THE ANTIOXIDANT AND FREE-RADICAL SCAVENGING ACTIVITIES OF TAMARIX BOVEANA AND SUAEDA FRUTICOSA FRACTIONS AND RELATED ACTIVE COMPOUND

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Abstract

The antioxidant potentials of the ethyl acetate and the methanolic fractions of the halophytes *Tamarix boveana* Bunge and *Suaeda fruticosa* L. were established by using radical-scavenging assays, namely $DPPH^{\bullet}$ scavenging and $ABTS^{\bullet+}$ scavenging, in addition to total polyphenol content assay. These fractions showed very interesting dose-dependent free radical scavenging properties in all samples. In particular, the ethyl acetate fraction of *T. boveana* exhibited a strong antioxidant activity toward DPPH and $ABTS^{+}$ radicals reaching 100 % of inhibition. To search the active principle responsible for this activity, this extract was further fractionated by repeated chromatography on silica gel. Interestingly, Naringenin was found to be responsible for the major share of the unregistered activity in the company of other compounds participating synergistically. An important correlation between antioxidant capacities of the other fractions and their total phenolic contents was revealed.

Keywords: *Tamarix boveana, Suaeda fruticosa,* antioxidant activity, naringenin, total phenolic content

Introduction

Oxidative stress is involved in the pathology of cancer, arteriosclerosis, malaria and rheumatoid arthritis, and could play a role in neurodegenerative diseases and ageing processes. On the other hand, lipid oxidation, initiated by free radicals, is one of the major factors for food deterioration during processing and storage (Thitileadecha et *al*., 2008). Food deterioration is also associated with unfavorable effects. Significant changes can occur in product flavor, color, and texture and finally can lead to loss of nutritive value or complete spoilage. Synthetic antioxidants have been widely used in the food industry to prevent oxidative damage. However, their use can involve toxic side effects (Cornwell et *al*., 1998). Natural antioxidants, especially plant phenolics, flavonoids, tannins and anthocyanidins, are safe and bioactive (Mohsen and Ammar, 2009). Consequently, in recent years, considerable attention has been paid to explore the potential antioxidant property of plant extracts or isolated products of plant origin, which may be used for human consumption (Chua et *al*., 2008). Numerous crude extracts, from plant materials rich in phenolics, are increasingly of interest in the food industry, because they can delay oxidative degradation of lipids and thereby improve the quality and nutritive value of food and can prevent diseases related to oxidative stress (Rios et *al*., 2009).

Halophytes are naturally salt-tolerant plants that may be potentially useful for economical applications as new sources of natural antioxidants in dietary food (Meot-Duros et *al.,* 2008). As, halophytes are known for their ability to withstand in salt condition and quench the toxic accumulated reactive oxygen species (ROS), responsible for cellular damage, metabolic disorders, and senescence processes (Menezes et *al.,* 2004), since they are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components (Ksouri et *al.,* 2008).

The halophytes *Tamarix boveana* and *Suaeda fruticosa* belong to *Tamaricaceae* and *Chenopodiaceae* families respectively. Species of both families show a wide range of biological activity of multiple interests, suggesting a great pharmacological and biotechnological potential. They hold promise as sources of chemical leads for the development of new drugs. In fact, *Tamarix* species are employed in traditional medicine as astringent, aperitif, stimulus of perspiration and diuretic (Gaston, 1998).

They are useful again in leucoderma, spleen trouble, eye disease and various liver disorders (Ksouri et *al.,* 2009). Hepatotonic and stimulant properties were also revealed additionally to antimicrobial activities (Ksouri et *al.,* 2009; Sultanova et *al.,* 2001). In particular, *T. boveana* was well known for its antimicrobial and anti-insecticidal properties (Saidana et *al.,* 2005, 2007, 2008 a et b et 2010).

In another part, *S. fruticosa* has a hypoglycemic effect (Benwahhoud et *al.,* 2001), a hypercholesterolaemic resistance (Bennani-Kabchi et *al.,* 1999), and it is specific with its black wool dyeing (Le Floc'h, 1983). Seeds oil of *S. fruticosa* is considered good quality oil and could be used in human consumption (Weber et *al.,* 2007).

Furthermore, several studies proved antioxidant activities of several *Tamarix* species such as *T. gallica* (Ksouri et *al.,* 2009), *T. ramosissima* (Sultanova et *al.,* 2001), *T. hispida* (Sultanova et *al.,* 2004) and *T. nilotica* (Raha et *al.,* 2009) and *Suaeda* species such as *S. salsa* (Zhao and Zhang, 2006; Chang-Quan et *al.,* 2007), *S. japonica* (Keisuke and Sakae, 2010), *S. australis* (Huang et *al.,* 2010), *S. monoica* and *S. maritima* (Thirunavukkarasu et *al.,* 2010).

These promising results encourage us to search a potential antioxidant activity of several fractions of *T. boveana* and *S. fruticosa* aerial parts, and specify the principle active responsible for the whole or part of the eventual activity.

Material and Methods

Plant material

The saltcedars *T. boveana* and *S. fruticosa* were collected respectively from Sebkhet El Kalbia (35°50' North and 10°15' East of Tunisia), and Sebkhet Monastir (35°46' North and 10°59' East), in flowering stage. Voucher specimens are deposited in the herbarium of the Laboratory of Natural Substances Chemistry and Organic Synthesis, Faculty of Sciences, Monastir, Tunisia.

Preparation of extracts

Dried and finely powdered *T. boveana* and *S. fruticosa* aerial parts (3600 g, 2480 g respectively) were immersed in MeOH and extracted by maceration four times at room temperature for 20 days.

Crude extract of each halophyte was filtered then evaporated to dryness under reduced pressure at 40 °C. The MeOH extracts of *T. boveana* and *S. fruticosa* (651 g, 320 g respectively) were defatted with petroleum ether. Removal of the solvent from the petroleum ether solutions gave oily fractions (25.6 g, 46 g respectively) and first methanolic residues. Extraction of the residues with $CHCl_3$ yields chloroform fractions (42.2 g, 43 g respectively) and second methanol residues. Extraction of the second residues with EtOAc yields EtOAc fractions (19.9 g, 15 g respectively) and third methanolic residues (510.4 g, 147 g respectively). Each fraction (oily fraction F_1, chloroformic fraction F_2, ethyl acetate fraction F_3 and third methanolic residue F_4) of each halophyte was taken to dryness under vacuum and stored at 4 °C until tests.

Isolation and identification

The EtOAc fraction of *T. boveana* (9 g) was fractionated once more sequentially on a silica gel column using $CHCl_3$, $CHCl_3$-EtOAc and EtOAc-MeOH at different proportions as elution system. 335 fractions of 100 ml each were collected and combined into 16 main groups named A-P. The fraction O (294 mg) was further subjected to repeated column chromatography on silica gel, eluted with EP/ $(CH_3)_2CO$ (60:40). These procedures led to the isolation and purification of a compound A (50 mg), as a white precipitate. The identification of this compound was based on NMR spectra. ^{1}H, ^{13}C NMR and two-dimensional NMR spectra were obtained with Bruker WP300 spectrometer at 300MHz for 1H NMR and 75 MHz for 13C NMR. Measurements were made in $CDCl_3$ at 27°C. In ^{1}H NMR spectra, chemical shifts were reported using TMS as an internal standard. In the ^{13}C NMR spectra, chemical shifts were reported as δ (ppm) values relative to the carbon signal of $CDCl_3$. EIMS spectra (70 eV) was obtained with HP 59712 spectrometer. Hxcoqf and inv4lplrndqf heteronucleair pulse programs were used respectively for CHCorr and HMBC experiments; Cosyqf 45 was used for COSY experiment. For column chromatography SDS silica gel (70-200mesh) was used. The purity of the natural compound was controlled by thin layer chromatography (TLC, Merck silica gel plates 60F-254). Solvents, EP, $CHCl_3$, EtOAc, $(CH_3)_2CO$ and MeOH used for extraction were purchased from SDS.

Antioxidant activity

Chemicals

All solvents were of analytical grade purity. The chemicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH); 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium carbonate, Folin–Ciocalteu's phenol reagent and Catechin were acquired from Sigma, while potassium persulfate (di-potassium peroxodisulfate) was purchased from Merck.

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay

Free-radical scavenging activities of ethyl acetate and methanolic fractions of the both saltcedars *T. boveana* and *S. fruticosa,* in addition to the compound A, isolated from *T. boveana* ethyl acetate fraction, were measured. The method of Choi et *al.,* (2002) was used, basing on hydrogen donating or radical scavenging ability, against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Different dilutions were prepared in triplicate. Then 0.5 mL of each dilution was added to 0.5 mL of DPPH 60 µM. The mixture was allowed to stand for 30 min at room temperature before measuring the absorbance at 520 nm. Inhibition was expressed as the

percentage of DPPH radicals decrease using the equation: Inhibition (%) = (1- A sample /A DPPH) * 100, where A sample was the absorbance of the fraction or the pure compound (dissolved in ethanol) read after 30 min, and A DPPH was the absorbance of equal volume of DPPH and ethanol read after 30 min.

IC50 values denoted the concentration of sample which was required to scavenge 50% of DPPH radicals (Fujita et *al.,* 1998). IC50 of the sample was determined from the graph of antioxidant activities (%) of the tested fractions (mg) or pure compound applied at different concentrations. Trolox® was used as a standard.

$ABTS^{\bullet+}$ scavenging assay

$ABTS^{\bullet+}$ scavenging assay was carried out following a modified method of Yu et *al.,* (2002). Radical cation was prepared by oxidizing 100 µL of ABTS commercial solution with 0.1 mg of $K_2S_2O_8$. The mixture was diluted in 100 mL of deionised water. ABTS solution was diluted with phosphate buffered saline (PBS, pH 7.4) to an absorbance of about 0.800 (+/- 0.050) at 734 nm. 10 µL of ethyl acetate and methanolic fractions of *T. boveana* and *S. fruticosa,* either to the isolated compound, were added at different concentrations to 990 µL $ABTS^{\bullet+}$ solution and the absorbance was noted after 5, 10, 15, 20 and 30 min of initial mixing at room temperature using PBS as blank.

The antioxidant activities were compared to Trolox equivalent content in 1mM sample.

Results were also expressed as Trolox® equivalent antioxidant capacity (TEAC) as described by Re et *al.,* (1999), measured after 30 min of the beginning of the reaction.

Determination of total phenol content

The total phenolic content of the fractions was determined spectrophotometrically, using the Folin-Ciocalteu method as described by Singleton and Rossi (1965), with some modifications. For this purpose, an aliquot of 100 µL of diluted fractions (1mg/ mL) were added to 750 µL Folin–Ciocalteu phenol reagent (diluted 10 times with distilled water), followed after 5 min by addition of 750 µL of saturated sodium carbonate solution (6%). The mixture was stirred and measured at 725 nm after staying 90 min in the dark. A blank sample consisting of water and reagents was used as a reference. Catechin was used as an analytical standard for total phenol quantification. The analysis was performed in triplicate and the contents of phenolic compounds in fractions were expressed as mg of Catechin equivalents per g of dry weight (mg CE/g DW).

Statistical analysis

All data on all bioassays were the averages of triplicate analyses. The data were recorded as means ± standard deviations. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by the Student's *t*-test, with *p* values of <0.05 being regarded as significant.

Results

Identification of the pure natural compound

Naringenin, $C_{15}H_{12}O_5$, was identified by comparison of its physical and spectral data with those previously reported (Breitmaier and Voelter, 1990; Fatope et *al.*, 2003). It gave the expected molecular ion at *m/z* 272 $[M]^+$ in the EIMS. Table 1 showed the ^1H and ^{13}C NMR spectral data characteristics of this compound. After the assignments of the UV, IR, 1D and 2D NMR spectral data, it was possible to establish that is a 4',5,7-trihydroxyflavanone.

Table 1. ^1H and ^{13}C NMR of naringenin

	δ_H (*J* in Hz)[a]	δ_C DEPT [b]
1		
2	5.35	79.1 CH
3	3.12	42.6 CH_2
	2.70	
4		196.4
5		163.5
6	5.88	95.6 CH
7		166.9
8	5.88	94.7 CH
9		164.1
10		101.9
1'		129.7
2'	7.31	127.6 CH
3'	6.82	114.9 CH
4'		157.6
5'	6.82	114.9 CH
6'	7.31	127.6 CH

[a] Assigned by ^1H-^1H COSY; [b] Protonated Carbons assigned by HSQC

Antioxidant activity

Various fractions obtained from the methanol extracts of *T. boveana* and *S. fruticosa* were tested for their free radical scavenging effects, additionally to a pure compound isolated from the ethyl acetate fraction of *T. boveana,* identified as naringenin.

Radical-scavenging activities on 1, 1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$)

Used as reagent, DPPH obviously offers a convenient, easy and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (Tominaga, 2005). The DPPH method (Choi, 2002) consists in the reaction of DPPH a stable free radical, which accepts an electron or hydrogen radical to become a stable molecule, and, accordingly, is reduced in presence of an antioxidant.

The scavenging abilities of *T. boveana* and *S. fruticosa* different fractions and naringenin the isolated compound, on DPPH radical were shown in Table 2.

Table 2. DPPH radical scavenging activities (%) of T. boveana and S. fruticosa fractions, naringenin, the isolated compound and Trolox, the standard (Mean[a] ± SD[b]).

Concentrations (mg/mL)	*T. boveana*		*S. fruticosa*		Naringenin	Trolox
	EtOAc	MeOH	EtOAc	MeOH		
0.15	90.01±0.03	90.07±0.00	34.81±0.01	75.12±0.00	0±0.01	94.7±0.02
0.31	97.16±0.00	100±0.00	66.44±0.03	95.28±0.01	60.76±0.03	94.84±0.02
0.62	93.44±0.00	100±0.00	87.88±0.02	91.17±0.01	82.01±0.02	94.91±0.01
1.25	96.72±0.00	95.71±0.00	86.63±0.01	94.17±0.00	89.10±0.01	95.39±0.00
2.50	100±0.00	100±0.00	80.63±0.00	87.19±0.00	89.78±0.03	95.32±0.02
5.00	100±0.00	100±0.00	100±0.00	93.13±0.00	90.46±0.01	94.98±0.04
10.00	99.84±0.04	99.94±0.00	100±0.00	90.87±0.05	90.60±0.02	94.45±0.06
IC_{50}	**0.08**	**0.08**	**0.23**	**0.10**	**0.28**	**0.07**

[a]Values are means of three replicate determinations; [b]SD: standard deviation

All fractions have shown strong scavenging activities on DPPH radicals. Additionally, the lower values of IC50 proved high antioxidant activities.

MeOH fractions of the both saltcedars *T. boveana* and *S. fruticosa,* as well as EtOAc fraction of *T. boveana,* exhibited a very power antioxidant profile (97.16, 100.00 and 95.28 % respectively) at a low concentration (310 µg/mL). EtOAc fraction of *S. fruticosa* scavenged DPPH radicals in a dose dependent manner. Indeed, 34.81% of DPPH$^{\bullet}$ was scavenged at 0.15 mg/mL, and reached 100.00 % at 5 mg/mL.

T. boveana showed a higher performance in scavenging radicals at low concentrations comparatively to *S. fruticosa.* 90.01 and 90.07 % of antioxidant activity were unregistered after application of EtOAc and MeOH fractions of *T. boveana* respectively, while only 34.81 and 75.12 % were noted after application of *S. fruticosa* ones.

The isolated natural compound, identified as naringenin, presented a strong antioxidant activity (82.01 %) at a low concentration (620 µg/mL), and increased according to concentrations rise. 90.60 % of scavenging free radicals was reached at a concentration of 10 mg/mL, showing the

importance of this compound as a powerful natural antioxidant. The value of the CI_{50} of naringenin was 282 µg/mL (Table 2).

$ABTS^{\bullet+}$ scavenging assay

In ABTS radical cation scavenging method, activities of the tested fractions and natural compound were expressed as percentages of free radical inhibitions as function of time. The TEAC assay is widely used to assess the total amount of radicals that can be scavenged by an antioxidant, i.e. the antioxidant capacity (Arts et *al.*, 2004). Thus, the antioxidant power was also converted to Trolox equivalents antioxidant activity (TEAC). The antioxidant activity results of the ethyl acetate and the methanolic fractions of both saltcedars *T. boveana* and *S. fruticosa* and naringenin, isolated from *T. boveana* ethyl acetate fraction, were elucidated in Table 3 and Trolox equivalents in Table 4.

The ethyl acetate fraction of *T. boveana* had an important antioxidant activity at 5 mg/mL (99.46 %) after 30 min of contact with $ABTS^{\bullet+}$.

This activity depended with time, indeed only 55.91% of radicals were scavenged after 5 min of application of this fraction and reached 99.46 % after 30 min. At 10 mg/mL, all radicals were inhibited (100 %) independently with time (Table 3).

Table 3. $ABTS^{\bullet+}$ cation radical scavenging capacity (%) of *T. boveana* and *S. fruticosa* fractions as a function of time and concentrations.

		mg/mL	5 min	10 min	15 min	20 min	30 min
Tamarix boveana	EtOAc	0.15	0.00± 0.01	0.00±0.01	0.00±0.04	0.00±0.03	0.00±0.06
		0.31	0.00±0.02	0.00±0.05	0.00±0.04	0.00±0.04	0.00±0.07
		0.62	0.00±0.04	0.00±0.05	0.00±0.06	0.00±0.05	2.69±0.08
		1.25	0.00±0. 05	0.00±00.10	0.00±0.07	0.00±0.06	0.00±0.08
		2.50	11.02±0.02	16.51±0.05	18.17±0.07	24.30±0.01	34.19±0.07
		5.00	55.91±0.09	82.90±0.00	93.12±0.03	95.38±0.04	99.46±0.01
		10.00	96.24±0.04	100.00±0.01	100.00±0.00	100.00±0.00	100.00±0.00
	MeOH	0.15	11.84±0.07	3.38±0.11	3.32±0.13	0.00±0.11	0.00±0.11
		0.31	54.59±0.04	45.41±0.09	44.44±0.07	33.13±0.16	30.07±0.10
		0.62	69.26±0.03	50.66±0.04	40.70±0.04	25.60±0.03	21.44±0.01
		1.25	69.20±0.03	49.19±0.04	39.73±0.06	27.21±0.05	15.38±0.09
		2.50	77.66±0.03	55.68±0.05	46.26±0.09	35.81±0.07	20.65±0.06
		5.00	79.32±0.04	61.51±0.03	46.77±0.07	36.04±0.02	22.38±0.04
		10.00	82.73±0.09	62.32±0.08	47.38±0.11	39.21±0.10	25.20±0.10
Suaeda fruticosa	EtOAc	0.15	0.00±0.03	0.00±0.02	0.00±0.01	0.86±0.01	1.07±0.02
		0.31	0.62±0.02	1.36±0.02	2.34±0.02	3.29±0.02	4.19±0.03
		0.62	10.11±0.02	10.40±0.04	13.36±0.04	20.09±0.03	19.52±0.04
		1.25	26.51±0.08	32.51±0.09	36.62±0.09	38.96±0.08	42.50±0.08
		2.50	35.39±0.08	40.16±0.09	41.96±0.06	44.60±0.09	50.64±0.06
		5.00	54.95±0.01	61.45±0.01	65.68±0.03	67.88±0.00	68.61±0.01
		10.00	63.95±0.06	67.90±0.02	69.01±0.02	68.72±0.01	70.15±0.02
	MeOH	0.15	0.00±0.02	3.90±0.01	4.03±0.02	5.47±0.02	5.26±0.02
		0.31	8.34±0.02	10.40±0.03	12.29±0.02	10.89±0.03	12.95±0.03
		0.62	11.43±0.02	16.19±0.02	19.19±0.02	20.43±0.03	22.19±0.04
		1.25	14.14±0.07	17.51±0.08	21.41±0.09	19.24±0.07	24.91±0.02
		2.50	29.92±0.05	41.43±0.07	47.72±0.09	53.64±0.07	59.56±0.05
		5.00	57.83±0.04	68.68±0.02	70.05±0.01	71.37±0.00	71.68±±0.01
		10.00	70.49±0.02	70.12±0.03	71.06±0.01	73.80±0.01	74.02±0.01

Table 4. Radical cation scavenging activity of *T. boveana* and *S. fruticosa* different fractions and naringenin expressed as Trolox equivalent after 30 min of initial mixing.

TEAC		Concentrations (mg/mL)						
		0.15	0.31	0.62	1.25	2.50	5.00	10.00
T. boveana	**EtOAc**	0.01	0.01	0.08	0.01	0.83	2.39	2.41
	MeOH	0.01	0.73	0.53	0.38	0.51	0.55	0.62
S. fruticosa	**EtOAc**	0.04	0.11	0.48	1.03	1.22	1.70	1.66
	MeOH	0.14	0.32	0.54	0.61	1.44	1.80	1.71
Naringenin		1.41	1.83	1.78	1.79	1.91	1.88	1.91

On the other hand, the ethyl acetate fraction of *S. fruticosa* seemed to be more active against ABTS cation radicals at a lower concentration, where 50.64 % of radicals were scavenged at 2.5 mg/mL. This antioxidant activity was however limited to 68.72 and 70.15 % at 5 and 10 mg/mL respectively after 30 min of application of the ethyl acetate fraction.

The methanolic fraction of *T. boveana* appeared active even at feeble concentrations, indeed 54.58 and 69.26 % of $ABTS^{\bullet+}$ were scavenged at 310 and 620 µg/mL respectively after 5 min of its application. This antioxidant activity increased at the higher tested concentration (10 mg/mL) and reached 82.73%. It seemed that the antioxidant activity unregistered for the methanolic fraction of *T. boveana* depended strongly with the duration of reaction. Indeed 82.73 % of scavenging radicals unregistered at 5 min became only 25.20% at 30 min. Power of this antioxidant activity was limited then to an earlier time (Table 3).

The antioxidant activity of *S. fruticosa* methanolic fraction became interesting at a concentration of 2.5 mg/mL (59.55 % after 30 min) and reach 74.02 at 10 mg/mL (after the same time).

Naringenin, isolated from the ethyl acetate fraction of *T. boveana*, exhibited a strong scavenging efficiency toward ABTS cation radicals from the lower tested concentration (Fig. 1). In this fact, 76.12 % of $ABTS^{\bullet}$+ were scavenged at 150 µg/mL after 30 min of contact. The maximum of naringenin activity was reached at 310 µg/mL and steel constant until 10 mg/mL. The IC50 of naringenin was 282 µg/mL.

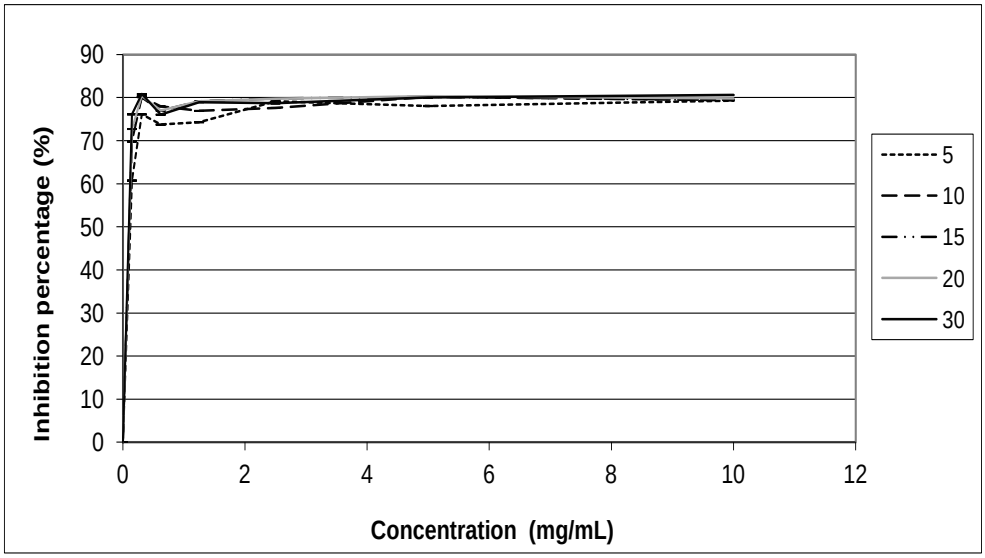

Figure 1. ABTS$^{\bullet+}$ cation radical scavenging capacity (%) of naringenin, the isolated compound from the ethyl acetate fraction of *T. boveana*, as a function of time (5 – 30 min) and concentrations.

The antioxidant activity of *T. boveana* methanolic fraction was the nearest to Trolox's one when applied at the majority tested concentrations, comparatively to the other tested fractions. The ethyl acetate fraction activity of *T. boveana* was the most important comparatively to Trolox activity at 10 mg/mL reaching 96.23 %, while the unregistered activity of Trolox was 99.83 % (Fig. 2).

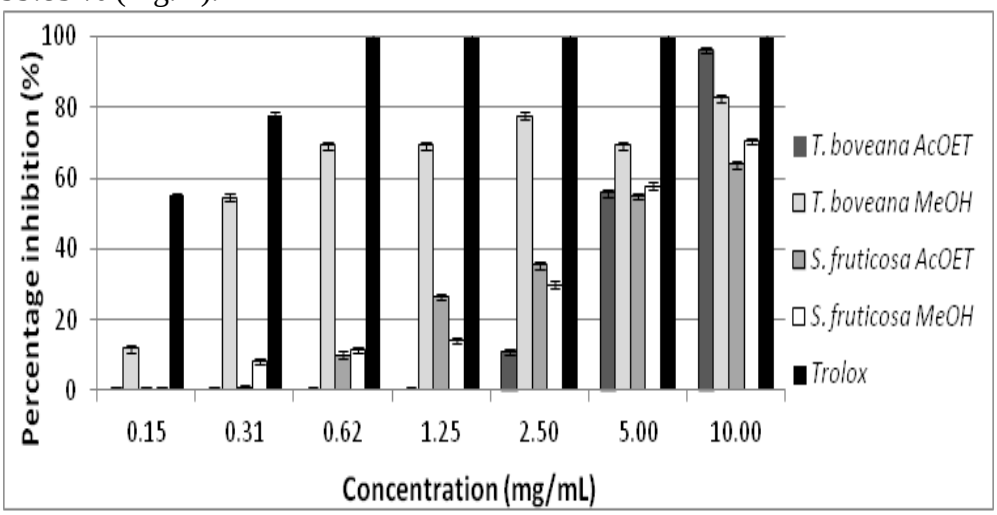

Figure 2. ABTS$^{\bullet+}$ cation radical scavenging capacity of *T. boveana* and *S. fruticosa* fractions, after 5 min of contact comparatively to Trolox.

The histograms presented in figure 3 showed a higher performance of naringenin comparatively to Trolox at the lower tested concentration (150 µg/mL). Naringenin exhibited an antioxidant activity of 60.76 %, while Trolox activity was 55 %. Trolox and naringenin antioxidant activities were similar at 310 µg/mL (about 78 %), but a better performance of Trolox at the higher tested concentrations (0.62 - 10mg/mL) was noted.

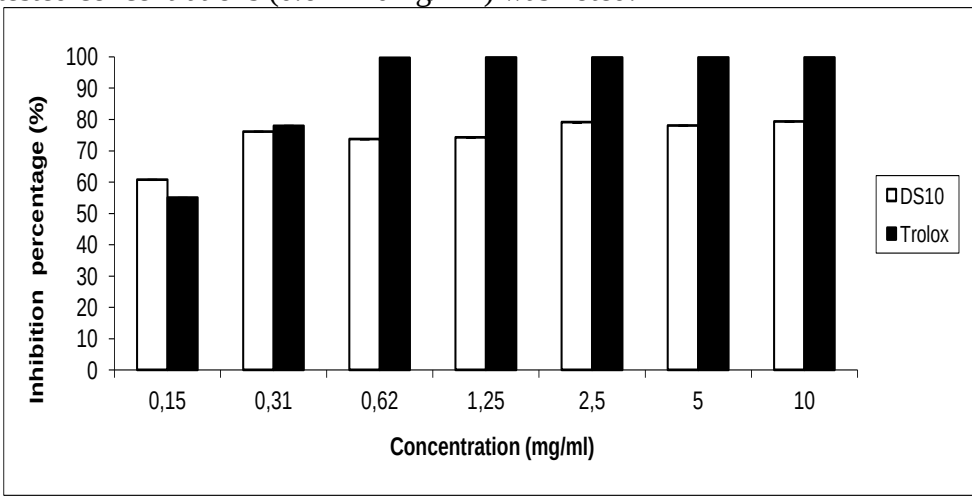

Figure 3. ABTS$^{\bullet+}$ cation radical scavenging capacity of naringenin, the isolated compound from the ethyl acetate fraction of *T. boveana*, after 5 min of contact comparatively to Trolox.

The TEAC of each fraction after 30 min of the reaction initiation was determined and plotted. Since TEAC is a measurement of the effective antioxidant activity of the fraction, a higher TEAC would imply greater antioxidant activity of the sample. It was observed that methanolic fraction of *T. boveana* had the highest TEAC of 2.41 mM, when applied at 10 mg/mL (Table 4). The TEAC values of the other fractions at this concentration ranged from 0.62 to 1.71 mM.

Comparatively to the tested fractions, Naringenin, isolated from the ethyl acetate fraction of *T. boveana*, showed important TEAC values, especially at the feeble tested concentrations.

The total phenolics (mg/g) of the aerial parts of *T. boveana* and *S. fruticosa* were determined by the Folin–Ciocalteu method from regression equation of calibration curve (y=4,2086x -0,001, R^2=0.9998) and expressed in catechin equivalents (CE). The content of phenolic compounds varied as function of the used solvent, closely dependent on the solvent polarity (Fig. 4).

The extraction with pure methanol showed the highest polyphenol content, reaching 237.53 and 259.63 mg CE/g DW for the methanolic fractions of *T. boveana* and *S. fruticosa* respectively.

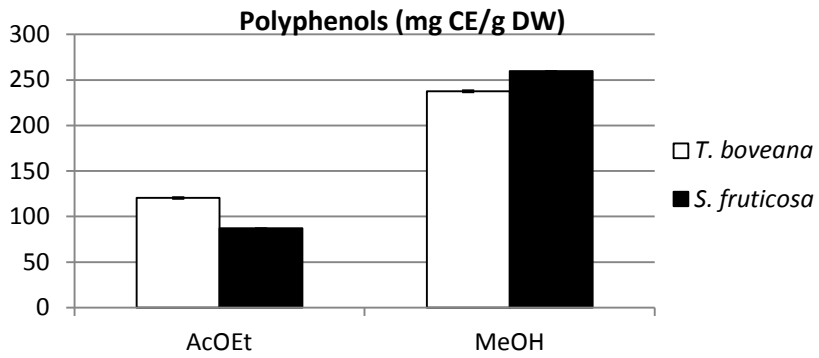

Figure 4. Total phenolic contents of *T. boveana* and *S. fruticosa* fractions (mg CE/g DW)

Correlation between the antioxidant activities (TEAC values) and total phenolic contents of the various tested fractions was studied and presented in figure 5. The correlation coefficient appeared very week in the case of the ethyl acetate and the methanolic fractions of both *T. boveana* and *S. fruticosa* (0.03) (Fig. 5a), but became very important and reached 0.95 when the case of *S. fruticosa* ethyl acetate fraction was eliminated (Fig. 5b).

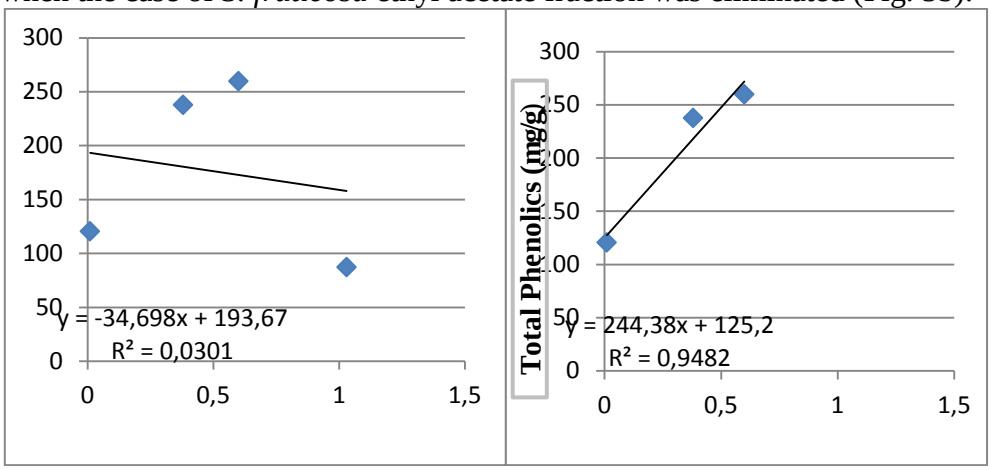

Figure 5. Correlation coefficient between total polyphenols and TEAC values, in the case of the methanol and the ethyl acetate fractions of both *T. boveana* and *S. fruticosa* (a), and in the case of the methanol and the ethyl acetate fractions of *T. boveana* and the methanol fraction of *S. fruticosa* (b).

Discussion

Interestingly, all tested samples from *T. boveana* and *S. fruticosa* exhibited high scavenging efficiencies toward DPPH and $ABTS^{+}$ radicals, reaching 100 % of inhibition.

Several species of the genus *Tamarix* appear active towards radicals. Indeed, the 2-AAF (2-acetyl aminofluorene) is a mediator of enzymatic anti-oxidation activities decrease, lipids peroxidation and formation of hydrogen

peroxide, favoring consequently liver carcinogenesis. The methanolic extract of *T. gallica* inhibited the oxidative damage caused by 2-AAF, by activating the hepatic anti-oxidizing enzymes of defense (Sehrawat and Sultana 2006). As well, *T. ramosissima* showed a significant antioxidant activity, where all the tested fractions and pure products seemed to be active (Sultanova et *al.*, 2001).

The 3α-(3″,4″-dihydroxy-trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid, isolated from the aerial part of *T. hispida*, seemed to be an excellent antioxidant compound (Parmar et *al.*, 1994).

Suaeda fruticosa is a member of the Chenopodiaceae, which belongs to the order Caryophyllales, Subclass Caryophyllidae. In Caryophyllales species, the red pigments intensified in high salinity correspond to betacyanins. These pigments are synthesized from tyrosine and constitute a class of secondary metabolites in these species (Clement and Mabry, 1996). Betacyanins together with betaxanthins (yellow pigments), are a group of chromoalkaloids known as betalains. Betalains have been used as natural additives for food, drugs and cosmetic products, and they are free radical scavengers and prevent active oxygen-induced and free radical-mediated oxidation of biological molecules (Chang-Quan et *al.*, 2007). *Suaeda fruticosa* betacyanin was identified as the citryl celosianin (Piattelli and Imperato, 1971).

The antioxidant activities in all investigated samples seemed to be proportional to the type of radical, the applied amount and the time of contact. The present study showed superiority of $DPPH^{\bullet}$ assay over $ABTS^{\bullet+}$. Indeed, 90 % of $DPPH^{\bullet}$ was scavenged by ethyl acetate and methanolic fractions of *T. boveana* at 150 µg/mL. While no antioxidant activity was noted against $ABTS^{\bullet+}$ when these fractions were applied at the same concentration. According to Aruoma (2003), more than one method of antioxidant testing should be employed to gain a fully comprehensive indication of antioxidant efficacy of test substances.

Naringenin, isolated from the ethyl acetate fraction of *T. boveana*, showed a powerful scavenging competence toward DPPH and $ABTS^{+}$ radicals reaching 90.60 and 80.60 % respectively at 10 mg/mL. At 310 µg/mL, 60.76 % of $DPPH^{\bullet}$ and 76.12 % of $ABTS^{\bullet+}$ were scavenged, demonstrating that even at a low concentration the unregistered antioxidant activity of naringenin steeled important and seemed to be comparable to that of Trolox especially at 150 and 310 µg/mL. The centres responsible for the antioxidant activity were: three hydrogen of the phenol groupings (OH), both hydrogen of the carbon 3 situated in α of the carbonyl group C=O, benzyl hydrogen of the carbon 2, as well as three benzyl hydrogen of the group CH_3 fixed to the atom 6'.

Furthermore, naringenin is qualified by its antioxidant properties, by

favoring the chelating of metals (Renugadevi and Prabu, 2010). Reactive oxygen species can prevent apoptosis by the inhibition of caspase-3, a marker of the apoptosis execution. The antioxidant action of naringenin can knock down this process and favor apoptosis.

Various other efficacies of naringenin has been reported to prevent gastric mucosal ulceration in animal models (Motilva et *al.,* 1994) and to act as a selective inhibitor of 5-lipoxygenase. Besides, naringenin could be an ideal therapeutic agent in the treatment of both cancer and fibrosis (Jin et *al.,* 2009).

Furthermore, this flavonoid compound suppresses hepatic glucose and then plays an important role in the hyperglycemia attenuation (Purushotham et *al.,* 2009). In the previous reports, supplemented diets with naringenin inhibited the experimentally induced atherosclerosis and hypercholesterolemia, which is a major risk factor of coronary heart disease (Jeon et *al.,* 2007).

All the inhibition percentages unregistered for the ethyl acetate fraction of *T. boveana* toward $DPPH^{\bullet}$, at different tested concentrations, were more important to those noted for naringenin, the purified compound from the cited fraction. Additionally, at 150 µg/mL, 90.01 % of DPPH radical scavenging was unregistered for the ethyl acetate fraction of *T. boveana*, contra 0 % for naringenin.

This result induced us to deduce the existence of other compounds than naringenin, which play an important role in the unregistered antiradical activity of the whole fraction. The ethyl acetate fraction of *T. boveana* contained then several compounds aging synergistically in the noted antiradical activity.

Contrarily, Naringenin showed an important antioxidant activity toward $ABTS^{+}$ radicals at feeble concentrations (0.15 - 2.5 mg/mL), while no activity was noted for the ethyl acetate fraction of *T. boveana* against these radicals. This result demonstrated that there are in the ethyl acetate fraction other compounds which inhibit naringenin activity. At high concentrations (5 and 10 mg/mL), this inhibition was blocked and naringenin get back its activity. Other compounds were also responsible for a share of the total activity noted at these concentrations, as known the activity of the ethyl acetate fraction was more important than the activity of naringenin, which is one of its compounds.

In fact, the interaction effects of diverse constituents present in the ethyl acetate fraction of *T. boveana* should be taken into consideration to account for its antioxidant activity.

The methanolic fractions of *T. boveana* and *S. fruticosa* showed the highest polyphenol content. Several studies showed that solvent natures, notably polarity, have significantly different extraction capacities for

phenolic compounds in plants (Parida et *al.,* 2004). Moreover, soil salinity may attribute a major influence on phenolic biosynthesis, and consequently a better antioxidant activity. In agreement with our findings, Parida et *al.* (2004) showed that polyphenol content increased significantly in *Aegiceras corniculatum* plants challenged with 250 mM NaCl.

In our study, in the case of methanolic fractions of both *T. boveana* and *S. fruticosa* and ethyl acetate fractions of *T. boveana*, the correlation coefficient between total polyphenols and TEAC values of the $ABTS^{\bullet+}$ quenching activity was highly significant (0.95), indicating that polyphenolics may play an important role in free radical scavenging activities of these fractions (Anagnostopoulou et *al.,* 2006). When we added the case of *S. fruticosa* ethyl acetate fraction, the correlation decrease significantly and reach 0.03, which indicated an unclear relationship between the antioxidant activity of this fraction and the total phenolics. Djeridane et *al.* (2006) explained this unclear relationship in several ways: the total phenolic fraction does not incorporate all the antioxidants, and synergistic interactions between the antioxidants in the mixture make the antioxidant activity not only dependent on the concentration, but also on the structure and the nature of the antioxidants.

Other literature reports also claimed that there is a low statistical correlation between the antioxidant activity and phenolic components. This can be explained as that different flavonoid and non-flavonoid subgroups exhibit various antioxidant activities (Di Majo et *al.,* 2008).

Conclusion

Naringenin, the natural compound isolated from the ethyl acetate fraction of *T. boveana*, exhibited a strong antioxidant activity by quenching the majority of DPPH and $ABTS^{+}$ radicals. The importance of this compound as active and effective principle in the treatment of several serious illnesses increases even more the therapeutic interest of the promising halophyte *T. boveana* and proves the utility of naringenin as natural antioxidant additive in foods. These results must encourage further to test naringenin in other biological and pharmacological assays and to isolate other substances potentially active compounds from this species and also from *Suaeda fruticosa* which showed a lot of interests in diverse economic and therapeutic domains.

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