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PHENOLICS CONTENTS AND *IN-VITRO* EVALUATION OF THE ANTIOXIDANT EFFECTS OF THE AERIAL PARTS OF THREE ALGERIAN *ATRIPLEX HALIMUS* L. ECOTYPES

Ould Kaddour Asma Souad[1,2]*, Bouzouina Mohamed[1]* and Lotmani Brahim[1]*

[1]Plant Protection Laboratory, Université abdelhamid IBN Badiss-Mostaganem, route nationale n°11, Kharrouba, 27000 Mostaganem, Algeria

[2]Ahmed Zabana, University Centre-Relizane, Bourmadia, BP 48000, W. Relizane, Algeria.

Abstract

Atriplex halimus L. "Guettaf", halophyte plants, is distributed in different geographical zones in Algeria. In this study, antiradical activities, polyphenols, flavonoïds, flavonols and carotenoïds contents of aerial parts (Leaves and Stems) from three Algerian sites (Mazagran, Biskra and Sig) were determined. Results revealed higher amounts of secondary metabolites and higher antioxidant capacities in leaves extracts unlike stems. Significant variation was found between ecotypes; Mazagran leaves and stems extracts are richer in TPC (279.33±1.17 and 68.11±7.76 mg GAE/g LE) and TFC (98.19±1.51and 64.35±0.41 mgRE/g LE, respectively). Great concentrations of flavonols and carotenoids were exhibited in Mazagran and Sig leaves extracts, respectively (34.87 ±1.32 mgRE/g LE and 73±00 µgβ CE/g LE). Like TAC, DDPH, ABTS and BCB tests demonstrated the higher antiradical capacity of Biskra stems extracts (IC_{50} = 497.46±37.53 TEµg/mL - 56.75±5.36 TEµ mol/mL and 17.38±4.17 BEµg/mL, respectively). Biskra *Atriplex halimus* can be used as source of natural antioxidant, in different fields such as animals' food and medicinal applications.

Key words: *Atriplex halimus* L. – Ecotypes - Bioactive compounds - Antioxidant activities

Introduction

Antioxidants are synthesized by living organisms (humans, plants and animals), and provided by nutrients for animals and humans (Durand *et al*, 2013), especially cultivated and spontaneous plants. Antioxidants showed protective role against biotic and abiotic stresses (oxidative stress), which cause overproduction of reactive oxygen species (ROS) (e.g. $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, $^1O^{\bullet}_2$, O_3) (Chaouche *et al*, 2013b; Mittler, 2002), in cells and cause oxidative damage to cellular biomolecules such as lipid, protein, RNA and DNA molecules, and can even lead to the oxidative destruction of cell in a process termed oxidative stress (Mittler, 2002). In parallel, ROS have a cell signaling role in many biological systems and controlling processes such as growth, development response biotic and abiotic, environmental stimuli and programmed cell, and play a vital role in maintaining human health (Hancock, 2001). Uncontrolled production of ROS and unbalanced mechanism of antioxidant protection system, can lead to many diseases (Chaouche *et al*, 2013b) In addition, oxidative stress affects products quality, and animal health (Durand *et al*, 2013). This mechanism is attenuated in cells by a large number of ROS detoxifying proteins (e.g. Superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PRX), as well as by non-enzymatic antioxidant such as ascorbic acid and glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols that are present in almost all sub-cellular compartments (Nuutila *et al.*, 2003).

Different varieties of plant species have high potential to be used in fodder species for livestock (Prache *et al.*, 2011) and phytotherapeutic applications (Bouzouina *et al.*, 2016), due to their potent bioactivity and relatively low toxicity (Kontogianni *et al.*, 2013).

Atriplex halimus Algerian species commonly known

***Author for correspondence :** E-mail : osouad70@yahoo.fr[1&2*], mohamed.bouzouina@univ-mosta.dz[1*], blotmani@yahoo.fr[1*]

as "Guettaf", xero-halophyte and nitrophilous species belonging to the Chenopodiaceae family, is autochthonous, grown over a wide range of soils and widely distributed in Algeria zones (Aouissat *et al.*, *2011*), and described as metal hyper-accumulator (Mesnoua et *al.*, 2016, Lotmani *et al.*, 2011a). This plant is often cultivated as forage because it tolerates severe conditions of drought, and it can grow up in very alkaline and saline soils (Talamati, 2001). *A. halimus* L. was subjected for economical utilization source; it has the property to produce an abundant foliar biomass even during unfavorable periods of year (Kessler, 1990), with a good nutritive value (Abdelguerif, 2004; Stringi, 1991). *A. halimus* L. can, also, contribute to marginal and degraded soils valorization and improvement of vegetable and animal productions, in several stepped areas (Le Houérou, 1992).

In traditional medicine, *A. halimus* minerals cocktail has antidiabetic effect, and reduce heart disease (Chichi *et al.*, 2014). In addition, this saltbush species is also used in anemia treatment and rheumatism (Aouissat *et al.*, 2011), parasites control effect in veterinary trials (Bayoumi and El-Shaer, 1992), antimicrobial activity against various Gram-positive and negative pathogenic bacteria (Abd El-Rahman *et al.*, 2011), and curative against urinary infection and lithiasis (Emam, 2011).

In Algeria, scientists were undertaken to the valorization of aerial parts of southern various/species, including *A. halimus* genus, and several studies reported *A. halimus* activities (Said *et al.*, 2007). In our knowledge, antioxidant activity of leaves and stems of Algerian saltbush has not been reported. Results can be assessed as the first report about antioxidant properties of *A. halimus* species. Thus, the present investigate aimed to scrutinize for the first time *In vitro* antioxidant activities, with radical scavenging DPPH, radical cation scavenging ABTS, β-carotene-linoleic acid bleaching (BCB) assay and total antioxidant activity (TAC) of three Algerian *A. halimus* populations hydro-ethanolic extracts (Biskra, Mazagran and Sig), as well as to assess separately, total phenolic compounds, total flavonoïds, flavonols, and Carotenoïds using colorimetric methods. Furthermore, correlation between compounds and antioxidant activities has been also examined.

Materials and methods

Plant material

Algerian *A. halimus* aerial parts (leaves and stems) were collected, randomly, from different localities: Sig (35°31'14'30"N 0°10'09'97"W, and 144m elevation), Mazagran (35°53'17'19"N 0°03'14'76"E, 58m elevation) and Biskra (34°55'42"N 5°38'58"E, and 198m elevation), during October 2016. Samples were shared dried (T°≤40°C), until weight stabilization, and conserved for further analysis.

Extract preparation

A. halimus L. leaves and stems (100g) were transferred to dark-colored flasks, and mixed with 70% (v/v) aqueous ethanol (Bourgou, 2016), at room temperature for 24h. Thereafter three extractions, macerates were homogenized, decanted and filtered through Whatman filter paper #1. Filtrates were evaporated under vacuum at 40^{0}C, using Buchi Rotavapor R 210. Resulting aqueous extracts were lyophilized and then stored at -20°C, awaiting analysis.

Extraction yields

Yields were expressed as a percentage of obtained extract weight relative to sample dried matter used for extraction, following Drosoua *et al*, (2015) equation:

Extraction yield % = mass of extract/ mass of dry matter × 100.

Bioactive compounds quantification

Total phenolic contents (TPC)

Reaction mixture was prepared by mixing 01 mL of ethanolic extract, 05mL Folin-Ciocalteu's reagent 10% and 04 mL of $NaHCO_3$ 7.5%. One hour later, in dark at room temperature, absorbance was determined using spectrophotometer at λ=765 nm. The same procedure was repeated using standard solution of Gallic acid (0-100 µg/mL) and calibration line was construed. Extracts phenolic contents were expressed in terms of Gallic acid equivalent per gram of lyophilized extract (mg/GAE/g LE) (Bouzouina *et al.*, 2016).

Total flavonoïds contents (FC)

Total flavonoïds contents in leaves and stems ethanolic extracts was determined by a colorimetric assay, using the method described by Ahn *et al.*, (2007), with slight modification. Briefly, 0.5 mL of Rutin with different concentrations (0-1000µg/mL) or ethanolic extracts was mixed with $AlCl_3$ (0.5 mL, 2%). After 10mn at room temperature, absorbances were measured at 430nm against the blank. Results were expressed as milligram of Rutin equivalents per gram of lyophilized extract (mg RE/g LE).

Total Flavonols contents (TFL)

Flavonols contents were determined by Liu *et al.*, (2010) method. Aliquot (1 mL) of ethanolic extract solution was mixed with 1 mL $AlCl_3$ (2 mg/mL) and 03mL sodium acetate (50 mg/mL). Absorbance at 440nm was recorded after 2.5h. Flavonols content was expressed as

mg of Rutin equivalents per gram of lyophilized extract (mg RE/g LE).

Carotenoïds contents

Total carotenes contents from ethanolic extracts were esteemed using the method described by Sass-Kiss *et al.*, (2005). Briefly, 100 mg from lyophilized extracts were homogenized with 10 mL of hexane/acetone/ethanol (2:1:1 v/v), and centrifuged (15 mn) at 2250 rpm. Obtained supernatant were adjusted to 10 mL with hexane. Solution absorbance was measured at 450nm. Total carotenoids content was expressed as β-carotene equivalents per gram of lyophilized extract (mg βCE/g LE).

Antioxidant activities determination

Total Antioxidant Capacity (TAC)

Total antioxidant capacities of samples were determined following ammonium molybdate reduction method, described by Chaouch et al. (2013a). Samples volume of 150 µL (200-1000 µg/mL) were added at 1.5mL reagent solution (4 mM ammonium molybdate, 0.6 M H_2SO_4 and 28 Mm Na_3PO_4), and incubated in water bath at 90°C for 90 mn. After cooling to room temperature, absorbance of reaction mixture was measured at 695nm. Ascorbic acid (100-500 µg/mL) was used as standard. TAC is expressed as equivalent of ascorbic acid (mg AAE/g LE).

DPPH scavenging assay

Hydrogen atom donation ability of chemical compounds in leaves and stems was measured on the basis to scavenge the 2, 2-diphenyl-1-picrylhydrazil free radical (DPPH) (Aouissat *et al*, 2011). Fifty microliter of each extract concentration was added to 5 mL methanolic DPPH solution 0.004% (m/v). After 30mn, absorbance was read against a blank at 517 nm. In the same conditions, standard curve was determined using trolox (200-1000 µg/mL). Results are expressed as µgTE/ mL of ethanolic extracts. Inhibition of DPPH radical was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = (\text{A blank - A sample/ A blank}) \times 100$$

A blank = Absorbance of control,

A sample = Absorbance of test compound.

The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

ABTS assay

ABTS assay was performed according to the method developed by Aarland *et al.* (2015). Working solution ($ABTS^{++}$) was prepared by mixing the same volume of two stock solutions 7 mM ABTS and 2.45mM potassium persulfate and let it react in dark for 12-16h. Previous solution was diluted by mixing 1 mL $ABTS^{++}$ solution with 60 mL ethanol to obtain an absorbance of 0.700±0.05 at 734 nm. The reaction mixture comprised 0.9 mL $ABTS^{++}$ and 0.1mL of extracts at a variety of concentrations (20-100mg/mL). After 15mn, absorbance was read at 734 nm. Trolox was used as standard (10- 100 µM/mL). Antioxidative activities of tested samples were calculated by determining decreases absorbance at different concentration by using the following equation:

$$ABTS^{+} \text{ scavenging effect (\%)} = \text{Abts (Ac-At)/Ac)} \times 100$$

Where: At and Ac are respective absorbance of tested samples and $ABTS^{+}$, was expressed as µM Trolox equivalents (TE) per gram of lyophilized extract. Scavenging ability was expressed as IC_{50} (TEµmol/mL).

Carotene/linoleic acid assay (BCB)

Inhibition of linoleic acid peroxidation was determined according Queiroz *et al.*, (2009). A stock solution of ß-carotene/linoleic acid mixture was prepared as follows: 2 mg β-carotene was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 mL of oxygenated ultra-pure water was added, then emulsion was vigorously shaken. Sample extract and Butylated hydroxytoluene standard (BHT) were prepared in ethanol. An aliquot (150 µL) of β-carotene/linoleic acid emulsion was mixed with 10 µL of various concentrations (20–100 mg/mL) of samples. After shaking the mixtures, tubes were placed in a water bath at 50°C for 120mn, and the absorbance was read at 470 nm. Readings of all samples were performed immediately and after 120 mn of incubation. A negative control consisted of 200 µL methanol instead of ethanolic extract or BHT was tested. Antioxidant activity of each extract was evaluated in term of β-carotene bleaching (BCB) using the following formula:

$$\beta\text{-carotene bleaching inhibition (\%)} = AA = [(A_A(120) - A_C(120))/(A_C(0) - A_C(120)] \times 100$$

Where $A_A(120)$ is the absorbance of the sample at t = 120 min; A_C (120) the absorbance of the control at t = 120 min and AC (0) the absorbance of the control at t =0 mn. These results were expressed as IC_{50} values BHT equivalent (BEµg/mL).

Statistical analysis

All measurements were performed in triplicate and expressed as mean±SD. Statistically significant data were compared using ANOVA and student's t-test, at the significance level $P<0.05$. Pearson's correlation coefficient was used to identify relationships between

bioactive compounds and antioxidant activities.

Results

Extract yields, total phenolic compounds, flavonoïds, flavonols and carotenoïds

Extraction yields

Extract yields determined from *A. halmus* leaves and stems are presented in Fig. 1. Extraction yields varied among ecotypes and plant parts. Sig ecotype shows the highest yield (leaf and stem extract), comparatively toBiskra and Mazagran extract ecotypes. Yields extract of leaves were higher than stems. However, yielding was found to be greatly different between leaves extracts; Sig (24.87%), Mazagran (21.25%) and Biskra (15%), and stems extracts; Biskra (13.25%), Sig (7.81%) and Mazagran (5.21%).

Bioactive compounds

TPC, TFC, FLC and CC compounds

Quantitative estimation of *A. halimus* phytochemicals was summarized in table 1. Significant variation in total phenolics contents among ecotypes was observed (F_2=1432.50; p=0); Maz?Bis?Sig. Further, TPC vary also according to studied organs (leaves and stems).Leave extracts appear to accumulate large quantity of phenolic compounds, compared to stems ($F_{2.1}$=1158.90; p=0). Leaves and stems concentrations varied from 85.23±1.80 to 279.33±1.17 and 50.41±5.80 to 68.11±7.76 mgGAE/g LE, respectively. The highest total phenolic content was observed on MazL (279.33±1.17 mg GAE/g DE), followed by SigL (162.21±1.48 mg GAE/g LE),and BisL (85.23±1.80 mgGAE/g LE). In addition, stems aqueous ethanolic extracts from Biskra and Mazagran samples were found to be equal (68.11±7.76 and 66.40±0.69 mg GAE/g LE, respectively). The less value was revealed on SigS (50.41±5.80 mg GAE/g LE).

Total flavonoids (TFC) and flavonols (FLC) contents exhibited significantly different data (p<0.05). MazL and BisL were displayed highest amounts in flavonoids (98.19±1.51and 56.71±1.63 mgRE/gLE, correspondingly), followed by SigL (55.02±0.14 mgRE/g LE). Concentrations in stems part were founded in the following order: MazS?SigS?BisS. Like above, leaves extracts revealed high total flavonols content than stems extracts. Mazagran leaves showed relatively the highest concentration (34.87±1.32 mgRE/g LE), than Biskra leaves (30.20±0.92 mgRE/g LE) and Sig leaves (16.59±0.77 mgRE/g LE). In addition, the important quantity of flavonols was also revealed in Mazgran stems (11.23±0.52 mgRE/g LE), comparatively to Sig (5.28±0.10 mg RE/g LE) and Biskra stems (1.54 ±0.07 mgRE/g LE).

Moreover, significant difference in carotenoïds contents between ecotypes was detected (F_2=532.44; p=0); (Sig?Bis?Maz). Furthermore, leaves extracts were exhibited a high content of total carotenoïds pigments comparatively to stems extracts ($F_{2.1}$=357.15; p=0). Sig leaves was revealed great amount of TCC (73µgβCE/g LE) followed by BisL (41 µgβCE/g LE) and MazL (17 µgβCE/g LE). Conversely, stems extracts of Biskra and Sig were showed statistically equal quantity (16 and 17±3.00 µgβCE/g LE) than Mazagran stems (10 µgβCE/g LE).

In-vitro antioxidant activity

Antioxidant potential of different aerial part extracts and standards can be estimated by four techniques as presented in fig.1 (A and B), tables 2 and 3.

Total antioxidant capacity (TAC)

Total antioxidant capacity was esteemed by the formation of phosphomolybdenum complexes. This capacity was based on the reduction of Mo (VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. Obtained data (Fig. 2A) revealed the highest TAC of Biskra ecotype leave extract, while the lowest capacity was found in MazL followed by SigL. Additionally, MazS extract indicated slightly higher antioxidant activity than BisS. Nevertheless, SigS represented the less antioxidant capability (Fig. 2B), comparatively with last both extracts.

Table 1: Total phenolics, flavonoïds, flavonols and carotenoïds contents of*A. halimus* leaves and stems

Locates of *A. halimus*		TPC (mgGAE/g LE)	TFC (mgRE/g LE)	TFLC (mgRE/g LE)	TCC (µgβCE/gLE)
Biskra	**Leaves**	85.23±1.80^{c}	56.71±1.63^{c}	30.20±0.92^{b}	41±0.00^{b}
	Stems	66.40±0.69^{d}	14.86±0.89^{e}	1.54 ±0.07^{f}	17±3.00^{d}
Mazagran	**Leaves**	279.33±1.17^{a}	98.19±1.51^{a}	34.87 ±1.32^{a}	33±00^{c}
	Stems	68.11±7.76^{d}	64.35± 0.41^{b}	11.23±0.52^{d}	10±00^{e}
Sig	**Leaves**	162.21±0.14^{b}	55.02±0.14^{c}	16.59±0.77^{c}	73±00^{a}
	Stems	50.41±5.80^{e}	40.47±0.31^{d}	5.28±0.10^{e}	16±00^{d}

Values in average (n=3) ±SE. Averages, mg GAE/g LE: mg of Gallic acid equivalent per g. of lyophilized extract;mg RE/g LE: mg of Rutin equivalent per g. of lyophilized extract and µg βCE /g LE: µg of carotene equivalent per g. of lyophilized extract. Averages, in the same column, with different letters (a-f) are significantly different values (p< 0.05).

Radical scavenging activities (DPPH), radical cation

Table 2: Antioxidant activities based on, DPPH, ABTS and BCB assay of aerial parts of *A. halimus*

Test groups	Concentration (mg/ml)	BisL	BisS	MazL	MazS	SigL	SigS
DPPH%	20	19.33±0.02	23.04±0.02	11.14±8.37	14.33±3.4	3.42±3.47	13.38±0.04
	40	43.06±0.01	30.46±00	25.80±7.07	25.80±2.1	7.17±2.51	22.71±0.01
	60	54.77±0.00	39.84±0.02	38.37±4.98	38.17±0.4	16.50±3.55	25.52±0.00
	80	68.70±0.01	53.25±0.03	47.43±9.35	52.89±0.5	22.02±6.13	34.27±0.00
	100	78.50±0.01	67.64±0.07	64.49±7.94	64.29±0.2	33.79±3.67	37.27±0.03
ABTS %	20	36.36±2.96	17.16±2.07	17.90±2.40	8.48±2.03	27.56±3.15	11.79±2.94
	40	57.59±1.70	27.07±4.81	20.67±00	25.39±3.23	40.22±2.50	14.45±2.01
	60	81.26±8.76	37.77±0.46	26.93±1.91	34.52±1.92	53.57±00	23.42±1.45
	80	94.29±2.22	46.01±4.39	29.89±3.29	39.10±0.53	63.85±2.42	34.91±7.49
	100	96.44±1.67	62.97±0.20	43.18±5.40	45.56±3.10	73.31±3.19	39.84±4.48
BCB %	20	14.84±0.50	14.09±0.67	0.22±0.33	21.72±1.2	17.31±0.17	00
	40	36.77±2.08	38.49±0.33	9.68±0.76	26.56±1.00	27.53±0.73	14.52±0.7
	60	46.88±0.60	44.09±1.33	48.60±1.33	32.47±0.3	57.10±1.04	16.77±1.8
	80	55.27±0.60	54.09±1.17	74.09±1.01	35.81±0.5	61.29±1.26	20.43±0.2
	100	86.34±1.71	56.02±0.17	76.99±0.83	45.81±00	88.92±0.44	59.46±0.5

Data expressed as mean as (% I): Percentageinhibition of radical scavenging

scavenging (ABTS) and β-carotene-linoleic acid bleaching activities (BCB)

Antioxidant capacities of *A. halimus* extracts was subsequently evaluated using DPPH, ABTS and β-carotene bleaching test. Table 2 revealed that each extract was found to be dose dependent scavenging activity on DPPH, ABTS and inhibited*β*-carotene oxidation process. Additionally, significant difference between antioxidant capability of samples tested and standards curves expressed as IC_{50} were detected (table 3). Great antioxidant effect basedon DPPH and ABTS assays were exhibited by Biskra leaves and stems extracts at 100mg/mL (78.50±0.01, 67.64±0.07 and 96.44±1.6, 62.97±0.20%, respectively). In contrast to Mazagran extracts (leaves and stems), the same average antioxidant capacity were observed by previous tests DPPH (64.49±7.94 vs 64.29±0.2%) and ABTS (43.56±5.40, 45.56±3.10%, respectively) (table 2). Exceptionally, DPPH quenching ability from Sig extracts (leaves and stems) did not exceed 37% at maximum concentration, and leaves extract was revealed more discoloration of $ABTS^{+}$ (73.31±3.19%) compared to stems extract (39.84±4.48%). The kinetics of β-carotene degradation shows better antioxidant activity of leaves extracts from sig (88.92±0.44%) followed by Biskra extracts (86.34±1.71%) and Mazagran extracts (76.99±0.83, 45.81%), than stems which showed moderate capacity to inhibit linoleic acid oxidation. Values were founded in the following order: SigS (59.46±0.5%) ? BisS (56.02±0.17%) ? MazS (45.81%).

Table 3: Leaves and stems extract antioxidant activities of DPPH, ABTS and BCB assays, expressed as IC_{50}

Locates of *A. halimus*		DPPH (TEµg/mL)	ABTS (TEµmol/mL)	BCB (BEµg/mL)
Biskra	**Leaves**	845.64±10.75^{c}	59.75±0.97a	28.28±1.31^{ab}
	Stems	497.46±37.53^{a}	56.75±5.36a	17.38±4.17^{a}
Mazagran	**Leaves**	553.86±3.00^{ab}	na	33.75±21.06^{b}
	Stems	501.85±45.91^{a}	na	na
Sig	**Leaves**	na	53.91±2.05a	45.56±1.20^{c}
	Stems	na	na	38.85±18.77^{b}
Standard		579.94±0.7^{b}	59.76±2.47a	15.38±2.33^{a}

Results are expressed as means ± standard deviations (n = 3). Values with same column, with different case letters (a, b, c) are significantly different ($p<0.05$). DPPH radical scavenging activities are expressed as IC_{50}, TEAC, trolox equivalent antioxidant capacity, expressed as µg trolox equivalents (TE)/mL; ABTS radical cation scavengingare expressed as IC_{50}, TEµmol/mL; BCBβ-carotene-linoleic acid bleaching activitiesare expressed as IC_{50}, BHT Butylatedhydroxytoluene expressed as µg BHT equivalents (BE)/mL.

Comparatively to standards (table 3), significant difference was showed between all extracts and trolox standard based on DPPH test (F_4= 68.33; p=0): Biskra Stems and Mazagan extracts (leaves and stems) were expressed superior scavenging effect than trolox, in contrast to Biskra leaves extract. ABTS radical cation scavenging highlighted no significant difference between whole extracts in terms of trolox, authentic standard. In added, ecotypes extracts and positive control (BHT) were revealed statistically significant difference inretarding ability or inhibiting β-carotene oxidation may be described as a free radical scavenger (F_5=3.11; *p?*0.05). Biskra stems was also exhibited strong

Table 4: Pearson's correlation coefficients between bioactive compounds and antioxidant activities (r)

Bioactive compounds from *A. halimus*				
Antioxidant activities	**TPC**	**TFC**	**FLC**	**BCC**
DPPH	0.506	0.548	0.546	0.151
ABTS	0.137	0.284	0.496	0.337
BCB	0.464	0.682	0.687	0.732
TAC	0.448	0.642	0.522	0.711

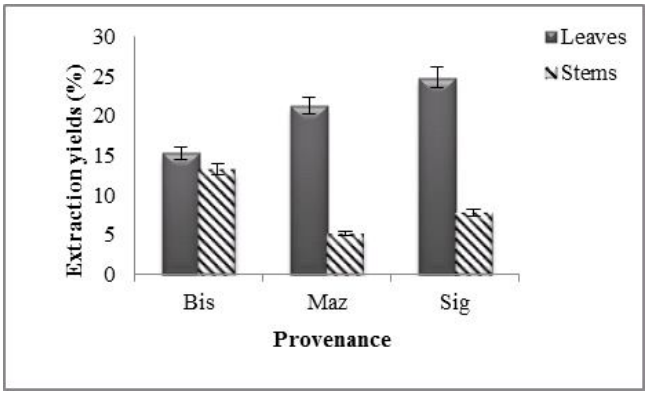

Fig. 1: Extraction yields of *Atriplex halimus* (%)

capacity of inhibiting bleaching of β-carotene than BHT standard, while Biskra leaves showed average inhibitory activity; lowest capacities of different extracts aerial parts from Sig and Mazagran leaves were displayed relatively to standard BHT.

Correlation between bioactive compounds and antioxidant activities

Results (table 4) indicate moderate correlation between total phenolics, flavonoids, flavonols compounds from all extracts and DPPH assays with values coefficients (r= 0.506 for TPC, r=0.548 for TFC and r=0.546 TFLC), carotenoid contents do not have a consistent correlation with DPPH(r=0.151); weak correlation appeared between ABTS and flavonoids (r=0.284) as well as total phenolic contents (r =0.137) contrarily to TFLC and TCC, where the positive correlation was observed with (r= 0.496 for TFLC) followed by TCC (r=0.337). Important positive correlation was seemed between TCC (r=0.732), TFLC (r=0.687), TFC (r=0.682) and ß-carotene bleaching methods. Found results revealed high amount of total phenolic in all extracts for not correspond to high total antioxidant capacity. Significant correlation was showed among TCC (r=0.711) and TAC tests shadowed by TFC (r=0.642) and less correlation with TPC (r=0.448).

Discussion

Secondary metabolites contents and antioxidant proprieties of *Atriplex halimus* L, from three Algerian areas were studied. In our knowledge, no data in the literature has been published on Biskra, Sig and Mazagran *A. halimus* regions. According Bennhammou *et al*, (2014), *A. halimus* methanolic extracts yields (leaves and stems) from Bechar area are similar to those obtained from Mazagran and Sig samples, and different to Biskra extracts. Differences showed probably influenced by increasing water concentration in the solvent, because of water percentage in solvent intensified yields extraction; mixture can extract polar and non-polar chemicals [20].

Our study demonstrated that *A. halimus* from Algeria was important source of phytocomponents, including phenolic compounds, flavonoids, flavonols and carotenoids, and are commonly distributed in plant parts. These compounds constitute a class of secondary plant metabolites with potential antioxidant activity [19]. Secondary metabolites varied among ecotypes (Bis, Maz, Sig), and between plant parts (leaves and stems). Extremely higher bioactive compounds were detected in leaves extracts. Extracts leaves from Mazagran, Biskra and Sig were revealed higher total phenolic concentrations than methanolic extracts of *A. halimus* from Bechar and

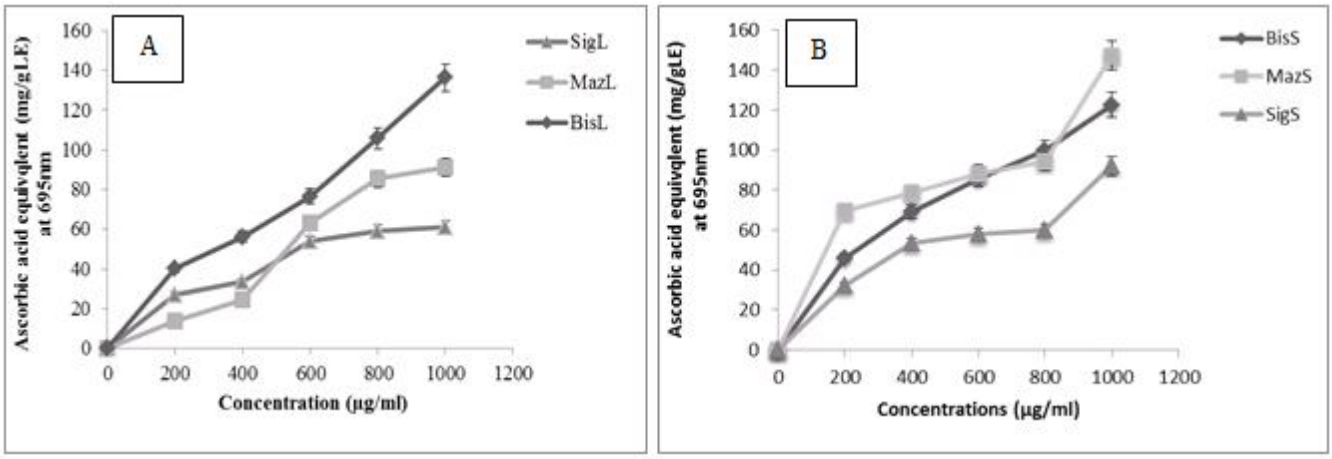

Fig. 2: Leaves (A) and stems (B) total antioxidant capacities of *A. halimus* extracts

Telmcen (Benhammou *et al*, 2014; Chikhi *et al*, 2014; Ksouri *et al*, 2011; Meot-Duros and Magné, 2009). However, total phenolic amounts in stems extracts from western samples (Biskra) are in agreement with Benhammou *et al*, (2014). Compared to chenopodiaceae species family, earlier results were lower than those reported elsewhere for *Halimione portulacoides* (Vilela *et al*, 2014), *Anabasis articulate* (Benhammou *et al*, 2013), and *Sesuvium portulacastrum* (Slama *et al*, 2015). Nonetheless, modulation of extracts yields and distribution from previous bioactive constituents may be attributed to numerous factors in particular, environmental conditions such as salinity and drought (Ksouri *et al*, 2011; Falleh *et al*, 2012), period and intensity of stress and plant age or developmental stage (Theerawitaya *et al*, 2014). Ebrahimzadeh *et al*. (2008) showed that previous variations were influenced by differences in plant matrix. Additionally, genotypic factors appeared to be control accumulation of these compounds in the plant (El-Waziry, 2007). Also, differences can be attributed to the extraction process (khoddami *et al*, 2013; hasmida *et al*, 2014) and the solvent used (Mohammedi and Atik, 2011; Jain *et al*, 2014; Sanda, 2015).

Antioxidant activities can be measured using numerous methods, *In vitro*, assays, such as ability to scavenge free radicals (DPPH and ABTS), inhibition of linoleic acid peroxidation and total antioxidant activities. Indeed, Nuutila *et al*. (2003) suggest that a single method is not recommended for evaluation of antioxidant activities of different plant products, due to their complex composition. Agreeing to the precedent results, it was observed that *A. halimus* from different locations may reflects a relative variation in antioxidant ability from compounds in extracts to act as reducing agents; TAC, reduce $ABTS^{d+}$, DPPH free radical and retarding the oxidation of β-carotene *In vitro* systems. Total antioxidant activity was showed in a concentration dependent manner from all extracts tested. Mazagran and Biskra extracts (leaves and stems) presented higher activities than Sig extracts. This antioxidant capability may be due to the presence of TCC, TFC. Though, this study confirmed the theory from Cai *et al*. (2004), where greatest antioxidant activities from plant sources are derived from phenolic-type compounds. This theory, do not always correlate with the presence of large quantities of phenolics, were confirmed by investigation of Ouchikhi *et al*. (2011). Hence, all antioxidant assays (DPPH, ABTS and BCB) tested as Biskra locate was exhibited their antioxidant capability. Mazagran and Sig extracts respond differently against antioxidant methods, where Mazagran area was mostly non-reactive and did not reach 50% inhibition of radicals (ABTS and BCB), and ABTS test was revealed the scavenging capacity of leaves Sig in contracts to BCB assays. Biskra stems and Mazagran extracts (leaves, stems) were showed a great antiradical activity than synthetic antioxidant (standard trolox) based on DPPH assay.

Results of our exploration are in disagreement with the findings of Benhammou *et al*. (2009), who showed higher DPPH quenching ability in methanolic leaves extracts; lowest than other halophyte and xero-halophyte species methanolic leaves extracts of *Limoniastrum monopetalum* (162µg/mL) and *Frankenia thymifolia* (99 µg/ml) (Megdiche *et al*, 2011), and methanolic leaves and stems extracts of *Sesuvium Portulacastrum* (90 vs 190 µg/mL) (Slama *et al*, 2015). It seems that aerial parts extracts from *A. halimus* possess hydrogen donating aptitudes to act as antioxidant. DPPH quenching capacity was probably attributed to the presence of phenolics. Bylka *et al*. (2001) suggest that this capacity is due to the abundance of flavonols (keapfero, quercetin); main class of *Atriplex* species.

Antioxidant activity evaluation of ABTS assay and DPPH method showed similar activity mechanism. Nonetheless, ABTS method is more reliable than DPPH method due to solubility of ABTS reagent in both aqueous solvents (Tewo *et al*, 2007). For this reason, ABTS assay is better than DPPH assay when applied to a variety of plant foods containing hydrophilic, lipophilic, and high-pigmented antioxidant compounds (Folgelel *et al*, 2011). Biskra extracts results (leaves, stems) and Sig leaves extract, suggesting its potency as a natural source of antioxidants, may be possibly due to the presence of favonols in both extracts. Comparatively, Mazagran extracts (leaves and stems) and stems of Sig extracts were mostly non-reactive and did not reach 50% inhibition of radicals at tested concentrations.

Identical scavenging linoleate-derived free radicals capabilities detected between Biskra stems extract and BHT standard, in contrast to leaves Mazagran, Biskra and sig (leaves, stems) extracts. This assessment is not only explained by the presence of TCC but also by TFC and TFLC constituents, with important positive correlation between these bioactive constituents and BCB assay. Findings are in agreement with other reports (Bylka *et al*, 2011; Cai *et al*, 2006). Because of the β-carotene is similar to an oil-in-water emulsion system, β-carotene bleaching assay only provides an indication of the level of lipophilic compounds (Miraliakbari and shahidi, 2008), such as unsaturated fatty acids (palitoleic, oleic, linoleic and linolenic acids) present in *A. halimus* species (Emam, 2011).

Findings seem to indicate that antioxidant activities depend on structure and concentration of active molecules present in tested vegetable (Popovici *et al*, 2009; Karamak *et al*, 2005). According to Theerawitaya *et al.* (2014), Hussain *et al.* (2015) and Wang *et al.* (2015), environmental factors, period, stress intensity and vegetative or developmental stage and plant parts influence reactive oxygen species accumulation (ROS) in plants, and induce variation in synthesized secondary metabolites quantities and qualities and consequently variability of antioxidant power.

Conclusion

This research allowed showing that the content of secondary metabolites vary according to the species ecotype and the plant part. Anti-free radical power does not always depend on the high concentration of antioxidants. *Atriplex halimus* antioxidant capacity from Biskra depend on the presence of flavonols bioactive compounds as well as flavonoids, which give the particularity of substituting synthetic antioxidants in agro-food industries, phytotherapy and even in livestock feeding for preventing damages that can be caused by oxidative stresses.

References

Aarland, RC, C. Morales Sanchez, F. Parra Bustamante, J.M. Villa Hernandez, L. Diaz. and F.A. Sanchez (2015). Pharmacological and phytochemical study of medicinal plants used in Mexican folk medicine. *Indian J. Traditknowle*, **14(4)**: 550-557.

Abd El-Rahman, H.H,M.I. Mohamed, A.E.A. Gehad. and I.M. Awadallah (2011). *Int. J. Agr. Biol*, **8**: 766.

Abdelguerfi, A. and M. Abdelguerfi-Laouar (2004). Les ressources génétiques d'intérêt fourrager et/ou pastoral: diversité, collecte et valorisation au niveau méditerranéen. *Cah. Options. Mediterr*, **62**: 29-41.

Ahn, M.R, S. Kumazawa, Y. Usui, J. Nakamura, M. Matsuka, F. Zhu. and T. Nakayama (2007). *Food Chem,* **101**: 1383–1392.

Aouissat, M, D.J. Walker, K. Hcini, M. Belkhodja & E. Correal, (2011). Osmolyte concentrations in *Atriplex halimus* L. and *Atriplex canescens* (Pursh) Nutt. Adapted to salinity and low temperature (Chenopodiaceae). *Anales Biologia*, **33**: 117-126.

Bayoumi, M.T. and H.M. El-Shaer (1992). Impact of halophytes on animal health and nutrition, in: Halophytes as a resource for livestock and for rehabilitation of degraded lands, Kluwer, Squires, V.R, A.T. Ayoub (eds.), Academic Publishers, Dordrecht, Netherlands, 267

Benhammou, N, F.A. Bekkara and T.K. Panovska (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *C. R. Chim*, **12**: 1259–1266.

Benhammou, N, N. Ghambaza, S. Benabdelkader, F. Atik-Bekkara and T. Kadifkova Panovska (2013). Phytochemicals and antioxidant properties of extracts from the root and stems of *Anabasis articulate*. *International Food Research Journal,* **20(5)**: 2057-2063.

Benhammou-Belyagoubi, N, L. Benyagoubi and F. Atik Bekkara (2014). Phenolic contents and antioxidant activities *In vitro* of some selected Algerian Plants. *Journal of Medicinal Plant Research,* **8(40)**:1198-1207.

Bourgou, S, R. Serairi Beji, F. Medini and R. Ksouri (2016). Effet du solvant et de la méthode d'extraction sur la teneur en composés phénoliques et les potentialités antioxydantes d'*Euphorbia helioscopia*. Journal of new sciences, *Agriculture and Biotechnology*, **28(12)**: 1649-1655.

Bouzouina, M, N.O. Amari, A. Berkani, B. Lotmani, M. Hanafi and A. Lazizi (2016). Total Phenolics Contents, Flavonoïds Contents, and Fatty Acids Compositions in *Thymelaea hirsuta* L. Aerial Parts, Grown in Western Algeria. *Advances in Environmental Biology*, **10(1)**: 16-22.

Bylka, W, M. Stobiecki and R. Franski (2001). Sulphated flavonoid glycosides from leaves of *Atriplex hortensis*. *Acta Physiologiae Plantarum*, **23(3)**: 285-290.

Cai, Y, M. Sun, J. Xing, Q. Luo and H. Corke (2006). Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Science*, **78**: 2872.

Cai, Y, Q. Luo, M. Sun and H. Corke (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Science*, **74**: 2157-2184.

Chaouche, T.M, F. Haddouchi, R. Ksour, F. Medini, I.A. El-Haci, Z. Boucherit, F.Z Sekkal and F. Atik-Bekara (2013a). Antioxidant activity profiling by spectrophotometric methods of phenolicextract of *Prasium majus* L. *Free Radicals and Antioxidants*, **3**: 43-46.

Chaouche, T.M, F. Haddouchi, R. Ksouri, F. Medini and F. Atik-Bekara (2013b). *In vitro* evaluation of antioxidant activity of the hydro-methanolicextracts of *Juniperus oxycedrus subsp. oxycedrus*. *Phytothérapie*, **11**:244-249.

Chikhi, I., H. Allali, M.E.A. Dib, H. Medjdoub and B. Tabti (2014). Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin-induced diabetic rats. *Asian Pac. J. Trop. Dis*, **4**: 181-184.

Deng, Y, Y. Zhao, O. Padilla-Zakour and G. Yang (2015). Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. *Industrial Crops and Products*, **74**: 803–809.

Do, Q.D., A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji and Y.H. Ju (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. *Journal of Food and Drug Analysis*, **22**: 296-302.

Drosoua, C., K. Kyriakopoulou, A. Bimpilas, D. Tsimogiannis and M. Krokida (2015). A comparative study on different extraction techniques to recovered grape pomace polyphenols from vinification by products. *Industrial Crops and Products,* **75**: 141-149

Durand, D., M. Damon and M.Gobert (2013). Le stress oxydant chez les animaux de rente: Principes généraux oxidative in farm animals: General aspects ; *Cahier de nutrition et de diététique*, **48(5):** 218-224.

Ebrahimzadeh, M.A., F. Pourmmorad and S. Hafezi (2008). Antioxidant activities of Iranian corn silk. *Turk J. Biol.*, **32**: 43–9.

El-Waziry, A.M. (2007). Nutritive Value Assessment of Ensiling or Mixing Acacia and Atriplex Using *In vitro* Gas Production Technique. *Res J Agric. Biol. Sci.*, **3**: 605.

Emam, S.S. (2011). Bioactive constituents of *Atriplex halimus* plant. *J. Nat. Prod.,* **4**: 25-41.

Falleh, H., I. Jalleli, R. Ksouri, M. Boulaaba, S. Guyot, C. Magné and C. Abdelly (2012). Effect of salt treatment on phenolic compounds and antioxidant activity of two Mesembryanthemum edule provenances. *Plant Physiol. Biochem,* 52: 1–8.

Flogelel, A., D.O. Kim, S.J. Chung, S.I. koo and O.K. Chun (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of food composition and analysis*, **24(7)**: 1043-1048.

Hancock, J.T., R. Desikan and S.J. Neill (2001). Role of reactive oxygen species in cell signaling pathways. *Biochemical Society Transactions*, **29(2)**: 345-349.

Hasmida, M.N., A.R. Nur Syukriah, M.S Liza and C.Y. Mohd Azizi (2014). Effect of different extraction techniques on total phenolic content and antioxidant activity of *Quercus infectoria* galls. *International Food Research Journal,* **21(3)**: 1075-1079.

Hussain, T, H.W. Koyro, B. Huchzermeyer and M.A. Khan (2015). Eco-physiological adaptations of *Panicum antidotale* to hyperosmotic salinity: water and ion relations and anti-oxidant feedback. *Flora,* **212**: 30–37.

Jain, A., R. Ranade, P. Pritam, N. Joshi, S.L. Vavilala and A. Jain (2014). A comparative study of antioxidant activity, total phenolic and flavonoid contents in different parts of *Helicteres isora* L. *American Journal of Life Sciences*, **2(5)**: 292-302.

Karamac, M., A. Kosiñska and R.B. Pegg (2005). Comparison of radical-scavenging activities for selected phenolic acids. *Pol. J. Food Nutr. Sci.*, **14/55 (2)**: 165-170.

Kessler, J.J. (1990). *Atriplex* forage as a dry season supplementation feed forsheep in the Montane Plains of the Yemen Arab Republic. *Journal of Arid Environments,* **19**: 225-234.

Khoddami, A., M.A. Wilkes and T.H. Roberts (2013). Techniques for analysis of plant phenolic compounds molecules, **18**: 2328-2375.

Kontogianni, V.G., G. Tomic, Nikolic. I, A.A. Nerantzaki, N. Sayyad, S. Stosic- Grujicic *et al.,* (2013). Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and antiproliferative activity. *Food Chemistry*, **136**: 120-129.

Ksouri, R., K.W. Megdiche, I. Jallali, A. Debez, C. Magné, I. Hiroko and C. Abdelly (2011) Medicinal halophytes: potent source of health promoting biomolecules with medical: nutraceutical and food applications. *Crit. Rev. Biotechnol,* **32**: 1-38.

Le Houérou, H.N. (1992). The role of saltbushes (*Atriplex* spp) in arid land rehabilitation in the mediterranean Basin: a review. *Agroforestry. Syst*, **18**: 107-148.

Liu, Y, B. lu, L. Xu *et al.* (2010). The antioxidant activity and hyperlipidemic activity of total flavonoids from the fruit *Rosa laevigata* Michx. *Naturel Science*, **2 (3)**: 175-183.

Lotmani, B., L. Fatarna, A. Berkani, J. Rabier, P. Prudent and I. Laffont-Schwob (2011a). Saltbush, *Atriplex halimus*, tolerant to high concentrations of lead, zinc, and copper for phytostabilization of heavy metal-contaminated soils. *The European Journal of Plant Science and Biotechnology.*

Megdiche, K.W., F. Chaouachi, R. M'Rabet, F. Medini, Y. Zaouali, N. Trabelsi, R . Ksouri, E. Noumi and C. Abdelly (2011). Antioxidant and antimicrobial properties of *Frankenia thymifolia* Desf. fractions and their related biomolecules identification by gas chromatography/ mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC). *Academic Journal*, **5 (24)**: 5754-5765.

Meot-Duros, L. and C. Magné (2009). Antioxidant activity and phenol content of *Crith-mum maritimum* L. leaves. *Plant Physiol. Biochem*, **47**: 37–41.

Mesnoua, M., E. Mateos-Naranji, J.M. Bacia-Pierdras, J. Alberto, Pérez-Romero, B . Lotmani and S. Redondo-Gmez (2016). Physiological and biochemical mechanisms preventing CD toxicity in the hyperaccumulators *Atriplex halimus* L. *Journal of plant physiology and biochemistry,* **106**: 30-38.

Miraliakbari, Shahidi (2008). Antioxidant activity of minor components of tree nut oils. *Food Chem*, **111**: 421-7.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.,* **7**: 405-410.

Mittler, R., S. Vanderauwera, M. Gollery and F. Van Breusegem (2004). Reactive oxygen gene network of plants. *Trends Plant Sci.*, **9**: 490-498.

Mohammedi, Z. and F. Atik (2011). Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarixaphylla* L. *International Journal of Pharma and Bio Sciences,* **2**: 609-615.

Nuutila, A.M., R. Puupponen-Pimia, M. Aarni and K.M. Oksman-Caldentey (2003). Comparison of antioxidant activities of

onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chemistry,* **81**: 485–493.

Ouchikh, O., T. Chahed, R. Ksouri. *et al.,* (2011). The effects of extraction method on the measured tocopherol level and antioxidant activity of L. nobilis vegetative organs. *Food Comp Anal*, **24**: 103.

Popovici, C., I.Saykova and B. Tylkowski (2009). Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. *Revue de génie industriel,* **4** : 25-39.

Prache, S., P. Gatellier, A.Thomas, B. Picard and D. Bauchart (2011). Comparison of meat and carcass quality in organically reared and conventionally reared pasture-fed lambs. *Animal,* **5(12)**: 2001-2009.

Queiroz, Y.S., E.Y. Ishimoto, D.H.M. Bastos, G.R. Sampaio and A.F.S. Elizabeth Torres (2009). Garlic (*Allium sativum* L.) and ready-to-eat garlic products: *In vitro* antioxidant activity. *Food Chemistry*, **115**: 371–374.

Said, O., K. Khalil, S. Fulder and H. Azaizeh (2007). Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. *J. Ethnopharmacol,* **83**: 251-265.

Sanchez-Moreno, C., J.A. Larrauri and F. Saura-Calixto (1998). A procedure to measure the antiradical efficiency of polyphenols. *Science of Food and Agriculture*, **76**: 270.

Sanda, M.A., G. Zengin, A. Aktumsek and Y.S. Cakmak (2015). Evaluation of antioxidant potential of two Daphne species (*D. gnidioides* and *D. pontica*) from Turkey. *Emirates Journal of Food and Agriculture*, **27(6):** 488-494.

Sass-Kiss, A., J. Kiss, P. Milotay, M.M. Kerek and M. Toth-Markus (2005). Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International,* **38**:1023-1029.

Slama, I., R. M'Rabet, R. Ksouri, O. Talbi, A. Debez and A. Chedly (2015). Water deficit stress applied only or combined with salinity affect physiological parameters and antioxidant capacity in *Sesuvium portulacastrum*. *Flora*, **213**: 69–76.

Stringi, L., G. Amato and L. Gristina (1991). *Agric. Mediterr*, **121**: 16.

Talamali, A. (2001). Polygamy in *Atriplex halimus* L. (Chenopodiaceae). *Compt Rendus Acad Sci III Sci Vie, French,* **324(2)**: 107-113.

Teow, C.C., V.D. Truong, R.F. McFeeter, R.L. Thompson, K.V. Pecota and G.C. Yencho (2007). Antioxidant activities, phenolic and b-carotene contents of weet potato genotypes with varying flesh colours. *Food Chem,* **103:** 829-838.

Theerawitaya, C., T. Samphumphaung, S. Cha-um, N. Yamada and T. Takabe (2014). Responses of Nipa palm (Nypa fruticans) seedlings, a mangrove species, to salt stress in pot culture.*Flora*, **209: 597–603.**

Vilela, C., S.A.O. Santos, D. Coelho, A.M.S. Silvab, C.S.R. Freirea, C.P. Netoa and A.J.D. Silvestre (2014). Screening of lipophilic and phenolic extractives from different morphological parts of *Halimione portulacoides*. *Industrial crops and products*, **52**: 373– 379.

Wang, Y., Y. Li, H. Xue, H.W. Pritchard and X. Wang (2015). Reactive oxygen species-provoked mitochondria-dependent cell death during ageing of elm (*Ulmus pumila* L.) seeds. *Plant Journal*, **81**: 438–452.