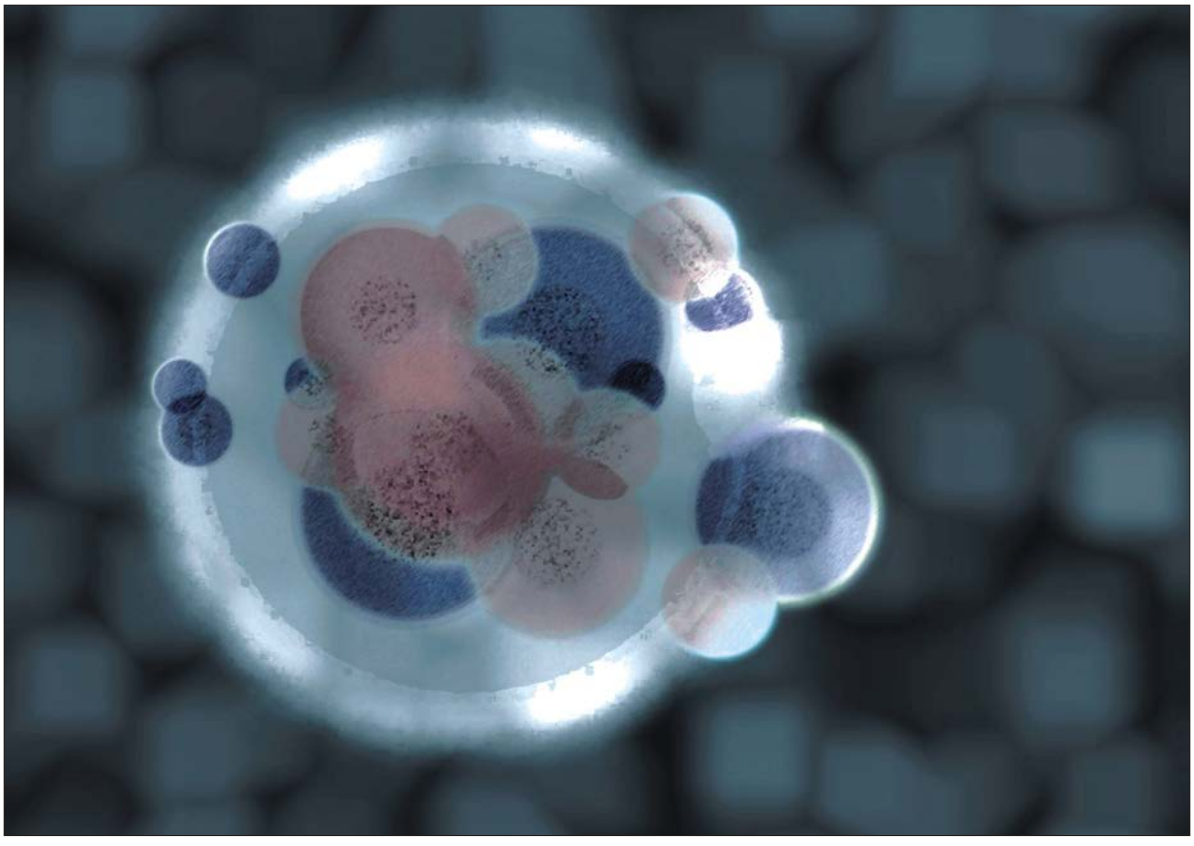

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Screening of the Antioxidant Potential of Some Algerian Indigenous Plants

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Abstract: In order to gain more knowledge about local medicinal plants, fifty two plants harvested in different regions of Algeria were screened for their antioxidant potential by means of two complementary assays, namely inhibition of DPPH radical and β-carotene bleaching and compared with authentic antioxidants, with respect of their total content of phenolic compounds and flavonoids. The seasonal variation of the antioxidant activity was also studied on *Tetraclinis articulata* grown in natural habitat, in order to determine the best time for harvesting this plant. Plant antioxidant activity showed best correlation with both phenolics and flavonoids contents. The flavonoids are the most important antioxidant agents as the correlation shown between these two parameters. Phytochemical screening showed a wide variety in the phytoconstituents profiles of the plants studied with phenolics predominance. Antioxidant activity assays showed that some extracts are very active comparatively with an authentic and common substance like BHA, synthetic antioxidant widely used in agro-alimentary industry. These extracts may be potential sources for isolation of natural antioxidants.

Key words: Medicinal plants, antioxidant activity, bioautography, phenolics, seasonal variation

INTRODUCTION

The medicinal plants are largely used either for the prevention, or for the curative treatment of several diseases. Among the properties behind these virtues, the antioxidant activity holds a place of first order (Cai *et al*., 2004; Manach *et al*., 2005; Russo *et al*., 2005; Boskou, 2006; Bandyopadhyay *et al*., 2007; Trichopoulou *et al*., 2007; Rahman, 2008).

Currently, several molecules isolated from the medicinal plants are used in the manufacture of drugs such as Taxol, an anti-cancer agent, isolated from the American yew (*Taxus brevifolia* Nutt., Taxaceae). Many of the medicinal plants contain a broad spectrum of phytochemical substances which are sources of natural antioxidants such as α-tocopherol, phenolic acids, flavonoids and tannins. In addition to their antioxidant activities, these compounds have other biological properties like anti-inflammatory; antimicrobial and anti-cancer activities (Lee *et al*., 2004). The use of these natural substances is not limited to the therapeutic field but also with the industrial field because they are more preferred instead of using of synthetic antioxidants such as BHT (Butylated-hydroxytoluene), BHA (Butylated-hydroxyanisol) and propyl gallate (PG) (Barreira *et al*., 2008). Today, the identification of new sources of natural substances is one of the most active fields of research in the world. It is also a privileged topic research of the Laboratory of plant Biochemistry and Natural substances of the University of Oran where this work was achieved. Abundance of medicinal plants in Algeria is attractive but much of these plants remain unknown on the phytochemical field, as well as on their biological properties justifying their use in traditional practice. Our objective is thus to establish a scientific base to these traditional uses by experimental studies in the laboratory as first step, basing on the hypothesis which point out that the medicinal virtues of these plants are mainly due to their antioxidant activity. This work lies within the scope of continuation of our published works describing some of biologically active extracts from Algerian plants (Boulenouar *et al*., 2009; Benamar *et al*., 2010).

MATERIALS AND METHODS

Plant material: The plant material studied in this research consists of various parts of 52 Algerian plants (Table 1). The majority of these plants were harvested between June 2007 and June 2008, from various areas of Algeria. The botanical identification of the plants is carried out by one

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Table 1: Antioxidant activity, total phenolic contents and flavonoids contents of 48 active extracts issued from 38 Algerian's species

Family/species	Used part	Bioautography		IC_{50}*	Total phenolics contents**	Flavonoids contents***
		DPPH	β-carotene			
Apiaceae						
Ammodaucus leucotrichus Coss. et Dur.	aerial parts	+	+	45.73±1.07	46.77±5.84	15.48±0.68
Ammodaucus leucotrichus Coss. et Dur.	Roots	-	-	nd	nd	nd
Deverra scoparia L.	Aerial parts	-	-	nd	nd	nd
Thapsia garganica L.	Leaves	+	+	90.72±0.98	47.64±1.43	18.13±0.47
Thapsia garganica L.	Root's bark	+	+	78.52±1.15	95.88±1.09	23.98±7.65
Thapsia garganica L.	Root's central part	-	-	nd	nd	nd
Apocynaceae						
Solenostemma argel (Del.) Hayne	Leaves	-	-	nd	nd	nd
Aristolochiaceae						
Aristolochia baetica L.	Seeds	-	-	nd	nd	nd
Asclepiadaceae						
Pergularia tomentosa L.	Leaves	-	+	183.45±85.71	42.46±3.09	11.80±0.48
Asteraceae						
Scorzonera undulata Vahl.	Roots	+	-	95.72±4.46	57.54±9.94	30.25±0.094
Warionia saharae Benth. et Coss.	Leaves	+	+	51.03±4.13	56.34±3.13	19.31±0.39
Atractylis humilis L.	Roots	+	+	74.30±0.46	63.91±5.00	16.83±3.66
Atractylis humilis L.	Leaves	+	-			
Perralderia coronopifolia Coss.	Flowers	+	+	56.65±1.49	99.35±5.85	29.80±5.33
Perralderia coronopifolia Coss.	Leafy stems	-	-	nd	nd	nd
Berberidaceae						
Berberis vulgaris L.	Branch bark	+	+	40.33±0.20	121.76±8.82	23.92±0.63
Borraginaceae						
Cynoglossum cheirifolium L.	Leaves	+	+	80.32±1.57	48.38±1.24	17.47±0.90
Brassicaceae						
Zilla macroptera Coss.	Aerial parts	+	-	nd	nd	nd
Caryophyllaceae						
Herniaria mauritanica Murb.	Aerial parts	-	+	nd	nd	nd
Chenopodiaceae						
Atriplex halimus L.	Leaves	-	-	30.12±6.9	16.50±0.89	16.41±2.24
Atriplex halimus L.	Roots	-	-	nd	nd	nd
Fredolia aretioides Coss. et Dur.	Leaves	-	-	nd	nd	nd
Fredolia aretioides Coss. et Dur.	Roots	+	+	53.79±0.63	110.92±6.34	16.98±3.14
Haloxylon scoparium Pomel.	Leafy stems	+	+	54.53±0.90	163.16±7.05	38.90±7.42
Cladoniaceae						
Cladonia rangiformis Hoffm.	Thallus	+	+	214.08±83.05	36.76±2.24	10.09±0.20
Corallinaceae						
Corallina officinalis L.	Thallus	-	-	nd	nd	nd
Cucurbitaceae						
Citrillus colocynthis Schrad.	Seeds	+	-	118.93±25.61	37.42±2.22	12.44±0.06
Citrillus colocynthis Schrad.	Fruit flesh	+	-	349.49±57.45	32.24±5.22	11.37±0.71
Cupressaceae						
Juniperus phoenicea L.	Leaves	+	+	21.98±0.57	199.66±7.32	48.54±10.65
Cynomoriaceae						
Cynomorium coccineum L.	Aerial parts	-	-	13.47±2.26	75.69±3.65	39.19±2.07
Cynomorium coccineum L. (EtOH)	Aerial parts	+	+	4.09±0.61	406.38±1.99	109.47±33.79
Ephedraceae						
Ephedra altissima Desf.	Aerial parts	+	+	12.01±0.23	107.18±1.39	41.27±2.23
Ephedra major Host.	Aerial parts	+	+	30.83±0.48	130.15±7.51	21.69±1.09
Euphorbiaceae						
Euphorbia guyoniana Boiss. et Reuter.	Aerial parts	+	+	44.00±2.11	54.90±3.46	21.49±1.34
Fabaceae						
Acacia raddiana Savi.	Trunk bark	+	+	7.36±0.19	266.82±9.14	115.36±11.28
Acacia raddiana Savi.	Leaves	+	+	17.11±1.55	72.34±6.26	21.52±2.02
Astragalus gombo Coss. et Dur	Aerial parts	-	+	nd	nd	nd
Cassia aschrek Forssk.	Leaves	-	-	nd	nd	nd
Vigna radiata L. R. Wilcz.	Seedling	-	-	nd	nd	nd
Globulariaceae						
Globularia alypum L.	Flowers		+	25.50±1.32	103.75±7.91	40.87±0.50
Globularia alypum L.	Roots	+	+	19.40±0.12	210.79±9.22	66.82±6.56
Globularia alypum L.	Leaves	+	+	24.43±0.85	112.01±6.16	42.31±4.35
Globularia alypum L. (MeOH)	Leaves	+	+	16.23±0.24	147.16±3.40	99.64±12.60
Lamiaceae						
Ajuga iva ssp. *pseudo-iva*	Aerial parts	+	+	358.49±10.21	61.38±4.92	17.23±0.87
Ajuga iva ssp. *pseudo-iva* (MeOH)	Aerial parts	-	-	nd	nd	nd

Table 1: Continued

Family/species	Used part	Bioautography		IC_{50}*	Total phenolics contents**	Flavonoids contents***
		DPPH	β-carotene			
Prasium majus L.	Leaves	+	+	64.47±4.32	71.39±2.71	32.34±6.75
Rosmarinus officinalis L.	Leaves	+	+	13.24±0.70	168.19±12.51	116.98±17.00
Teucrium polium L.	Leaves	+	+	26.47±1.08	81.22±2.41	79.64±1.58
Moraceae						
Ficus ingeus Miq.	Leaves	+	+	46.79±0.21	115.06±8.95	37.29±4.46
Ficus salicifolia Vahl.	Leaves	+	-	nd	nd	nd
Myrtaceae						
Myrtus nivellei Batt. et Trab.	Leaves	+	+	4.90±0.52	242.68±9.79	28.53±4.51
Oleaceae						
Olea lapperini Batt. et Trab.	Leaves	+	+	28.85±1.26	172.95±7.26	72.94±9.44
Pinaceae						
Cedrus atlantica (Endlicher) Manetti	Leaves	+	-	24.83±1.99	97.09±3.29	27.60±1.67
Poaceae						
Cymbopogon citratus (D.D.) Stapf.	Leaves	-	-	136.78±13.13	62.99±3.93	34.49±4.80
Ramalinaceae						
Ramalina panizei De Not.	Thallus	-	-	nd	nd	nd
Rhamnaceae						
Zizyphus lotus L. Desf.	Fruits flesh	-	-	nd	nd	nd
Zizyphus lotus L. Desf.	Seeds	-	-	nd	nd	nd
Zizyphus lotus L. Desf.	Root's bark	+	+	9.14±0.72	192.09±8.50	68.64±4.91
Zizyphus lotus L. Desf.	Leaves	+	+	12.29±0.29	158.16±5.32	54.75±2.79
Zizyphus lotus L. Desf.	Root's central parts	+	+	13.37±0.46	98.76±9.46	32.64±2.01
Rutaceae						
Ruta chalepensis L.	Leaves	+	+	61.41±9.38	66.95±6.84	16.69±0.23
Salvadoraceae						
Salvadora persica L.	Leaves	-	+	nd	nd	nd
Santalaceae						
Osyris quadripartita Salzm.	Leaves	+	+	4.80±0.53	438.99±7.52	72.09±1.60
Osyris quadripartita Salzm.	Immature fruits	+	+	39.20±1.30	109.49±11.07	16.95±1.93
Solanaceae						
Solanum sodomaeum L.	Seeds	+	+	83.85±1.35	39.55±0.69	17.11±1.98
Withania frutescens L. Pauquy	Leaves	+	-	42.59±3.94	48.29±3.55	18.79±0.62
Thymeliaceae						
Thymelia hirsuta L.	Aerial parts	+	+	32.27±1.65	77.91±1.85	40.82±4.51
Thymelia microphylla Coss. et Dur.	Leafy stems	-	-	nd	nd	nd
Zygophyllaceae						
Fagonia cretica L.	Aerial parts	-	-	nd	nd	nd
Peganum harmala L.	Seeds	+	+	111.33±13.48	72.78±2.51	15.24±1.76
Standards						
Quercetin				1.66±0.21		
Ascorbic acid				2.66±0.07		
BHA				4.15±0.25		

*Antioxidant activity expressed as μg mL^{-1}. **Total phenolics contents expressed as mg gallic acid/g of freeze-dried extract. ***Flavonoids contents expressed as mg catechin g^{-1} of freeze-dried extract. nd: Not determined, +: Positive result, -: Negative result. Data expressed as Mean±SD from triplicate experiments. NB: All the extracts were obtained by aqueous extraction then freeze-dried, except contrary mention

of us (Pr Abderrazak Marouf). The fresh plants were dried, either with the free air, in the shade, or in a drying oven at 50°C during 24 h then crushed using a ball mill to obtain a fine powder.

Extraction: All the samples are extracted by heat reflux with water distilled 10% (p/v) during 30 min. This procedure was repeated successively three times with fresh solvent each time, followed by filtration. Filtered extracts were mixed and lyophilized to dryness. Some of these plants are extracted by maceration with ethanol (aerial parts of *Cynomorium coccineum*). The roots central parts of *Zizyphus lotus* are macerated by methanol during 24 h, or by refluxing with the methanol (aerial parts of *Ajuga iva* ssp. *pseudo-iva* and leaves of *Globularia alypum*).

For the liquid-liquid fractionation assay, it has used solvents with increasing polarity (chloroform, ethyl acetate and n-butanol) in order to separate antioxidant compounds contained in the crude aqueous extract of *Myrtus nivellei* according to their polarity. The residual aqueous extract is concentrated by lyophilization. All the lyophilized powders were collected and stored at -20°C until used. On the other hand, the macerated, the organic fractions and the methanolic extracts were evaporated by rotary vacuum evaporator.

Preparation of the samples: The samples are prepared at a concentration of 2 mg mL^{-1} in MeOH and then homogenized using an ultrasonic bath until complete dissolution.

Chemicals: All the standard substances and reagents are of analytical grade.

Determination of the antioxidant activity by Thin Layer Chromatography (TLC) bioautography: The biological tests used to detect the presence of the antioxidant compounds in plants extracts is based on the principle of the reduction of DPPH and the oxidation inhibition of the β-carotene. To apply these two tests, we deposited the same quantities (25 μL) of each extract on the plate of silicagel 60 F254 (Merck). The development of TLC plates is carried out in the following system: ethyl acetate, formic acid, acetic acid, distilled water (100: 11: 11: 26). After migration and drying of chromatograms, the plates are pulverized with 0.2% DPPH (2, 2'-Diphényl-1-picrylhydrazyl, Sigma) in methanol (Dominguez *et al*., 2005). Bands with the DPPH scavenging activity were observed as white yellow bands on a purple background. Concerning the β-carotene test, the antioxidant zones appear yellow orange on a white background after pulverization with 0.05% β-carotene in chloroform (Dominguez *et al*., 2005).

DPPH spectrophotometric assay: This method was applied only to extracts having shown the antiradical activities with bioautography assay. The antioxidant activity of various extracts was tested *in vitro* according to the method of Blois (1958) with little modifications using DPPH, which is a stable free radical, soluble in methanol. It has an intense purple color with a maximum absorption at 517 nm. The methanolic solution of DPPH (1950 μL) at a concentration of 6.10^{-5} M was mixed with 50 μL of different extract concentrations (10-20-30-40-50 μg mL^{-1}). Then, the mixture was incubated at room temperature in darkness for 1h. The absorbance of each extract containing DPPH was read at 517 nm using a UV–vis spectrophotometer (8500P Double-Beam spectrophotometer). The curves which represent the percentage of DPPH radical inhibition as a function of concentrations allowed to calculate the extract concentration necessary to decrease DPPH radical concentration by 50% (called IC_{50}). The percentage of inhibition (PI) of the extract is calculated according to the following equation:

$$PI = \frac{\text{A neg. control-A sample}}{\text{A neg. control}} \times 100$$

where, A neg.control is the absorption of the negative control solution (containing only DPPH), A sample is the absorption in the presence of the plant extract in DPPH solution.

The results are expressed by the means of three measurements±standard of deviation. The value of the lowest IC_{50} of extract expresses the strongest activity of this extract.

The ascorbic acid, quercetin and the BHA were used as the positive controls. Each experiment is repeated three times for statistical analysis of the results.

Phytochemical analysis by TLC: The identification of the phytoconstituents of the active extracts was achieved in TLC plates in normal phase where the stationary phase used is Silicagel 60 F_{254} (0.25 mm thickness) on an aluminum support (Merck). The mobile phase employed differs from one identification to another as described previously (Benamar *et al*., 2010), according to the polarity of phytoconstituents. The chromatograms are evaluated under UV at 254 and 365 nm and visible before and after revelation to detect phenolic acids, coumarins, flavonoids, lignans, quinones, anthracenic derived, saponins, terpenes, sesquiterpenes lactones, alkaloids and cardiotonic glycosides.

Total phenolics contents assay: The total phenolics content is estimated by colorimetric assay, using the Folin-Ciocalteu reagent as reported previously (Benamar *et al*., 2010).

Flavonoids contents assay: Flavonoids contents determination of the different samples was carried out according to the same procedure described previously (Benamar *et al*., 2010).

Statistical analysis: The statistical analysis was carried out by software Microsoft Excel 2007. The results were represented by the means of three measurements for the antioxidant activity, the total phenolics and flavonoids contents with its standard deviation.

RESULTS

Antioxidant activity by bioautography: In this study which related to 72 extracts of 52 Algerian plants (68 aqueous extracts, methanolic extracts of leaves of *Ajuga iva*, leaves of *Globularia alypum* and roots of *Zizyphus lotus* and ethanolic extract of *Cynomorium coccineum*), much of them are famous as medicinal ones, 45 extracts of 36 plants belonging to 25 families appeared active by the DPPH assay carried out by bioautography (Table 1). This test gives several anti-radical spots for various extracts among which aqueous extracts of *Osyris quadripartita* (leaves), *Myrtus nivellei*, *Globularia alypum* (leaves and roots), *Acacia raddiana* (bark), ethanolic extract of

Cynomorium coccineum (aerial parts); the ethyl acetate and the butanolic fractions, resulting from liquid-liquid partitioning of the crude aqueous extract of leaves of *Myrtus nivellei*, present the most active bands.

For β-carotene assay, the antioxidant potential is highlighted also by the appearance of several active spots like aqueous extracts of the leaves of *Osyris quadripartita* L. which showed the greatest number of bands (5 active products).

DPPH reduction assay: The DPPH reduction by spectrophotometric assay highlighted 48 active extracts from 38 plant species, among which ethanolic extract of *Cynomorium coccineum*, aqueous extracts of *Osyris quadripartita*, *Myrtus nivellei*, barks of *Zizyphus lotus* and *Acacia raddiana* which present the lowest values of IC_{50}, going from 4.09 to 9.14 μg mL^{-1} (Table 1).

It was shown that the ethanolic extract of *Cynomorium coccineum* presents activity very close to that of the BHA, synthetic antioxidant widely used in agro-alimentary industry, (IC_{50} = 4,09 and 4,15 μg mL^{-1}, respectively).

After liquid-liquid fractionation of *Myrtus nivellei*, the spectrophotometric assay of the DPPH showed that the aqueous extract and all the fractions are active and able to reduce DPPH radical. Classified by decreasing order (Table 2), this activity is as follows: AcOEt > BuOH > $CHCl_3$ > H_2O. The activity of the initial crude aqueous extract is between those of BuOH and $CHCl_3$.

Phytochemical results by TLC: The phytochemical TLC profiles inform us on the richness of these extracts in flavonoids, phenolic acids, lignanes, coumarins, terpenoids, sesquiterpenes and cardiotonic glycosides. The extracts having the strongest activities gave spots from different phytochemical constituents, revealed active ones by bioautography like the case of the ethanolic extract from *Cynomorium coccineum*, the aqueous extracts of the leaves of *Osyris quadripartita* and *Myrtus nivellei* which containing one flavonoidic band for each extract, for *M. nivellei*, an active spot is identified as terpenoid.

Phenolics and flavonoids assay: The total phenolics contents of the studied extracts vary greatly and are between 16.50±0.89 in *Atriplex halimus* and 438.99±7.52 mg of gallic acid equivalent per gram of freeze-dried matter in *Osyris quadripartita* (Table 1).

The flavonoids contents vary between 10.09±0.20 in the thallus of *Cladonia rangiformis* and 116.98±17 mg of catechin equivalent per gram of freeze-dried matter in the leaves of *Rosmarinus officinalis* (Table 1). The highest phenolics and flavonoids contents were detected in leaves of *Osyris quadripartita*; ethanolic extract of *Cynomorium coccineum*; bark of *Acacia raddiana*; leaves of *Myrtus nivellei*; roots of *Globularia alypum*; bark of *Zizyphus lotus*; leaves of *Juniperus phoenicea*; *Olea lapperini*; *Rosmarinus officinalis*; *Tetraclinis articulata*; *Haloxylon scoparium* and *Zizyphus lotus*. These same plants have a strong antioxidant activity. The lowest phenolics contents were found in *Atriplex halimus*; the fruits flesh and seeds of *Citrullus colocynthis*; the thallus of *Cladonia rangiformis*; seeds of *Solanum sodomaeum*; leaves of *Pergularia tomentosa*; fruits of *Ammodaucus leucotrichus*; leaves of *Thapsia garganica*; *Withania frutescens*; *Cynoglossum cheirifolium* and *Ajuga iva*, as well as the aqueous phase of *Myrtus nivellei*.

Seasonal variation of the antioxidant activity, phenolics and flavonoids contents of *Tetraclinis articulata*: According to our results, the strongest accumulation of phenolic compounds and the antioxidant activity in *Tetraclinis articulata* leaves are observed during the spring (April) (Table 3). IC_{50} values are varying between 9.51±0.52 and 29.86±1.45 μg mL^{-1}. The capacity of DPPH radical scavenging by the aqueous extract of the leaves of *Tetraclinis articulata* during the various seasons is due to their richness in active components like phenolics and flavonoids.

Table 2: IC_{50} values*, total phenolics contents** and flavonoids contents*** of the fractions issued from the liquid-liquid fractionation assay of the crude extract of *Myrtus nivellei*

Fraction	IC_{50}*	Total phenolics contents	Flavonoids contents
Chloroform	53.50±2.05	81.700±2.445	25.122±1.368
Ethyl acetate	3.08±0.40	521.220±6.167	74.159±2.735
n-butano	4.40±0.43	393.410±15.679	66.022±1.804
Water	64.84±5.09	35.441±0.830	13.641±0.233

*IC_{50} expressed as μg mL^{-1}. **Total phenolics contents were expressed as mg gallic acid/g of freeze-dried extract. ***Flavonoids contents were expressed as mg catechin g^{-1} of freeze-dried extract. Data expressed in Mean±SD from triplicate experiments. NB: all the fractions are active by the 2 tests of bioautography

Table 3: IC_{50} values*, total phenolics contents** and flavonoids contents*** of *Tetraclinis articulata*

Month, year	IC_{50}	Total phenolics contents	Flavonoids contents
June, 2007	13.527±1.233	163.691±3.208	48.736±1.714
November, 2007	12.796±1.045	155.166±3.637	56.501±7.831
January, 2008	29.860±1.455	111.103±6.248	27.392±4.133
April, 2008	9.519±0.521	206.187±16.612	65.184±7.242

*IC_{50} expressed as μg mL^{-}, **Total phenolics contents were expressed as mg gallic acid g^{-1} of freeze-dried extract. ***Flavonoids contents were expressed as mg catechin g^{-1} of freeze-dried extract. Data expressed in Mean±SD from triplicate experiments

Correlation between the antioxidant activity with the phenolics and flavonoids contents: The antioxidant activity of the extracts and the phenolics content showed a good correlation ($R^2 = 0.79$).

The antioxidant activity of the fractions resulting from the liquid-liquid partitioning of the aqueous extract of *Myrtus nivellei* exhibits also a good linear correlation with their total phenolics ($R^2 = 0.99$) and with the flavonoids contents ($R^2 = 0.95$).

A good correlation also exists between the phenolics contents and the seasonal fluctuations of the antioxidant activities ($R^2 = 0.96$) and between the flavonoids contents and the seasonal fluctuations of the antioxidant activities of *Tetraclinis articulata* ($R^2 = 0.97$).

DISCUSSION

Antioxidant activity by TLC bioautography: The greatest number of bands exhibited by bioautography in the active extract like the leaves of *Osyris quadripartita* L. explains why these extracts have a good antioxidant capacity. Thus, it can be suggested that the antioxidant activity recorded by DPPH reduction spectrophotometric assay is the result of the total activities of various components present in this extract as it was previously pointed by Wang *et al.* (2008) who shown that the antioxidant capacity of the essential oil of *Rosmarinus officinalis* results from the co-operation of its constituents. The extracts those present the lowest IC_{50} having the highest antioxidant activities as shown in the Table 1.

This activity can be explained by the presence of the phenolic compounds whose antioxidant activity is well established. Indeed, several studies reported that the antioxidant activity of the plants which have therapeutic properties is due to the presence of natural substances mainly phenolic compounds (Virgili *et al.*, 2001).

According to our results, it also showed that the ethanolic extract of *Cynomorium coccineum* and the methanolic extract of *Globularia alypum* have the higher antioxidant effect than the aqueous extracts of the same parts. These results can be explained by the low phenolic compounds content in aqueous extracts (Table 1) (Tachakittirungrod *et al.*, 2007; Yen *et al.*, 2008). For the same plant, the antioxidant activity is very variable in one part to another. Thus, the roots of *Globularia alypum* exhibited the higher activity compared to that of the leaves and to that of the flowers. For *Zizyphus lotus*, the activity of the bark is higher than that the leaves and that of the roots. The antioxidant activities of the bark of *Acacia raddiana* and the leaves of *Osyris quadripartita* are respectively twice stronger than in the leaves and nine times more than in the fruits (Table 1). This antioxidant activity different according to the species and the parts from the same species, would be directly related to their different composition in phenolic compounds (Wojdyło *et al.*, 2007). The antiradical capacity of the seed and fruit extracts is low and would be explained by the weakness in phenolic compounds of these parts. This weakness is compensated by their high percentage of carbohydrate component which contribute for a big part to extracts (Maisuthisakul *et al.*, 2007). On the other hand, the barks present a larger activity compared to the other parts because their richness of tannins and procyanidines. Similar results were found by Souza *et al.* (2008) with various species. It should be noted that the antioxidant potential of the extracts depends not only on the concentration of phenolic compounds but also of their nature. This is illustrated by the presence of same contents of phenolic compounds in the leaves of *Acacia raddiana* which is rich in antioxidant condensed tannins (Downs *et al.*, 2003) and *Peganum harmala* but the IC_{50} value of *Acacia raddiana* is 6.5 times weaker than that of seeds of *Peganum harmala*. It was also shown that the antioxidant capacity of some olive species depends on quality as well as quantity of the phenolic compounds (Boskou *et al.*, 2006).

The results of the fractionation are comparable with those found by Yen *et al.* (2008) for *Cuscuta chinensis*. The IC_{50} value of AcOEt is lower than that of the BHA. This difference of the activity in these extracts is explained by the difference in the proportions of phenolic compounds and the flavonoids being given the polarity different from this extracting.

The whole of these results gives a scientific justification to the traditional use of the medicinal plants studied in the treatment of diseases such as the diabetes; cardiovascular diseases and cancer. Among the plants having an antioxidant property and which are recognized antidiabetic plants: *Ajuga iva*, *Citrillus colocynthis*, *Fredolia aretioides*, *Globularia alypum*, *Haloxylon scoparium*, *Juniperus phoenicea*, *Rosmarinus officinalis*, *Peganum harmala*, *Teucrium polium*, *Tetraclinis articulata*, *Zizyphus lotus* (Bnouham *et al.*, 2002). *R. officinalis* is used in the treatment of the cardiovascular diseases (Gonzalez-Tejero *et al.*, 2008) and *Euphorbia marginata* Pursh; *Globularia alypum* L., *Osyris quadripartita* Salzm. in the treatment of cancer (Graham *et al.*, 2000; Abdelwahed *et al.*, 2007).

Phenolics and flavonoids assay: Some phenolic compounds like the flavonoids are endowed with anti-carcinogenic and anti-mutagenic activities (Chung *et al.*, 1998). These activities could be related to the antioxidant activity and can play a big role in the prevention and treatment of cancer (Chung *et al.*, 1998).

The results in Table 1 showed that plants those had high phenolics contents giving stronger antioxidant activities. In agreement with Boskou *et al.* (2006), Djeridane *et al.* (2006), Wojdyło *et al.* (2007), Biglari *et al.* (2008) and Yen *et al.* (2008), these results suggest that the antioxidant capacity of the plants is related to their phenolics and flavonoids contents. It is also noticed that the phenolics contents were different from part of the plant to another. Such is the case of *Globularia alypum* and *Zizyphus lotus*. It is noticed that the extracts which have the strong phenolic compounds contents, contain also high flavonoids contents. Thus, the latter would be the principal representatives of the phenolic compounds pool. For example, the contents of both phenolic compounds and flavonoids in *Rosmarinus officinalis* are respectively of 168.19±12.51 and 116.98±17.00 mg g^{-1}. These results are rather comparable with those found by Hui-Yin *et al.* (2007) with the same type of extract (185.04 and 141.2 mg g^{-1}).

Seasonal variation of the antioxidant activity, phenolics and flavonoids contents of *Tetraclinis articulata*: The antioxidant activity as well as contents of phenolics and flavonoids responded to seasons in a similar manner, reaching their maximum at spring. The variation in the antioxidant activity of *Tetraclinis articulata* during the different seasons was in agreement with those of Gardeli *et al.* (2008) working on the methanolic extracts of *Pistacia lentiscus* L. and *Myrtus communis* L. and Tsai *et al.* (2008) working on the aqueous extract of the leaves of *Pennisetum purpureum*. In the same context, Hussain *et al.* (2008) showed that both of the content and the chemical composition of essential oils of the aerial parts of *Ocimum basilicum* L. fluctuate according to the seasons. It is well established that the phytochemical profile of a plant is directly related to the conditions of the environment such as the climate, the geographical location, the temperature, the photoperiod, the vegetative stage, etc. These factors influence the synthesis pathways of the active compounds of the plant (Tsai *et al.*, 2008). However, this seasonal trend in overall antioxidant capacity can't be extrapolated to all species of plants. There is several reports were the highest antioxidant capacity is observed in other periods (Toor *et al.*, 2006; Chen *et al.*, 2009).

Correlation between the antioxidant activity with the phenolics and flavonoids contents: Correlation analysis revealed that a higher amount of phenolics contents was correlated with higher antioxidant activity. These results are in agreement with the previous findings that the phenolic compounds contribute in the antioxidant activity of several Chinese medicinal plants (Cai *et al.*, 2004; Yen *et al.*, 2008). The result of the correlation between the antioxidant activities with the flavonoids explained that these later were the antioxidant agents of first order as testifies the good correlation between these two parameters.

CONCLUSIONS

The results of the experimental studies have enabled us to better knowledge of the antioxidant capacity and the phytochemical profile of the tested plants. The antiradical activity was undertaken with an aim to evaluate the antioxidant potential and thus, to predict the pharmacological potential of these plants which are considered by their medicinal status and used in traditional practice for various affections.

It comes out from our results that the aqueous extracts of the leaves of *Osyrus quadripartita*, *Myrtus nivellei*, *Tetraclinis articulata*, *Ephedra altissima*, *Zizyphus lotus*, *Rosmarinus officinalis*, barks of *Acacia raddiana* and *Zizyphus lotus,* the ethanolic extract of *Cynomorium coccineum* and the methanolic extract of the roots of *Zizyphus lotus* have a good antioxidant activity. The results of the biological tests show that some extracts are very active compared to authentic and known substance like the BHA, synthetic antioxidant widely used in agro-alimentary industry. So, these extracts are potential sources for isolation of the natural antioxidant. It is the case of the ethanolic extract of *Cynomorium coccineum*. The antioxidant potential is variable of one part to another, but, in all the cases, proportional to the phenolic compounds content in these parts.

Spectrophotometric assay supports the results of the phytochemical analysis by TLC in the direction where we observe a correlation between antioxidant activity and the presence of phenolic compounds.

The results of the liquid-liquid fractionation of *Myrtus nivellei* give an antioxidant activity in all the fractions but in direct relationship with the both phenolics and flavonoids contents. The ethyl acetate fraction which has the strongest activity contains also the highest phenolics and flavonoids contents.

The follow-up of the seasonal variations of antioxidant activity, phenolics and flavonoids contents of *Tetraclinis articulata* shows a notable increase in the antioxidant activity (the weakest IC_{50}) correlated with both the phenolics and the flavonoids contents (the highest contents) for April period.

The whole of the results obtained in this study makes it possible to consider the plants with strong antioxidant activity as potential sources of natural antioxidants able

to be used with a therapeutic or industrial aim as alternative for the synthetic products which are known for their multiple disadvantages. It would be thus judicious to continue this work by the isolation of the active principle(s) responsible for this activity and their biological evaluation by *in vivo* tests on animal models as well as the determination of their possible toxicity, as precondition to their clinical evaluation.

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REFERENCES

Abdelwahed, A., I. Bouhlel, I. Skandrani, K. Valenti and M. Kadri *et al*., 2007. Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus* Confirmation by microarray expression profiling. Chemico-Biol. Interact., 165: 1-13.

Bandyopadhyay, M., R. Chakraborty and U. Raychaudhuri, 2007. A process for preparing a natural antioxidant enriched dairy product (Sandesh). Food Sci. Technol., 40: 842-851.

Barreira, J.C.M., I.C.F.R. Ferreira, B.M.P.P Oliveira and J.A. Pereira, 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem., 107: 1106-1113.

Benamar, H., W. Rached, A. Derdour and A. Marouf, 2010. Screening of algerian medicinal plants for acetylcholinesterase inhibitory activity. J. Biol. Sciences, 10: 1-9.

Biglari, F., A.F.M. AlKarkhi and M.E. Azhar, 2008. Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. Food Chem., 107: 1636-1641.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Bnouham, M., H. Mekhfi, A. Legssyer and A. Ziyyat, 2002. Medicinal plants used in the treatment of diabetes in Morocco Int. J. Diabetes Metab., 10: 33-50.

Boskou, D., 2006. Sources of natural phenolic antioxidants. Trends Food Sci. Technol., 17: 505-512.

Boskou, G., F.N. Salta, S. Chrysostomou, A. Mylona, A. Chiou and N.K. Andrikopoulos, 2006. Antioxidant capacity and phenolic profile of table olives from the Greek market. Food Chem., 94: 558-564.

Boulenouar, N., A. Marouf and A. Cheriti, 2009. Effect of some poisonous plants extracts on *Fusarium oxysporum* f. sp. Albedinis. J. Biol. Sci., 9: 594-600.

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 Sci., 74: 2157-2184.

Chen, Y., Y. Jiang, J. Duan, J. Shi, S. Xue and Y. Kakuda, 2009. Variation in catechin contents in relation to quality of 'Hang Zhi Xiang' Oolong tea (*Camellia sinensis*) at various growing altitudes and seasons. Food Chem., 119: 648-652.

Chung, K.T., T.Y. Wong, C.I. Wei, Y.W. Hang and Y. Lin, 1998. Tannins and human health: A review. Crit. Rev. Food Sci. Nutr., 38: 421-464.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660.

Dominguez, M., A. Nieto, J.C. Marin, A.S. Keck, E. Jeffery and C.L. Cespedes, 2005. Antioxidants activities of extracts from *Barkleyanthus salicifolius* (Asteraceae) and *Penstemon gentianoides* (Scrophulariaceae). J. Agric. Food Chem., 53: 5889-5895.

Downs, C.T., P.M. McDonald, K. Brown and D. Ward, 2003. Effects of *Acacia* condensed tannins on urinary parameters, body mass and diet choice of an *Acacia* specialist rodent, *Thallomys nigricauda*. J. Chem. Ecol., 29: 845-858.

Gardeli, C., V. Papageorgiou, A. Mallouchos, K. Theodosis and M. Komaitis, 2008. *Essential oil* composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts. Food Chem., 107: 1120-1130.

Gonzalez-Tejero, M.R., M. Casares-Porcel, C.P. Sanchez-Rojas, J.M. Ramiro-Gutierrez and J. Molero-Mesa *et al*., 2008. Medicinal plants in the Mediterranean area: Synthesis of the results of the project Rubia. J. Ethnopharmacol., 116: 341-357.

Graham, J.G, M.L. Quinn, D.S. Fabricant and N.R. Farnsworth, 2000. Plants used against cancer–an extension of the work of Jonathan Hartwell. J. Ethnopharmacol., 73: 347-377.

Hui-Yin, C., L. Yuh-Charn and H. Chui-Lan, 2007. Evaluation of antioxidant activity of aqeous extract of some selected neutraceutical herbs. Food Chem., 104: 1418-1424.

Hussain, A.I., F. Anwar, S.T.H. Sherazi and R. Przybylski, 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108: 986-995.

Lee, J.C., K.Y. Lee, J. Kim, C.S. Na, N.C. Jung, G.H. Chung and Y.S. Jang, 2004. Extract from *Rhus verniciflua* stokes is capable of inhibiting the growth of human lymphoma cells. Food Chem. Toxicol., 42: 1383-1388.

Maisuthisakul, P., M. Suttajit and R. Pongsawatmanit, 2007. Assessment of phenolic content and free radical-scavenging capacity of some *Thai indigenous* plants. Food Chem., 100: 1409-1418.

Manach, C., G. Williamson, C. Morand, A. Scalbert and C. Rémésy, 2005. Bioavailability and bioefficacy of polyphenols in humans: I. Review of 97 bioavailability studies. Am. J. Clin. Nutr., 81: 230S-242S.

Rahman, I., 2008. Antioxidant therapeutic advances in COPD. Ther. Adv. Respir. Dis., 2: 351-374.

Russo, A., V. Cardile, L. Lombardo, L. Vanella, A. Vanella and J.A. Garbarino, 2005. Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* Sweet roots in human tumor cell lines. J. Ethnopharmacol., 100: 323-332.

Souza, J N.S., E.M. Silva, A. Loir, J.F. Rees, H. Rogez and Y. Larondelle, 2008. Antioxidant capacity of four polyphenol-rich Amazonian plant extracts: A correlation study using chemical and biological *in vitro* assays. Food Chem., 106: 331-339.

Tachakittirungrod, S., S. Okonogi and S. Chowwanapoonpohn, 2007. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leave extract. Food Chem., 103: 381-388.

Toor, R.K., G.P. Savage and C.E. Lister, 2006. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. J. Food Compos. Anal., 19: 1-10.

Trichopoulou, A., S. Soukara and E. Vasilopoulou, 2007. Traditional foods: A science and society perspective. Trends Food Sci. Technol., 18: 420-427.

Tsai, P.J., S.C. Wu and Y.K. Cheng, 2008. Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. Food Chem., 106: 27-32.

Virgili, F., C. Scaccini, L. Packer and G. Rimbach, 2001. Cardiovascular Disease and Nutritional Phenolics. In: Antioxidants in Food, Pokorny, J., N. Yanishlieva and G. Rimbach (Eds.). Woodhead Publishing Ltd., Cambridge, pp: 87-99.

Wang, W., N. Wu, Y.G. Zu and Y.J. Fu, 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem., 108: 1019-1022.

Wojdyło, A., J. Oszmianski and R. Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940-949.

Yen, F.L., T.H. Wua, L.T. Lin, T.M. Cham and C.C. Lin, 2008. Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*. Food Chem., 108: 455-462.