrates), one of the major peculiarities lies in their ability to produce very diversified so-called secondary substances which contain molecules usable by man in pharmacology or agroalimentary [6]. In this

study, the *Atriplex nummularia* of the chenopodiaceae family which is a halophyte forage plant used as a cattle feed and which adapts to the steppic and saharan environment, was chosen. In previous studies, its antioxidant properties have been mentioned.

There are about 400 species of *Atriplex* in the world, mostly in temperate zones, subtropical and Mediterranean zones between 20 ° and 50 ° N latitude [7].This genus includes 48 wild species in the Mediterranean Basin [8] , these species are the most interesting and endearing in the dry and salty regions. Some species are spontaneous in Algeria, and there are some other species including *Atriplex nummularia, Atriplex ceneriensis, Atriplex leucoclada, Atriplex polycarpa, Atriplex canariensis* in this country [9]. *Atriplex nummularia* at (200 and 400 mg / kg) has been proven to have an antihyperglycaemic effect compatible with the drug glibenclamide [10]. Active ingredients of *Atriplex halimus* provided reliable results as an alternative antibacterial therapy against a highly encountered family of beta-lactamase bacteria (BLSE) [11]. High antifungal activities were detected in the genera of *Atriplex Alphitonia*, and the seeds of *Atriplex nummularia* [12]. Isolation and extraction of the secondary metabolites of *Atriplex leucoclada* and *Atriplex nummularia* were studied for pharmacological actions that possessed anti-fungal, anti-ulcerative, anti-colitic effects, and molluscicidal activity [13]. The current study because of investigating the antioxidant efficacy of this species and certain kinds of the same family and evaluating their antioxidant properties of polyphenolic compounds (polyphenols, flavonoids and condensed tannins) can be considered as a part of the research on natural antioxidants.

MATERIALS AND METHODS

Preparation of extracts

The *Atriplex nummularia* leaf samples were taken on July 18th from a natural population in the south of "Ain Skhouna, Saïda Algeria". The leaves were separated and dried in a hot air dryer at 45 °C. The samples were ground to a fine powder, and packaged before being used in solvent extraction [14]. solid-liquid and liquid-liquid extraction have been the most commonly used procedures before the analysis of simple phenolic compounds in natural plants. These are still the most used techniques, mainly because of their ease of use, efficiency, and great applicability [14].

Solid liquid extraction

Four extraction hydroalcoholics with increasing polarity were used to extract phenolic compounds. 5g of powder was macerated with 4x25ml of different mixtures of solvents, ethanol (80:20, v / v), methanol (80:20, v / v) v), acetone (80:20, v / v) and deionized water with magnetic stirring for 72h at temperature of (0±4 ° C) by adding a reducing agent (sodium metabisulfite in the middle of extraction to ensure the protection of polyphenols) [6]. The extracts were filtered through Whatman No. 1 filter paper. And the marcs were again macerated with the same solvents until the exhaustion of the extractions.

Liquid liquid extraction

The four filtrates were dried under the reduced pressure at 50° C using a rotary vacuum evaporator. The first series of purification of the overall extracts was necessary with an apolar solvent (petroleum ether 2 x 25 ml to remove the oils, and the chlorophyll pigments, and waxes), [15], and the second series of purification with dichloromethane 2x25ml for the hydro-acetonic extract was used to extract the majority of the glycosides [16], and the diethyl ether 2x25ml was used for the hydro-ethanolic extracts , hydro-methanol and water to extract the free gene linked to glycosides [16], then they were evaporated dried under the reduced pressure at 50 ° C [15].The third series of extraction was used for extracting the phenolic compounds by two solvents at intermediate polarity (ethyl acetate for the first two extracts, hydro-acetonic hydro-ethanolic), and (n-butanol for the third extract), then they were concentrated in vacuum at 50 ° C, and preserved at temperature of 4 ° C. Finally, four fractions were obtained: the fraction diethyl ether ethyl acetate (F / DieEA), the fraction dichloromethane ethyl acetate (F / DcmEA), the fraction diethyl ether n-butanol (F / n- BUOH), the aqueous fraction (F / Aqueous).

Determination of total polyphenols contents (TPP)

The total content of phenolic compounds in the extract was determined using the folin- ciocalteu (FC) reagent according to the method described by [17], with some modifications, a diluted extract of 0.5ml of concentration 1 mg / ml or gallic acid standard solutions (50-300 ug/L), was prepared beforehand in a mixture (methanol / water, 50:50). The absorbance of the solution was measured by the spectrophotometer (OPTIZEN3220 UV) at 765 nm, the results were expressed in milligrams equivalent to gallic acid per each gram of extract (mg GAE /gE).

Determination of total Flavonoids contents (FLV)

The total Flavonoid content was determined by the colorimetric assay of the aluminum chloride according to the method described by [18], with small modifications, by using 0.5 ml of 1 mg ml^{-1} of each concentration. The absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer (OPTIZEN3220 UV). The total flavonoid dosage was expressed in milligrams of quercetin equivalent per gram of extracts (mg QE/ gE).

Determination of condensed tannins (CT). Proanthocyanidins

In this study, the vanillin-HCl method of [19] was followed. A calibration curve was constructed using (+)-catechin, and the contents were estimated in milligrams equivalent (+)-catechin per gram of extracts (mg CE / gE).

Free Radical-Scavenging Activity Using (DPPH Assay)

The DPPH scavenging activity was determined using the method of [20] with a slight modification. For each antioxidant, different concentrations were tested (0.1 mg / ml-1 mg / ml). Gallic acid, ascorbic acid and quercetin were used as reference materials. The amounts of absorbance at 515 nm were recorded against 95% methanol. Initial DPPH absorbance was (0.632 ± 0.47). The DPPH inhibition percentages of the test sample and references were calculated by the following formula:

$$\%\ Inhibition = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Where A_{control}: the absorbance of the control; A_{sample}: the absorbance of the test samples. For each concentration, the test was repeated 3 times, and the EC_{50} values were determined graphically by linear and non-linear regression.

Ferric-reducing antioxidant power (FRAP)

The ferric reduction determination procedure has been adapted from [21] with a slight modification, 0.5 ml of each of the extracts at 1 mg / ml concentration was considered. The colored solution was read at 700 mm against a blank using a UV-VIS spectrophotometer. The results were expressed in milligram equivalent Quercetin per each gram of the extract (mg QE/ gE).

Total antioxidant capacity of the Mo (VI) reducing assay (TAC)

The amounts of total antioxidant capacity (TAC) were estimated by the method described by [22]. Except that the sample dose was tripled. 3.0 ml of each extract at 0.5 mg / ml concentration was considered. The absorbance was measured at 695 nm against a blank. By replacing the sample with gallic acid under the same conditions mentioned above, the total antioxidant capacity (TAC) was expressed in milligrams equivalent of gallic acid per each gram of extract (mg GAE / gE).

***β*-carotene bleaching assay (BCB)**

The antioxidant activity was determined according to a slightly modified version of the β- carotene bleaching method [23]. The negative control consisted of having 500 ul of methanol instead of extract or the synthesized antioxidant. For the positive control, the sample was replaced by the BHT. The coefficient of antioxidant activity (CAA) was calculated according to the following equation:

$$CAA\% = [(A_{S120} - A_{C120})/(A_{C0} - A_{C120})] \times 100$$

Where A_{S120} : the absorbance of the antioxidant at t = 120 min A_{C120}: the absorbance of the control at t =120 min and A_{C0}: the absorbance of the control at t =0 min. The test was repeated 3 times, and EC_{50} values were determined graphically by the non-linear regression.

Statistical analyzes

All the tests were carried out in triplicate, and the results were expressed in (mean ± standard error), by using two software of statistical analysis (IBM SPSS Statistics v 24 and Sigmaplot v12.5 and (a software of treatment and of scientific data analysis) Origin pro v 9.0.

RESULTS AND DISCUSSIONS

The yields of the hydroalcoholic and aqueous extractions were calculated according to the extraction yield formula given by [24]:

$$R\% = ((M_{extract})/(M_{sample}) \times 100)$$

The fractions examined showed the highest yield was that of the aqueous fraction (8,560±0,445%), and the lowest yield was that of F/DcmEA (3.853 ± 0.219) % (Table 1)**.**

Table 1. Yields of leaf extracts of Atriplex nummularia.

Fractions	Yields%	Mass (g/5ML)
F/ DieEA	$6{,}200 \pm 0{,}144^{b}$	$1{,}55 \pm 0{,}036^{b}$
F/ DcmEA	$3{,}853 \pm 0{,}219^{c}$	$0{,}963 \pm 0{,}054^{c}$
F/n-BUOH	$5{,}946 \pm 0{,}456^{b}$	$1{,}486 \pm 0{,}114^{b}$
F/Aqueous	$8{,}560 \pm 0{,}445^{a}$	$2{,}140 \pm 0{,}111^{a}$
F. Stat of ANOVA	31,195	31,195
Sig : intergroup	$P < 0{,}000$	$P < 0{,}000$

mass values and yields are the (mean ± SE) of the three test repetitions. The letters (a, b, c) indicate the homogeneous subsets of the Tukey multiple comparison (HSD) test P <0.05.

Determination of the contents of phenolic compounds

The intention of this study was based on a large family that has been very widespread in the photochemical environment, which provided us with an overview of the phenolic substances in the leaves of *Atriplex nummularia*.

The results of the extractions showed that the hydroalcoholic and aqueous fractions had different contents of the polyphenols, flavonoids, and tannins, depending on the nature of the solvent and the extraction method. And, they determined a significant difference between the averages in the analysis of variance at P <0.05.

The comparative study of the extrapolated total polyphenol contents of a linear regression gallic acid calibration curve indicating: Y = 0.0035X + 0.0113, r^2 = 0.9948 showed that the content of F / DieEA at 23.437 ± 0.251 ug GAE/gE was three times higher than that of the F/n-BUOH 8.961 ± 0.908 ug GAE/gE and that of the F / aqueous 7.342 ± 0164 ug GAE/gE, but almost similar to that of the F / DcmEA 19.723 ± 0.190 ug GAE/gE, so no significant difference was noticed between F / n-BUOH and F/Aqueous.

In parallel with the evaluation of total polyphenols, the flavonoids were calculated using the following linear regression equation: Y = 0.0084X-0.0526, r2 = 0.9928, revealing that the equalities of two-to-two in the same groups ranged between 9,515 ± 0,277 mg QE/gE and 9,238 ± 0,119 mg QE/gE for F/DieEA and F/DcmEA; respectively, and a content of 7.690 ± 0.181 mg QE/gE and 6.896 ± 0.039 mg QE/gE for F/n- BUOH and the F/Aqueous; respectively as shown in Table 2. A study carried out by [25] showed that the ethanolic extract of the aerial parts of *Atriplex nummularia* had very high polyphenol and flavonoid contents including 144.4 + 6 ,11 mg GAE / gE and 81.0+ 2.33 mg QE/gE like the results of this study. [26] showed that halophytes in the salt response can cause variability in phenolic compound contents, for sustainability and species growth. The results of the subsequent work of [27] who evaluated the extracts of twelve medicinal and aromatic plants for their antiradical effect, and found that the levels of their plants in polyphenols and flavonoids varied between 4.3 ± 0.6 and 37.9 ± 2.1 mg GAE / g extract and 1.0 ± 0.1 and 13.8 ± 0.2 mg GAE / g extract respectively, were not compatible with the results of the current study, this proved that the polyphenol contents and in flavonoids extracts of *Atriplex nummularia* remained relatively high compared to the twelve medicinal plants tested. Besides the six chenopodiaceae of the same family of this plant studied by [28], it was shown that polyphenol contents varied between 62 ± 0.88 and 109.51 ± 0.34 mg of RU /g of extract, remained higher than the current research. So, the quantitative and qualitative variations of phenolic compounds in a plant acted under several factors:

- Environmental and climatic factors: drought geographical areas, soil, humidity.
- the time of harvest, the genetic heritage and the stage of growth of the plants [29].

The estimate of condensed tannin content was based on a standard curve of the equation: Y=0,0041X -0.0084, r^2=0.997, the results indicated that the highest tannin content was observed in F/DcmEA 17,251 ± 0,354 mg CE/gE followed by F/n-BUOH 8,227 ± 0,081 mg CE/gE and the F/aqueous 4,080 ± 0,406 Mg CE/gE, while the lowest content was recorded in the F/DieEA.

Table 2. The contents of the phenolic compounds of the four fractions of leaf extracts of Atriplex nummularia.

Fractions	Polyphenols [1]	Flavonoids [2]	Tannins [3]
F/ DieEA	23.437 ± 0.251^{a}	9.515 ± 0.277^{a}	2.617 ± 0.215^{d}
F/ DcmEA	19.723 ± 0.190^{b}	9.238 ± 0.119^{a}	17.251 ± 0.354^{a}
F/n-BUOH	8.961 ± 0.908^{c}	7.690 ± 0.181^{b}	8.227 ± 0.081^{b}
F/Aqueuos	7.342 ± 0.164^{c}	6.896 ± 0.039^{b}	4.080 ± 0.406^{c}
F. Stat of ANOVA	263.983	49.723	504.160
Sig : Intergroup	$P < 0.000$	$P < 0.000$	$P < 0.000$

The values of the phenolic compounds are (mean ± SE) of the three repetitions of the tests. [1:] Gallic acid equivalent (mg GAE / g extract); [2:] Quercetin Equivalent (mg QE / g extract);[3:] (+)-Catechin Equivalent (CE mg / g extract). The letters (a, b, c) indicate the homogeneous subsets of the Tukey multiple comparison (HSD) test P <0.05.

Another study showed that the rate of condensed tannin in young seedlings of *Atriplex nummularia* was higher in relation to the rate of *Atriplex halimus* because the latter has frequently synthesized hydrolysable tannins, condensed tannins and oxalates and phenols tannic [30]. Seasonal variations in the air temperature have had a limited effect on the biosynthesis of proanthocyanidins in grape berries [31]. High temperatures followed by night-time temperatures close to 16 ° C favored the accumulation of anthocyanins in the skin [32]. The results of this study were compared with the literature of [33] on the study of the methanolic raw extract of *Atriplex halimus* leaves and it was found that they were almost similar to the results of the current study. The total phenol content was 10.127 ± 2.244mg GAE/g MS, and the condensed tannins was 9,118 ± 0,684 mg CE/g MS, except for the flavonoids which were lower than its own 2,485 ± 0,017 mg CE/g MS. A work by [34] indicated that phenolic compounds of the aqueous extracts of leaves of *Atriplex canescens* contained 9.34±1.24 mg GAE/g MS of polyphenols and 2.09±0.08 mg QE/g MS flavonoids and 1.5±0.11 mg EC/g MS tannins, followed secondly by the methanolic extract with an amount of polyphenols of 8.67 ± 1.71 mg GAE/g MS and flavonoids of 1.90 ± 0.04 mg QE/g MS and tannins of 2.1 ± 0.19 mg CE/g MS. The study done by [35] for five coastal halophytes commonly used in traditional herbal treatments recorded a proanthocyanidin level of 0.2 - 0.6 EC g-1, compared with the high rates of F / DcmEA and F / n. -BUOH meant that tannins with high values could react as a natural antioxidant to various mechanisms, as well as neutralization of free cardinals in humans and animals. A similar trend in tannins was noted in [36] for root extracts of the vegetative halophyte Limonium delicatulum ranging from 0.2 ± 0.13 to 17.6 ± 7.67 mg EC / g MS indicated the physiological characteristics of halophytes that had the ability to produce bioactive substances, and could be used as food preservatives [37].Variations in the levels of phenolic compounds have been often considerable from one species to another and even inside a species, depending on the varieties considered [6]. Within a given plant species, these varieties might have very different phenolic equipment which is the characteristic of each of them [6]. Phenolic compounds were resulted from lignin and tannins plant residues; a part was synthesized by microorganisms by cyclization of certain aliphatic compounds (microbial melanins) [38].

DPPH trapping activity

The antioxidant activity has been based on the principle of the reduction of the free radical DPPH•(2,2-diphenyl-1-picrylhydrazyl, α, α-diphenyl-β-picrylhydrazyl), that is to say the passage of a violet color towards a yellow color by measuring its absorbance at 517 nm [39]. This absorbance will decrease when the hydrogen atom of the antioxidant compound serves an odd electron of the nitrogen atom of DPPH• formed, (2,2-diphenyl-1-picrylhydrazine , DPPH-H),[40] .The reaction study (antioxidant-DPPH•) showed free radical scavenging activity, which determined a significant difference between EC_{50} averages and DPPH•, and inhibition percentages were measured in an analysis of variance at $p < 0.05$. The inhibition percentages of the DPPH• reduction in the samples had no significance at low concentrations, but they increased hypothetically with increasing concentrations in the following order, F / DcmEA> F / DieEA> F / n-BUOH> F / Aqueous, with values as follows (15.96 ± 0.60 mg / ml, $r^2 = 0.851$); (13.11 ± 0.22 mg / ml, $r^2 = 0.788$); (11.78 ± 0.20 mg / ml, $r^2 = 0.683$); (10.96 ± 0.26mg / ml, $r^2 = 0.859$); respectively in (Table 3) . But this antioxidant activity always remained slower than the standards used as a reference, almost 20 times in the percentage of the anti-radical activity of minimal inhibition and 8 times in the percentage of the anti-radical activity of maximum inhibition, it was confirmed that the chemical reaction in the presence of vit C, and quercetin and gallic acid had started quickly, at certain times which would become a slow chemical reaction.

The accumulation in large quantities of the active forms of the superoxide radicals (02^{-}) and the oxygen peroxide (H_20_2) and hydroxyl (OH), during an abiotic saline stress was translated in a response and a cellular defense [41-43], and could develop a non-enzymatic low molecular weight antioxidant mechanism, such as polyphenols, flavonoids, anthocyanins and Ascorbic acid [44, 45]. The EC_{50} values were a functional inhibitory response corresponding to 50% of the dose of the concentration effect [46].

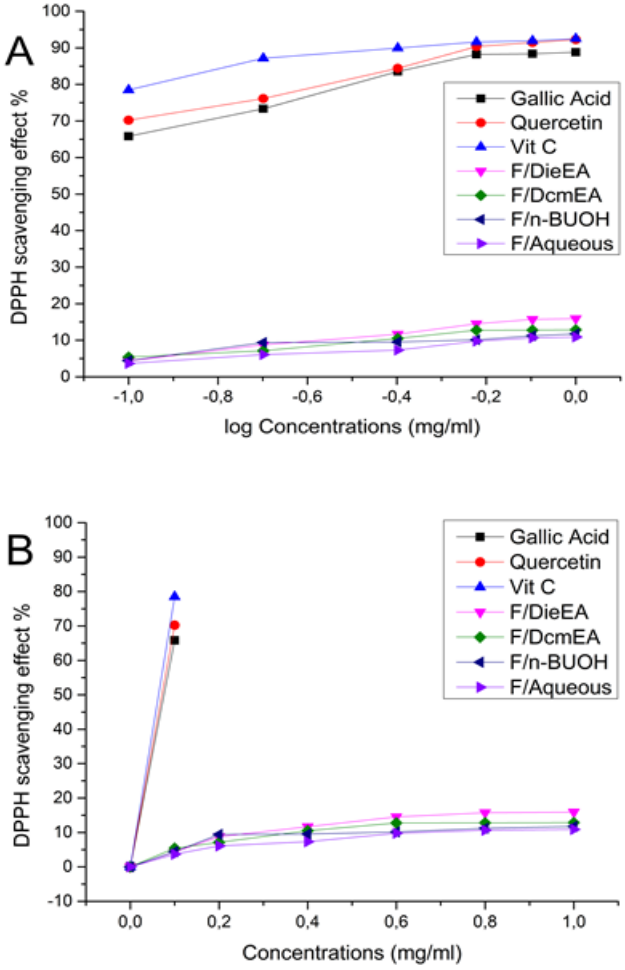

Figure 1. Antioxidant activity of different hydro alcoholic fractions with the three standards, VitC, Quercetin, Gallic acid. (A) reduction of the free radical DPPH• in linear regression. (B) reduction of the free radical DPPH• in non-linear regression.

Two models of the equations were posed in a comparison test, a linear regression equation and a logarithmic non-linear regression equation (dose-response). The complexity and impossibility of determining the EC_{50} for the majority of the samples on the logarithmic fit curve [47], except the DieEA fraction with (20,131 +0,717 mg / ml, r^2 = 0,978), allowed the researchers to put in experimentation a linear regression equation, in order to adjust straight lines for the three references, gallic acid, quercetin and vitC, which did not show any significant difference between the averages, through selecting the square sum of the vertical distance of the first two concentrations (0.1 mg / ml and 1 mg / ml).

Table 3. Inhibition percentages and EC_{50} values determined in extracts fractions Atriplex nummularia leaves compared with Gallic acid and Quercetin.

Fractions	**Minimal % inhibition DPPH·**	**Maximal %inhibition DPPH·**	**Linear regression equation**	**r^2**	**Logarithmic equation**	**r^2**
	0.1mg/ml	1mg/ml	EC_{50}(mg ml^{-1})	Test1	EC_{50}(mg ml^{-1})	Test1
Gallic Acid	$65,55 \pm 0,43^{c}$	$88,99 \pm 0,10^{b}$	$0,076 \pm 0,006^{d}$	1	$0,160 \pm 0,119^{b}$	0,998

Quecetin	$70,40 \pm 0,49^{b}$	$92,43 \pm 0,22^{a}$	$0,074 \pm 0,033^{d}$	1	$0,031 \pm 0,020^{b}$	0,998
Vit C	$76,85 \pm 0,86^{a}$	$92,73 \pm 0,14^{a}$	$0,064 \pm 0,007^{d}$	1	$0,012 \pm 0,018^{b}$	0,999
F/DcmEA	$4,29 \pm 0,07^{d}$	$15,96 \pm 0,60^{c}$	$3,073 \pm 0,088^{c}$	0,851	$20,132 \pm 0,717^{a}$	0,978
F/DieEA	$5,16 \pm 0,17^{d}$	$13,11 \pm 0,22^{d}$	$3,951 \pm 0,619^{b}$	0,788	-	-
F/n-BUOH	$4,22 \pm 0,12^{d}$	$11,78 \pm 0,20^{e}$	$4,747 \pm 0,708^{a}$	0,683	-	-
F/Aqueous	$3,77 \pm 0,16^{d}$	$10,96 \pm 0,26^{e}$	$4,666 \pm 0,859^{a}$	0,859	-	-
F. Stat of ANOVA	7084,976	49190,096	2124,523	-	762,005	-
Sig : Interg	$p < 0,000$	$p < 0,000$	$p < 0,000$	-	$p < 0,000$	-

The values of EC_{50} and %DPPH• are the (means ± SE) of the three test repetitions. The letters (a, b, c, d, e) indicate the homogeneous subsets of the Tukey multiple comparison (HSD) test. <0.05.

This facilitated the calculation of the EC_{50} value graphically. The results of this study confirmed those of [33]considering the determination of EC_{50} in butanol fractions and ethyl acetate of *Atriplex halimus* leaves. Also, the results of the current study approved the results recorded by [48] for the determination of the EC50 concentrations, of some Algerian plants of the order of (*Helianthemum lippii, Inula montana, Anabasis articulata, Cotula cinerea, Marrubium deserti, Thymelaea microphylla, Zygophylum album)* ranging from 66,284 to 6310,0041 mg antioxidant / g DPPH, these results remained inferior to those obtained in *Atriplex nummularia*. And the results of the work of [49] for the DPPH test on seeds of *Nigella sativa* showed that the IC_{50} value was 0.64 ± 0.08 mg / ml which was higher than the values of the four hydroalcoholic fractions tested for *Atriplex nummularia* ranging from 3.073 ± 0.088 to 4.666 ± 0.859 mg/ml. Overall, the results made it possible to know and value this plant particularly for its biological interest, in order to use its resources in the therapeutic field and widen perspectives in other potential tests of Gram-positive antimicrobial [50], anti-inflammatory, anticancer, antidiabetic agents. Although the first model (linear regression equation) confirmed the accuracy of the results of this study, the high DPPH• trapping activity was obtained in the F/DcmEA; the EC_{50} value was (3.073-0.088 mg / ml, r2 = 0.851), and the lowest DPPH• trapping activity was determined in F/aqueous (4.666 ± 0.859 mg / ml, $r^2 = 0.859$).

Evaluation of (FRAP)

The FRAP method was based on the ability of an antioxidant to give an electron to Fe (III), which caused the reduction of $Fe3^{+}$/ ferricyanide complex to $Fe2^{+}$ complex [51]. The multiple comparison between the different means was significant in the test of Tukey to p<0.05, and the change in absorbance was therefore directly related to the total reducing power or electron donor antioxidant present in the reaction mixture [52]. The results obtained from the Quercetin curve of the equation were: $Y = 0.0003X + 0.5096$; $r^2 = 0.9817$ showed the highest reductive antioxidant potency included in F/DcmEA (433,55±24,36 mgEQ/g E), followed by F/DieEA (261,33±8.81 mgQE/g E) and n-BUOH with a value of (29,11±6.75 mgQE/gE), as it was shown in (Table 4), but that of gallic acid at (1642.55 ± 22.20 mgQE / g E), always remained higher compared to the other hydroalcoholic fractions.

Table 4. reducing power (FRAP) and the total antioxidant capacity (TAC) of the different hydroalcoholic fractions of Atriplex nummularia with standard references, Gallic acid and Ascorbic acid at OD = 700 nm.

Fractions	**(FRAP)[E] test mgQE/gE**	**OD[F]**	**(TAC) [G] ,(PPM)[H] test mg GAE/gE**	**OD[I]**
F/DcmEA	$433,55 \pm 24,36^{b}$	0,635	$20,28 \pm 1,96^{b}$	0,087
F/DieEA	$261,33 \pm 8,81^{c}$	0,592	$16,22 \pm 0,50^{b}$	0,067
F/n-BUOH	$29,11 \pm 6,75^{d}$	0,522	$15,14 \pm 0,95^{b}$	0,067
F/Aqueous	-	0,420	$11,83 \pm 0,72^{b}$	*0,049*
Gallic acid	$1642,55 \pm 22,20^{a}$	1,005	-	-
Vit C	-	-	$790,54 \pm 7,49^{a}$	2,272
F. stat of ANOVA	1712,825	-	599,346	-
Sig. Interg	$p < 0,000$	-	$p < 0,000$	-

[E:] Ferric Reducing power (FRAP); [G:] total antioxidant capacity (TAC); [H:] phosphomolybdate, are the (means ± SE) of the three repetitions of the tests; [F, I:] The absorbance of the first test. The letters (a, b, c, d) indicate the homogeneous subsets of the Tukey multiple comparison (HSD) test at P <0.05.

The results of this study were compared with those of [34] which was done on *Atriplex canescens* where a low activity of iron reduction (OD = 1.04 and OD = 0.90) was shown for the crude methanolic and aqueous extracts of leaves and stems at a concentration of 10 mg / ml and higher activity (OD = 1.30) for the fraction of ethyl acetate of the leaves at a concentration of 2 mg / ml [53]; in another study done by [54] on the *Satureja calamintha* plant, there was an OD = 0.484 which was lower than the extracts of *Atriplex nummularia*, and it could be said that the reducing power of the active substances might assist as a significant indicator of a potential antioxidant activity [55].

Reducing of Mo (VI) to Mo (V)

The quantitative measurement of (TAC) reduction in Mo (VI) to Mo (V) which was done in gallic acid equivalent per gram of the extract in an established linear equation Y = 0.0028 X = 0.0192, r^2 = 0.9994, showed a (TAC) of the following descending order: Ascorbic Acid > F/DcmEA> F/DieEA >F/n-BUOH >F /Aqueous (table4), but there were no significant differences between the four fractions of extracts from the leaves of *Atriplex nummularia* because they were in the same homogeneous group, this could be interpreted by the fact that all the phenolic compounds TPP, FLV, CT, and others played a key role in the reduction of Mo (VI). Overall, the intergroup comparison and the presence of ascorbic acid as a reduction reference for Mo (VI) showed a clear significance at p <0.05 and a moderate value of (TAC) with a value of (790.54 ± 7.49 mg GAE / gE), 39 times greater than the four hydroalcoholic fractions of the organ investigates of *Atriplex nummularia*. In this study, the researchers made a comparison with the results performed by [53], for an assessment of the antioxidant capacity of the three fractions, ethyl acetate (0.241 mgAA / gMS); dichloromethane (0.110 mgAA / gMS) and butanolic (0.112 mgAA / gMS) of *Atriplex halimus*, which showed lower contents than the results of this study. Also, a comparision was made with another study carried out by [56] considering the aqueous and hydroalcoholic extract of the *aloysia triphylla* species, a CAT of 173.50 ± 0.044 and 270.14 ± 0.1mgEqAA / gE were revealed. It was difficult to compare this research with the other studies because of the different variety of plants; and also the use of different methods of extraction and solvent mixture reduced the credibility of agreements between the research studies.

β-Carotene assay

The BCB method has been based on the loss of the yellow color of β-carotene due to its reaction with the radicals formed by the oxidation of linoleic acid in an emulsion [57]. This method has been used frequently since β-carotene has a powerful antioxidant activity due to its important physiological composition [58, 59]. Hydroalcoholic fractions had a moderate ability to inhibit concentrations dependent on β-carotene discoloration. The results obtained from IC_{50} have been summarized in Table 5 with a clear significance between the IC_{50} averages at p < 0.05, except F / n-BUOH and F / aqueous which showed no significant difference between those. The smallest IC_{50} values indicated the strong inhibition seen in the F/DcmEA> F/DieEA> F/n-BUOH >F/Aqueous.

Table 5. IC_{50} determination of β-carotene bleaching with hydroalcoholic fractions of *Atriplex nummularia* and BHT.

Fractions	β -carotene IC_{50} ($mgml^{-1}$)	r^2 test1
F/DcmEA	$0{,}92 \pm 0{,}0175^{c}$	0,981
F/DieEA	$1{,}15 \pm 0{,}0041^{b}$	0,993
F/n-BUOH	$1{,}65 \pm 0{,}0249^{a}$	0,966
F/Aqueous	$1{,}74 \pm 0{,}0302^{a}$	0,947
BHT	$0{,}065 \pm 0{,}0014^{d}$	0,981
F. Stat of ANOVA	1222,377	-
Sig. Intergroup	$p < 0.000$	-

The β-carotene IC_{50} values are the (means ± SE) of the three test repetitions, the letters (a, b, c, d,) indicate the homogeneous subsets of the Tukey multiple comparison (HSD) test P <0.05.

Despite this confirmation, the positive control of the BHT would remain the most powerful among the extracts with a value of IC_{50} (0.065 ± 0,0014mg / ml). The antioxidant inhibitory similarity of β-carotene decolorization, was observed in the study done by [60] which was conducted on the three shoots (*Suaeda pruinosa, Suaeda mollis and Suaeda maritima*) with respective IC_{50} (540,540,1420) ug / ml, and was compared with the results

found in this study' hydroalcoholic fractions ranging from 0.92 ± 0.0175 mg / ml to 1.74 ± 0.0302 mg / ml, which were less active than those of [61] in the *Mesembryanthemum* genus with IC_{50} (250.210,205) ug / ml. This gave information about the importance of *Atriplex nummularia*, which has been one of the main active agents that can maintain β-carotene staining through its antioxidant effects.

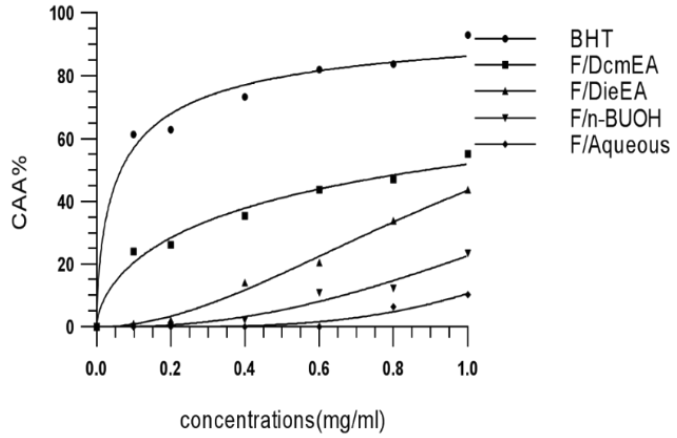

Figure 2. Antioxidant activities measured (dose-response) of hydroalcoholic fractions with BHT using β-carotene bleaching test / linoleic acid

CONCLUSION

According to the results of this study, all the phenolic compounds tested showed a considerable antioxidant activity, especially in the two fractions of F / DieAE and F / DcmAE by their content of polyphenols and high tannins. A significant antioxidant activity would occur with increasing sample concentrations, but there have been still other bioactive compounds to be evaluated based on their antiradical activity at different mechanisms. Atriplex nummularia can be mentioned as several halophytes for example, A. halimus, A. Farinosa, A. lentiformis, and A. hortesnsis, which can be a precursor base of the natural bioactive compounds which are able to break the antioxidants and neutralize the free radicals, and they can be used in multiple medicinal and industrial applications, and they are qualified to defy the abiotic constraints, tolerate the saline soles, and help to produce industrial materials based on bioactive issues, and replace the derivative synthetics of the pharmaceutical industries.

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