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Phenolic contents and antioxidant activities *in vitro* of some selected Algerian plants

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In order to find new sources of natural antioxidants, the methanolic extracts of eleven Algerian medicinal plants from eight botanical families were investigated for their *in vitro* antioxidant activity using total antioxidant capacity (TAC), reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical scavenging and β-carotene–linoleate bleaching. The total phenolic, flavonoid and proanthocyanidin contents were also measured. Most of these plants were analyzed for the first time for their antioxidant activities. Results showed that the plants *Pistacia atlantica*, *Thymelaea microphylla* and *Marubium deserti* exhibited higher phenolic content (133.74 to 285.95 mg gallic acid equivalents/g dry matter). The flavonoid and proanthocyanidin contents varied from 0.56 to 12.44 mg catechin equivalents/g dry matter and 1.42 to 25.02 mg catechin equivalents/g dry matter, respectively. The extracts were found to have different levels of antioxidant properties in the test models used. The medicinal plants with the highest antioxidant activities were *P. atlantica*, *Helianthemum lippii* (stem, leaf, and fruit), *Inula montana, Anabasis articulata* and *Sedum villosum*. A positive correlation, R^2 = 0.906, was observed between total flavonoid contents and TAC values, reflecting a high involvement of flavonoids in antioxidant activity, but no correlation was established between the five tests and the total phenolic and proanthocyanidin contents.

Key words: Antioxidant properties, *in vitro* tests, phenolics, flavonoids, proanthocyanidin, Algerian plants.

INTRODUCTION

Currently, the scientific society (biologists and chemists alike) highlights the tragic role of the uncontrollable oxidative processes, induced by the reactive oxygen species (ROS) formed *in vivo.* These oxidants are directly responsible for various pathological states of degenerative diseases such as atherosclerosis, coronary heart diseases, aging, cancer (Finkel and Holbrook, 2000), and other disorders, for example, Alzheimer and Parkinson's diseases, Down's syndrome, inflammation, viral infection, autoimmune pathology and digestive ulcers (Atawodi, 2005). ROS are indirectly implicated in lipid peroxidation in foodstuffs. Whatever the case, the risk is worsened as

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risk is worsened as these molecules accumulate in the human body, leading to a radical chain reaction which degrades vital biological molecules such as DNA, proteins, sugars and membrane lipids; this results in cell and tissue damages (Abdi and Ali, 1999).

Actually, natural antioxidants are the subject of much research and a new breath towards the exploitation of polyphenols in health and pernicious diseases (cancer), as well as in food industry in order to lengthen the shelf life of food products by reducing the harmful substances formed (Han et al., 2008). These compounds include flavonoids which are required for their biological properties, that is antioxidant, anti-inflammatory, anti-allergic and anticarcinogenic. It is worth noting the powerful effectiveness of these substances to stop the reactions, which generate the radical species and neutralize the reactive oxygen species, by one or more of the mechanisms like reducing the activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen (Ali et al., 2008). All these activities are mainly due to their phenolic structures with the presence of hydroxyl groups. In recent years, there has been a growth of interest in natural antioxidants, whence the number of commercially available drugs derived from plant sources is increasing.

The aim of this study was to evaluate, for the first time, the *in vitro* antioxidant properties of eleven medicinal plants from Algeria, using different methods, including the total antioxidant capacity, reducing power, β-carotene bleaching test and radical scavenging activities against DPPH and hydroxyl radicals. We also wanted to determine their total phenolic compounds, flavonoid and proanthocyanidins and investigate the relationship between these contents and the antioxidant activity.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu, sodium carbonate, sodium nitrate, aluminium chloride, sodium hydroxide,vanillin, sulfuric acid, sodium phosphate, ammonium molybdate, phosphate buffer, potassium ferricyanide, trichloracetic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, 3-t-butyl-4-hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, hydrochloric acid, thiobarbutiric acid, ethylenediaminetetraacetic acid (EDTA), iron (III) chloride ($FeCl_3$), β-carotene, linoleic acid and tween 40 were obtained from Sigma-Aldrich Chemie (Germany). Gallic acid, catechin, ascorbic acid, chloroform and methanol were from Merck (Darmstadt, Germany).

Sample preparation and extraction

The plant materials were collected from different localities of Algeria based on the information provided in the ethnobotanical survey and their abundance in nature. For each plant, the various data (scientific name, family, used organs, original habitat location and harvest period) are given in Table 1. The collected plants were identified by the Vegetable Ecological Laboratory, University of Tlemcen, Algeria. Voucher specimens for each plant have been deposited in the Herbarium of the Biology Department, University of Tlemcen, Algeria. One gram of plant material was ground to fine powder and extracted with 20 ml of methanol at room temperature for 48 h. After filtration through Whatman no. 0.45 filter paper, the solvent was evaporated under vacuum at 60°C. The residue was weighed and dissolved in methanol for further analysis. Eventually, the solutions were stored at -20°C (Benhammou et al., 2009).

Determination of total phenolic content

The total phenolic content of methanolic extracts was determined by spectrometry using Folin-Ciocalteu reagent assay (Singleton and Rossi, 1965). A volume of 200 µl of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 10-fold in water) and 0.8 ml of a 7.5% sodium carbonate solution in a test tube. The absorbance was read at 765 nm after 30 min on a Jenway 6405 UV-vis spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM) through the calibration curve with gallic acid ($y = 2.916x$, $R^2 = 0.997$). All the tests were carried out in triplicate.

Estimation of total flavanoid content

Total flavonoid content was determined by a colorimetric assay using a method described by Zhishen et al. (1999). Briefly, 500 µl of catechin standard solution with different concentrations or methanolic extracts was mixed with 1500 µl of distilled water in a test tube, followed by addition of 150 µl of a 5% (w/v) sodium nitrate solution at time zero. After 5 min, 150 µl of aluminium chloride solution to 10% (m/v) was added. After the incubation of 6 min at the ambient temperature, 500 µl of sodium hydroxide (1 M) was added. Immediately, the mixture was completely agitated in order to homogenize the contents. The absorbance was read at 510 nm and concentrations of flavonoids were deduced from a standard curve ($y = 5.140x$, $R^2 = 0.991$) and calculated in mg catechin equivalent (CE)/g dry matter (DM). Data are the mean ± SD results of triplicate analyses.

Quantification of proanthocyanidins

Proanthocyanidins were measured using the vanillin assay described by Julkunen-Titto (1985). To 50 µl of methanolic extract, 1500 µl of vanillin/methanol solution (4%, w/v) was added and the contents mixed. Then, 750 µl of concentrated hydrochloric acid was added and allowed to react at room temperature for 20 min. The absorbance at 550 nm was measured against a blank. The amount of proanthocyanidins was expressed as milligrams of catechin equivalents per gram of dry matter (mg CE/g DM) from the calibration curve ($y = 0.116x$, $R^2 = 0.996$). All the tests were carried out in triplicate.

Total antioxidant capacity

The total antioxidant capacity (TAC) of the plant extracts was evaluated by the phosphomolybdenum method of Prieto et al. (1999). An aliquot (0.3 ml) of plant extract was combined to 3 ml of

Table 1. Botanical (scientific name, family) data and harvest characteristics (location, period and plant parts) of eleven plants studies.

Botanical name	Family	Site of collection	Harvest period	Plant parts
Anabasis articulata Moq	Chenopodiaceae	Bechar	November, 2007	S
Atriplex halimus L	Chenopodiaceae	Bechar	April, 2008	S/L
Cotula cinerea Del	Asteraceae	Adrar	December, 2007	FP
Helianthemum lippii (L) Pers	Cistaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L/F
Inula montana L	Asteraceae	Oum El Alou (Tlemcen)	November, 2008	FP
Marrubium deserti De Noé	Lamiaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L
Pentzia monodiana Maire	Asteraceae	Ladjdar	November, 2007	FP
Pistacia atlantica Desf	Anacardiaceae	Aïn Fezza (Tlemcen)	May, 2007	F
Sedum villosum L	Crassulaceae	Woued Al Akhdar (Tlemcen)	April, 2007	FP
Thymelaea microphylla Coss et Dur	Thymelaeaceae	Aïn Ben Khelil (Naâma)	May, 2007	S/L
Zygophylum album L	Zygophyllaceae	Adrar	December, 2007	S

Stem: S; Leaf: L; Fruit: F; Flower part: FP.

the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. Later, the samples were allowed to cool to room temperature. The absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalents per gram of dry matter (mg AAE/g DM) from the calibration curve ($y = 4.671x$, $R^2 = 0.9869$).

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (mg/ml) in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 ml, $K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. IC_{50} value (mg/ml) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

DPPH free radical scavenging activity

The free radical scavenging activity was measured by a modified DPPH$^{\bullet}$ assay (Sanchez-Moreno et al., 1998). A solution of the extract prepared as describe above (50 µl) was added to 1950 µl of methanolic DPPH solution (0.025 g/L). The decreasing absorbance at 515 nm was monitored in order to reach constant values. The DPPH$^{\bullet}$ concentration in the reaction medium was calculated from the following calibration curve determined by linear regression:

$A_{515nm} = 24.41 \times [DPPH^{\bullet}]t + 0.0022$ $R^2 = 0.999$

Where $[DPPH^{\bullet}]t$ was expressed as mg/ml at *t* time.

The percentage of the remaining DPPH$^{\bullet}$ (% $DPPH^{\bullet}_{REM}$) at the steady state was calculated as follows:

$\%\ DPPH^{\bullet}_{REM} = 100 \times [DPPH^{\bullet}]_t / [DPPH^{\bullet}]_{t=0}$

Where $[DPPH^{\bullet}]t = 0$ and $[DPPH^{\bullet}]t$ are concentrations of DPPH$^{\bullet}$ at t=0 and t= t, respectively.

Using various antioxidant concentrations, it was possible to determine the amount of antioxidant necessary to halve the initial DPPH$^{\bullet}$ concentration (EC_{50}). EC_{50} is expressed in mg of dry extract per g of DPPH. The time needed to reach the EC_{50} concentration, noted T_{EC50}, was graphically determined. The antiradical efficiency (AE) was calculated as follows:

$AE = 1 / (EC_{50} \times T_{EC50})$

Hydroxyl radical scavenging assay

The effect of extracts on hydroxyl radicals was assayed by using the deoxyribose method (Halliwell et al., 1987). The reaction mixture contains the following reagents: 0.4 ml of phosphate buffer saline (50 mmol/L, pH 7.4), 0.1 ml of extracts solution, 0.1 ml of EDTA (1.04 mmol/L), 0.1 ml of $FeCl_3$ (1 mmol/L) and 0.1 ml of 2-deoxyribose (60 mmol/L). The reaction was started by the addition of 0.1 ml of ascorbic acid (2 mmol/L) and 0.1 ml of H_2O_2 (10 mmol/L). After incubation at 37°C for 1 h, the reaction was stopped by adding 1 ml of thiobarbutiric acid (TBA) 10 g/L follow-up by 1 ml of hydrochloric acid (HCl) (25%), then heating the tubes in a boiling water bath for 15 min. The contents were cooled and absorbance of the mixture was measured at 532 nm against reagent blank. Decreased absorbance of the reaction mixture indicates decreased oxidation of deoxyribose. The percentage inhibition of deoxyribose oxidation was calculated using the following equation:

Hydroxyl radical scavenging activity (%) $= [A_0 - (A_1 - A_2)] \times 100 / A_0$

Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract and the deoxyribose, A_2 was the absorbance in the presence of the extract without deoxyribose.

β-Carotene bleaching method

The antioxidant activity of methanolic extracts was evaluated using β-carotene-linoleate model system, as described by Moure et al. (2000). Two milligrams of β-carotene were dissolved in 10 ml chloroform and 1 ml β-carotene solution was mixed with 20 µl of purified linoleic acid and 200 mg of Tween 40 emulsifier. After evaporation of chloroform under vacuum, oxygenated distilled water (100 ml) were added by vigorous shaking. To an aliquot of 4 ml of this emulsion, 200 µl of extracts or the BHA were added and mixed well. The absorbance at 470 nm, which was regarded as t = 0 min, was measured immediately, against a blank consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50°C for a period of 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (A_{120}). For the positive control, sample was replaced with BHA. A negative control consisted of 200 µl methanol instead of extract or BHA. The antioxidant activity (AA) was calculated according to the following equation:

$$AA = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)}] \times 100$$

Were $A_{A(120)}$ is the absorbance of the sample at t = 120 min; $A_{C(120)}$ is the absorbance of the control at t = 120 min and $A_{C(0)}$ is the absorbance of the control at t = 0 min.

Statistical analysis

The results were analyzed using the Microcal Origin 6. All the data are expressed as means ± standard deviation (n = 3).

RESULTS

Extraction yields - total phenolic compounds, flavonoid and proanthocyanidins contents

The yield extracted from different parts of Algerian plants is reported in Table 2. In this experiment, the yields of extracts ranged from 3.43 to 33.43%. The highest extract yield was obtained by PAF extract, followed by *Atriplex halimus* (AHL) (24%) while the lower yield was recorded in *Helianthemum lippii* (HLS) (3.43%). These yields were higher in leaves compared to the other parts of the plant. The total phenolic contents of 11 medicinal plants were measured using the Folin-Ciocalteu method. The results were shown in Table 2. There is a large variation in total phenolic content of the plant species examined. The values varied from 3.77 ± 0.06 to 285.95 ± 10.25 mg GAE/g DM. The highest total phenolic content was observed in *Pistacia atlantica* (PAF), followed by the leaves of *Thymelaea microphylla* (TML) (257.40 mg/g) and *Marrubium deserti* (MDL) (235.18 mg/g) and their stems (201.64 and 133.74 mg/g, respectively). The total flavonoids and proanthocyanidins of the plant extracts were also measured (Table 2). For flavonoid content, the values ranged from 0.56 ± 0.03 mg CE/g for *Atriplex halimus* (AHS) to 12.44 ± 0.25 mg CE/g for PAF, whereas the levels of proanthocyanidins varied from 1.42 ± 0.17 to 25.02 ± 3.44 mg CE/g.

Assessment of antioxidant activities

In this study, we combined five complementary assays: TAC, reducing power, scavenging activity on DPPH and hydroxyl radicals and inhibition of β-carotene bleaching to evaluate the antioxidant activities of selected plant extracts.

Total antioxidant capacity (TAC)

The TAC of plant extracts was expressed as ascorbic acid equivalents. The phosphomolybdenum method 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 at acid pH. The PAF showed the highest TAC (45.51 ± 1.63 mg AAE/g DM) while the lowest capacity was found in AHS (1.64 ± 0.80 mg AAE/g DM). For the other extracts, the values were different, ranging from 6.14 ± 0.10 to 20.46 ± 0.06 mg AAE/g DM (Table 2).

Reducing power

The reducing properties are generally associated with the presence of reductones, which have capacity to donate an electron to free radicals and convert them into more stable. In Figure 1, all the extracts showed some degree of electron-donating capacity in a linear concentration-dependent manner. The best total reduction capability was observed for PAF extract (Abs $_{700nm}$ 0.88 at 0.25 mg/ml). All other extracts showed weak activities compared to the ascorbic acid (Abs $_{700nm}$ 0.81 at 0.1 mg/ml). As shown in Table 3, PAF extract possessed the strongest reducing power (0.13 ± 0.001 mg/ml), followed by HLF (0.25 ± 0.004 mg/ml), HLS (0.35 ± 0.009 mg/ml), SVFP (0.42 ± 0.004 mg/ml) and HLL extracts (0.47 ± 0.009 mg/ml). IC_{50} values of other extracts ranged from 0.52 ± 0.007 mg/ml for AAS to 4.56 ± 0.79 mg/ml for AHL. However, the ascorbic acid (0.06 ± 0.002 mg/ml) required to reduce the ferric iron was lower than the other phenolic extracts, indicating superior activity. The

Table 2. Extract yield, total phenolic (as gallic acid equivalents), total flavonoids (as catechin equivalents) and proanthocyanidins contents (as catechin equivalents) in investigated plants expressed in mg/g of dry matter.

Sample	Yield (%)	Total phenolics (mg GAE/g DM)	Flavonoids (mg CE/g DM)	Proanthocyanidins (mg CE/g DM)	TAC (mg AAE/g DM)
PAF	33.43 ± 2.70	285.95 ± 10.25	12.44 ± 0.25	3.06 ± 0.15	45.51 ± 1.63
TML	9.53 ± 2.34	257.40 ± 89.33	4.18 ± 0.04	6.91 ± 0.44	14.56 ± 0.69
TMS	7.45 ± 0.63	201.64 ± 4.65	2.98 ± 0.13	4.13 ± 0.31	16.85 ± 0.80
MDL	10.88 ± 0.17	235.18 ± 6.11	5.87 ± 0.20	25.02 ± 3.44	20.46 ± 0.06
MDS	7.18 ± 1.04	133.74 ± 27.35	3.17 ± 0.12	13.94 ± 1.37	13.41 ± 0.28
HLL	10.45 ± 0.71	60.95 ± 2.55	4.18 ± 0.20	10.37 ± 1.08	13.30 ± 0.59
HLF	9.20 ± 0.69	74.21 ± 1.18	3.57 ± 0.15	4.67 ± 0.34	17.41 ± 2.54
HLS	3.43 ± 0.56	46.09 ± 6.45	1.67 ± 0.02	9.29 ± 1.39	7.72 ± 0.39
AHL	24 ± 1.41	10.12 ± 2.24	2.48 ± 0.01	9.11 ± 0.68	11.51 ± 0.60
AHS	7.5 ± 0.70	3.77 ± 0.06	0.56 ± 0.03	1.42 ± 0.17	1.64 ± 0.80
AAS	9.36 ± 2.66	43.14 ± 0.63	4.85 ± 0.21	7.26 ± 0.36	13.99 ± 0.62
ZAS	14.30 ± 3.81	6.92 ± 0.63	1.61 ± 0.02	4.35 ± 0.57	6.14 ± 0.10
IMFP	10.19 ± 1.80	20.24 ± 1.06	6.52 ± 0.11	9.29 ± 0.69	18.99 ± 0.44
CCFP	15.79 ± 4.54	22.22 ± 0.41	3.93 ± 0.06	8.61 ± 0.18	17.19 ± 1.27
PMFP	6.93 ± 2.29	9.87 ± 0.82	2.29 ± 0.04	4.48 ± 0.47	10.45 ± 0.15
SVFP	6.76 ± 0.73	27.09 ± 0.97	2.96 ± 0.13	7.52 ± 1.87	13.01 ± 0.67

PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract. Values were the means of three replicates ± standard deviation (SD).

efficiency of iron reduction is inversely proportional to the IC_{50} value; it is in the ascending order, according to the following ranking: AA > PAF > HLF > HLS > SVFP > HLL > AAS> IMFP > MDS > TML > CCFP = TMS > AHS > PMFP > ZAS > AHL.

DPPH free radical scavenging activity

The scavenging effect of phenolic extracts on the DPPH radical expressed as EC_{50} values varied widely from 47.39 to 6310.04 mg antioxidant/g DPPH (Table 3). PAF was most efficient, with the lowest EC_{50} value of 47.39 mg/g, followed by HLF (66.28 mg/g), IMFP (66.96 mg/g), HLS (97.73 mg/g) and SVFP (99.02 mg/g). In contrast, ZAS has the lowest activity. However, the DPPH radical scavenging activity of different extracts was inferior to that of ascorbic acid (39.5 mg/g) and BHT (13.47 mg/g). To classify the antioxidant capacities of the various extracts, we used the antiradical efficiency (AE), a new parameter which involves the potency ($1/EC_{50}$) and the reaction time (T_{EC50}) (Sanchez-Moreno et al., 1998). The lower the EC_{50}, the lower the T_{EC50} and the higher the AE. As shown in Table 2, the ascorbic acid (4174.46×10^{-5}) was more powerful antioxidant than the investigated extracts, trolox (260.43×10^{-5}) and BHT (45.97×10^{-5}). The classification order of AE for the tested antioxidant was: AA > Trolox > BHT > MDS > PAF > CCFP > MDL > TMS > HLF > IMFP > SVFP > HLL > TML > AAS > HLS.

Hydroxyl radical scavenging assay

The scavenging abilities of different extracts and BHA on hydroxyl radical inhibition by the 2-deoxyribose oxidation method are shown in Figure 2. Each extract showing hydroxyl radical-scavenging activity was increased with increasing concentration of the extract sample. At a concentration 0.5 mg/ml, all the extracts showed good hydroxyl radical-scavenging activities above 35.60%. These percentages of inhibition exceeded 50%, except for ZAS (40.40%) and AHL (37.27) at 1 mg/ml. As shown in Table 3, HLS showed the highest hydroxyl radical scavenging activity (EC_{50} = 0.20 ± 0.08 mg/ml) compared to BHA (EC_{50} = 0.30 ± 0.05 mg/ml), while AHL showed the lowest activity (EC_{50} = 3.07 ± 1.68 mg/ml). For all the plant extracts, EC_{50} values varied from 0.30 ± 0.04 to 1.02 ± 0.12 mg/ml. The scavenging abilities on hydroxyl radical are in the following descending order: HLS > PMFP = BHA > AAS > HLF > SVFP > MDL > TMS > MDS

Table 3. Antioxidant properties of the extracts of the studied plants on reducing power, DPPH radical-scavenging assay, hydroxyl assay and β-carotene-linoleic acid assay.

Sample	Reducing power	DPPH assay			Hydroxyl radical scavenging assay	β-Carotene-bleaching method
	IC_{50} (mg/ml)	EC_{50}	Temps (T_{EC50}) (min)	AE $\times 10^{-5}$	EC_{50} (mg/ml)	EC_{50} (mg/ml)
PAF	0.13 ± 0.001	47.39[a]	255.93	8.24	0.83 ± 0.14	-
TML	0.73 ± 0.04	512.75[a]	1468.13	0.13	0.50 ± 0.06	1.18 ± 0.13
TMS	1.17 ± 0.08	830.15[a]	153.91	0.78	0.43 ± 0.06	2.95 ± 0.07
MDL	0.66 ± 0.007	396.33[a]	63.84	3.95	0.4 ± 0.11	-
MDS	0.71 ± 0.01	366.36[a]	18.57	14.70	0.46 ± 0.07	-
HLL	0.47 ± 0.009	144.10[a]	2766.49	0.25	0.46 ± 0.14	1.02 ± 0.15
HLF	0.25 ± 0.004	66.28[a]	1971.34	0.76	0.35 ± 0.02	3.07 ± 0.25
HLS	0.35 ± 0.009	97.73[a]	40330.40	0.02	0.20 ± 0.08	0.76 ± 0.13
AHL	4.55 ± 0.79	31.83[b]	-	-	3.07 ± 1.68	-
AHS	3.24 ± 0.23	20.58[b]	-	-	0.66 ± 0.43	-
AAS	0.52 ± 0.007	297.74[a]	10927.27	0.03	0.33 ± 0.06	2.60 ± 0.18
ZAS	3.95 ± 0.77	6310.04[a]	-	-	1.02 ± 0.12	-
IMFP	0.64 ± 0.004	66.96[a]	2017.09	0.74	0.52 ± 0.05	-
CCFP	1.17 ± 0.05	462.19[a]	50.73	4.26	0.66 ± 0.12	-
PMFP	3.64 ± 0.51	10.04[b]	-	-	0.30 ± 0.04	-
SVFP	0.42 ± 0.004	99.02[a]	1897.42	0.53	0.39 ± 0.16	0.97 ± 0.04
AA	0.06 ± 0.002	39.53[a]	0.61	4174.46	-	-
BHT	-	13.47[a]	161.52	45.97	-	-
Trolox	-	49.21[a]	7.80	260.43	-	-
BHA	-		-	-	0.30 ± 0.05	0.03 ± 0.005

PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract, AA, ascorbic acid. [a]EC_{50} concentration expressed as mg Antioxidant/ g DPPH. [b]EC_{50} concentration expressed as mg/ml after 30 min.

= HLL > TML > IMFP > AHS = CCFP > PAF > ZAS > AHL.

β-Carotene bleaching method

The results of the antioxidant effect of various extracts of plants on the autoxidation of linoleic acid are shown in Figure 3. At a concentration 3 mg/ml, HLL (97.64 %) exhibited the best efficiency in inhibiting the oxidation of linoleic acid. At 4 mg/ml, SVFP registered higher inhibition of bleaching of β-carotene (91.13%), followed by TML (88.09%) and HLS (86.82%); whereas the inhibition percentages of AAS, HLF and TMS were, respectively 65.13, 62.94 and 59.52%. For the other extracts, the percentages of inhibition varied between 8.61 and 40.05%, except for the AHS, which showed a very weak activity, lower than 5% at 4 mg/ml. According to EC_{50} showed in Table 3, HLS exhibited an interesting antioxidant activity (0.76 ± 0.13 mg/ml), followed by the SVFP (0.97 ± 0.04 mg/ml), HLL (1.02 ± 0.15 mg/ml) and TML (1.18 ± 0.13 mg/ml). EC_{50} values of AAS, TMS and HLF were 2.60 ± 0.18, 2.95 ± 0.07 and 3.07 ± 0.25 mg/ml, respectively. All the extracts were less effective compared to BHA (0.03 ± 0.005 mg/ml).

DISCUSSION

Our results are a contribution to the valorization of some medicinal plants from Algeria. According to our best knowledge, this is the first report focusing on the evaluation of antioxidant capacities *in vitro* and the quantification of total phenolics compounds, flavonoids and proanthocyanidins from different parts of selected medicinal plants. In this study, total phenolics were higher

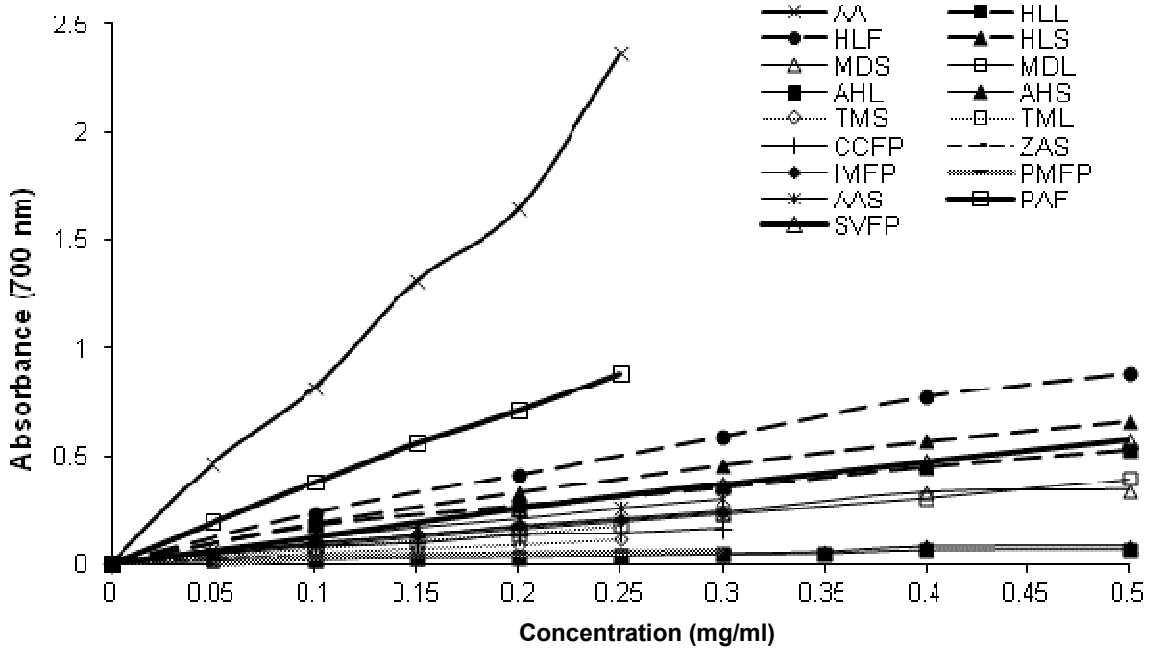

Figure 1. Reducing power as a function of methanolic extracts concentration.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

in fruits of *Pistacia atlantica*, followed by *Thymelaea microphylla* (leaf, stem) and *Marubium deserti* (leaf, stem). In comparative studies with other plant extracts, *M. deserti* (3.67 mg GAE/g DM) and *T. Microphylla* (10.80 mg GAE/g DM) showed the lowest phenolic contents (Djeridane et al*.,* 2010). These contents found in our study were also higher than those reported for others species of Jordanian plants (2.1 to 52.8 mg/g) (Alali et al., 2007) and some Algerian medicinal plants like *Thymelaea hirsute* (6.81 mg/g) (Djeridane et al., 2006).

For *Helianthemum lippii* extracts, the total phenolic contents obtained in the leaves, stems and fruits were higher than those deferred by Tawaha et al. (2007) in the methanolic (17.3 ± 1.0 mg GAE/g DW) and aqueous extracts (8.7 ± 3.0 mg GAE/g DW) from *H. ledifolium*. Alali et al. (2007) found the phenolic contents of 25 and 30.5 mg GAE/g DW for the methanolic and aqueous extracts of *H. lippii*. For the other extracts tested (*Atriplex halimus*; *Anabasis articulata; Zygophylum album; Inula montana*; *Cotula cinerea* and *Pentzia monodiana*), the contents ranged from 3.77 ± 0.06 to 43.14 ± 0.63 mg GAE/g DM. Our results were within the range of the values reported by Li et al. (2008) for 45 Chinese medicinal plants and Tawaha et al. (2007) for 51 Jordanian plants. These differences in total phenolic contents could be due to genotypic and environmental variations (climate, location, temperature, fertility and diseases) within species, plant part tested, harvesting time and extraction procedure (Shan et al., 2005).

According to the experimental data, PAF revealed a higher TAC, a strong activities to reduce iron and to scavenge DPPH and OH radicals but a very weak activity to inhibit the oxidation of β-carotene. This strong activity of *P. atlantica* extracts might be attributed to the inductive effect of the three hydroxyl groups in the gallic acid structure and other phenolic acids, such as the ρ-coumaric acid (Sanchez-Moreno et al., 1998; Benhammou et al., 2008). Moreover, the Anacardiaceae family and the Pistacia kind are characterized by the existence of myricetin and gallic acid-derived (5-O- galloyl; 3,5-O-digalloyl; 3,4,5-tri-O-galloyl) (Baratto et al., 2003). It has also been shown that vanillic, syringic, ferulic and ρ-coumaric acids as well as catechin show a strong positive correlation with the scavenging effects on DPPH and $ABTS^{+}$ radicals and the iron reducing power. Moreover, the gallic acid and catechin are narrowly correlated with the

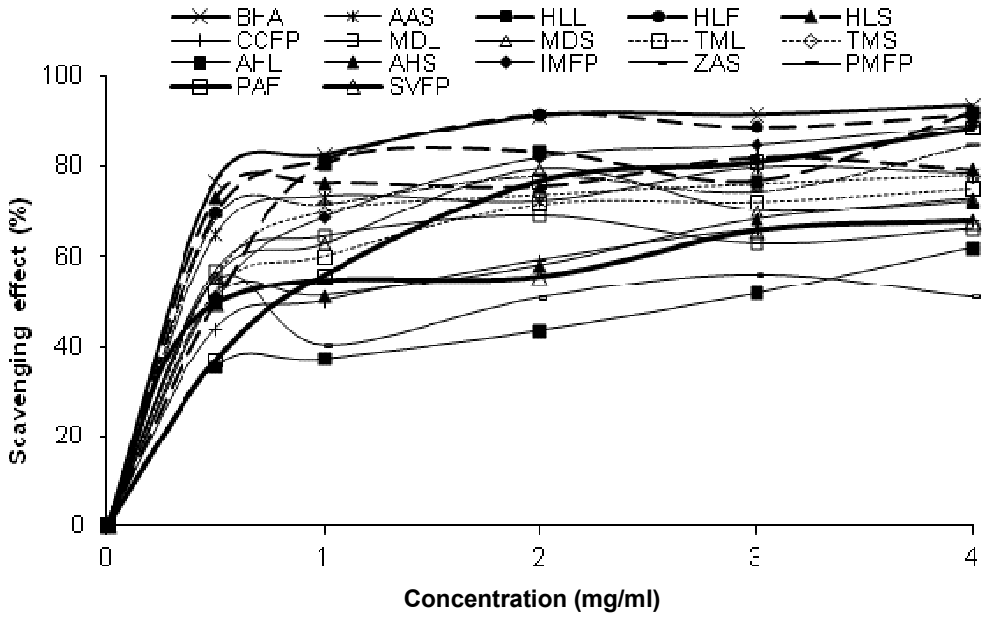

Figure 2. Scavenging effect of methanolic extracts of studied plants on hydroxyl radical compared to that of BHA.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

metal chelating activity and the inhibition of lipidic peroxidation (Tsai et al., 2007).

Considering its low TAC content, the leaves extract of *A*. *halimus* showed very low antioxidant activities to reduce the ferric iron and to quench DPPH and OH radicals. According to Benhammou et al. (2009), the ethyl acetate fraction of *A*. *halimus* exhibited an interesting antioxidant activities to scavenge DPPH (EC_{50} = 2.04 mg/ml) and to reduce iron (IC_{50} = 1.51 mg/ml). These capacities are attributed to the abundance of flavonols (kaempferol, quercetin) which constitute the main class of Atriplex species (Bylka et al., 2001).

In our study, the methanolic extracts of *H*. *lippii* also exhibited high antioxidant potency, which might be due to presence of flavonols. Similarly, Calzada et al. (1999) reported the presence of kaempferol and quercetin in methanolic extract of *H. glomeratum.* However, all other extracts presented different antioxidant capacities. Overall, antioxidant activities are more variable between species of a plant (inter-species) than within the same species (intra-species) (Ksouri et al., 2008).

The results obtained from the present study suggested that there was a negative correlation between phenolic contents and different antioxidant activity assays. The relationship between total flavonoid contents and total antioxidant capacity of extracts of different plants shows a significant relationship with coefficient correlation R^2 = 0.906 (Figure 4). It is well known that these compounds have powerful antioxidant capacities. The number and position of hydroxyl groups on the flavonoid nucleus enhances antioxidant activity. Substitution patterns in the B-ring and A-ring as well as the 2,3-double bond (unsaturation) and the 4-oxo group in the C-ring also affect antioxidant activity of flavonoids (Cai et al., 2006).

Conclusion

The antioxidant capacities and the total phenolic, flavonoid and proanthocyanidins contents of eleven selected medicinal plants growing in Algeria were evaluated. The results obtained showed that *P. atlantica*, *T. microphylla* and *M. deserti* could become a promising source of phenolic compounds. It has been found that plants like *P. atlantica, H. lippii, I. montana, Anabasis articulata* and *S. villosum* present the highest antioxidant capacities and may serve as valuable sources of natural antioxidants for further isolation and purification of

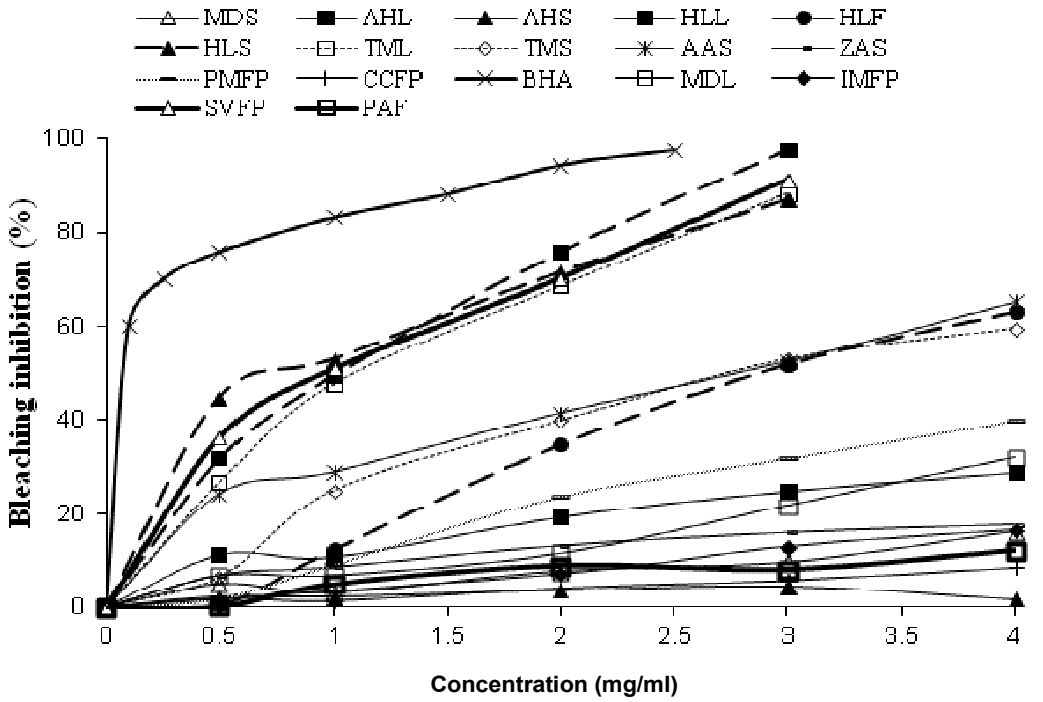

Figure 3. Inhibition (%) of lipid peroxidation of methanolic extracts of studied plants by the β-carotene bleaching method.
PAF, *Pistacia atlantica* fruit extract; TML, *Thymelaea microphylla* leaf extract; TMS, *Thymelaea microphylla* stem extract; MDL, *Marrubium deserti* leaf extract; MDS, *Marrubium deserti* stem extract; HLL, *Helianthemum lippii* leaf extract; HLF, *Helianthemum lippii* fruit extract; HLS, *Helianthemum lippii* stem extract; AHL, *Atriplex halimus* leaf extract; AHS, *Atriplex halimus* stem extract; AAS, *Anabasis articulata* stem extract; ZAS, *Zygophylum album* stem extract; IMFP, *Inula montana* flower part extract; CCFP, *Cotula cinerea* flower part extract; PMFP, *Pentzia monodiana* flower part extract; SVFP, *Sedum villosum* flower part extract.

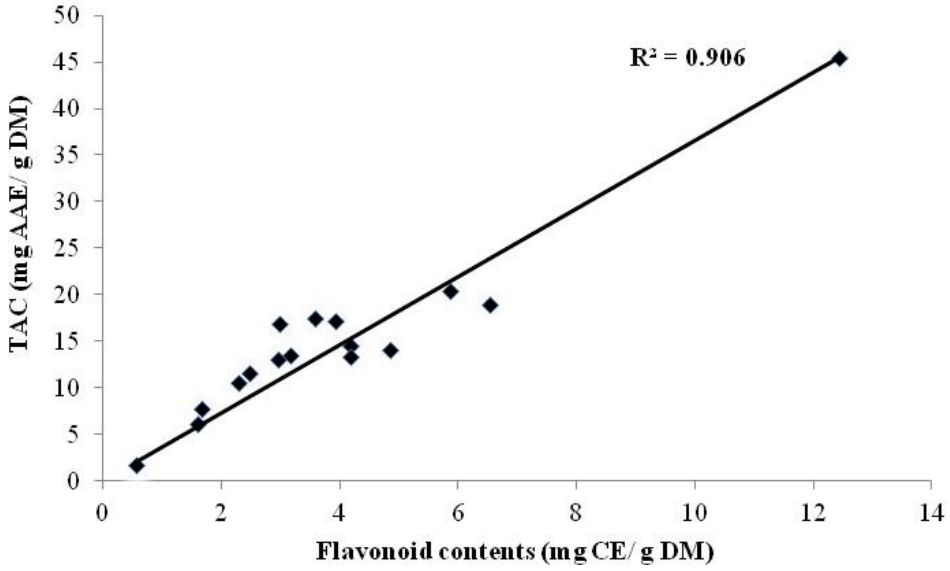

Figure 4. Correlation between the total antioxidant capacity expressed as ascorbic acid equivalent and flavonoids content.

antioxidant compounds. A strong correlation has been reported between TAC and the flavonoid contents, indicating that these compounds are the major contributor of antioxidant capacities of these plants.

Conflict of interests

The authors have not declared any conflict of interests

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