

## ANTIOXIDANT CAPACITY, MINERAL CONTENT AND ESSENTIAL OIL COMPOSITION FROM SELECT ALGERIAN MEDICINAL PLANTS

Hadjira Guenane<sup>1\*</sup>, Abdelaziz Gherib<sup>2</sup>, Boulanouar Bakchiche<sup>1</sup>,  
Ángel A. Carbonell-Barrachina<sup>3</sup>, Francisca Hernández<sup>4</sup>,  
Marina Cano-Lamadrid<sup>3</sup>

<sup>1</sup>University Amar Telidji-Laghouat, Faculty of Technology, B.P 37G, Laghouat  
03000, Algeria

<sup>2</sup>University Amar Telidji-Laghouat, Faculty of Sciences, B.P 37G, Laghouat  
03000, Algeria

<sup>3</sup>Universidad Miguel Hernández de Elche, Departamento Tecnología  
Agroalimentaria, Escuela Politécnica Superior de Orihuela, Carretera de Beniel,  
km 3,2, Orihuela, 03312-Orihuela, Alicante, Spain

<sup>4</sup>Universidad Miguel Hernández de Elche, Departamento de Producción Vegetal  
y Microbiología, Grupo de Fruticultura y Técnicas de Producción, Carretera de  
Beniel, km 3,2, Orihuela, Alicante 03312, Spain

\*Corresponding author: [guenane.hadjira@yahoo.fr](mailto:guenane.hadjira@yahoo.fr), [ha.guenane@lagh-univ.dz](mailto:ha.guenane@lagh-univ.dz)

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**Abstract:** The objective of the present study was to analyze the total antioxidant capacity, minerals contents of four plants (*Juniperus oxycedrus*, *Thymus capitatus*, *Laurus nobilis* and *Eruca vesicaria*) and chemical composition of the essential oils of the aerial parts of *T. capitatus*. Their antioxidant activity was assessed by DPPH, ABTS and FRAPS assays. Total phenol and flavonoid contents of the extracts were also determined. The results showed that the *L. nobilis* extract had the highest total phenolic and flavonoids contents ( $19.11 \pm 0.22$  mg GAE·g<sup>-1</sup> dw,  $4.47 \pm 0.12$  mg QE·g<sup>-1</sup> dw, respectively). The extract of *E. vesicaria* had the highest value of TEAC for scavenging DPPH, whereas *L. nobilis* extract was active for ABTS and FRAP. GC/MS analysis revealed that the essential oil from the aerial parts of *T. capitatus* contained thirty-seven compounds; *thymol* was the major constituent (82.79 %). Atomic absorption spectroscopy showed high levels of Ca, K, Mg and Fe, and trace amounts of Zn, Cu and Mn in all four extracts.

**Keywords:** ABTS, DPPH, FRAP, minerals, phenols, TEAC

## INTRODUCTION

Levels of free radicals and reactive oxygen species generated during metabolism and other activities that exceed the antioxidant capacity of a biological system cause oxidative stress [1] and play a role in heart disease, neurodegenerative diseases, cancer, and the aging process [2]. There is increasing evidence that oxidative damage is involved in the development of chronic, age-related degenerative disorders, and these can be mitigated by dietary antioxidants, thus lowering the risk of disease [3]. Antioxidants are compounds that at low concentrations delay or prevent oxidation of oxidizable substrates [4].

Apart from their health benefits, antioxidants are added to food to avoid oxidation normally initiated by free radicals formed during exposure of the food to environmental factors such as air, light, and heat [5]. Most antioxidants used for this purpose are currently manufactured synthetically (BHA, BHT), and they can cause side effects when ingested [6]. Strict governmental rules regarding food safety imposed the search for alternatives for food preservatives [7]. So, nowadays, a good alternative would be medicinal plants. Plants are a potential source of natural antioxidants. Major natural antioxidants and phytochemical antioxidants are secondary metabolites of plants [8] and include carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, and tocotrienols. Beta-carotene, ascorbic acid, and alpha-tocopherols are widely used antioxidants in the food industry [9].

*Thymbra capitata* (L.) Cav. [= *Thymus capitatus* (L.) Hoffm. Et Link. = *Coridothymus capitatus* Rch. f.] [10] grows widely in the Mediterranean basin and is typical of garrigues, dry slopes, and Mediterranean pine forests [11].

*T. capitatus*, called 'zaatr' in Algeria, is a commonly used spice and exhibits biological properties such as antibacterial, antiviral, and antioxidant activities [12]. *Thymus* is currently used only locally in traditional remedies, and it is mainly exploited for the quality of its essential oil, which is used in folk medicine as a decoction to treat colic, ulcers, and arterial hypertension [13]. Infusions and decoctions prepared from the leaves and flowers are used to treat diarrhea, and digestive and respiratory system disorders [14], and they have antimycotic, antioxidant, antibacterial, and spasmolytic actions [15, 16]. Essential oils and their components are currently of increasing interest because of their relative safety, their wide acceptance by consumers, and their exploitation for potential multi-purpose therapeutic uses.

*Juniperus oxycedrus* (Cupressaceae) is a shrub or small tree that grows wild in stony areas of the Mediterranean and Near East. Juniper berries are used as a spice, particularly in European cuisine, and also to give gin its distinctive flavor. According to one FAO (Food and Agriculture Organization) document [17] *J. oxycedrus* is also used to prepare oil of cade, also known as juniper tar. This oil has been used to treat chronic eczema and other skin diseases and the rectified oil has been used as a fragrance component in detergents, soaps, creams, and lotions [18]. *J. oxycedrus* was used in folk medicine to treat various diseases such as hyperglycemia, obesity, tuberculosis, bronchitis, and pneumonia [19].

*Eruca vesicaria* (rocket or arugula) is an economically important leafy vegetable commonly found in the Mediterranean region, southern Europe and Central Asia [20]. Beside its culinary uses, rocket is also considered a medicinal plant, the extract of both plant and seed possessing diversified therapeutic properties including

antihyperlipidemic, antihyperglycemic, antinephrolithiatic, antiulcer, antiscorbutic, stimulant, stomachic and diuretic [20 – 23]. In addition, rocket has shown great potential as a green manure for controlling pathogenic fungi and parasitic nematodes as it contains chemicals with high biocidal activity that mimic synthetic fumigants [24].

*Laurus nobilis* (Lauraceae), commonly known as bay is an evergreen tree distributed widely in the Mediterranean area and Europe [25]. Bay leaves are often used as a folk medicine for asthma [26], cardiac diseases [25], digestive disorder, diarrhea, and rheumatic pains [27]. These biological activities have been attributed to a wide range of phytochemical such as: non-polar flavonoids, sesquiterpenoid lactones, isoquinoline alkaloids and vitamin E, which that could be used as antioxidant and antimicrobial compounds [28].

For all the above reasons, the main aim of this work was to evaluate the functional properties of some Algerian medicinal plants. For that, the major volatile compounds of the *T. capitatus* essential oil and mineral content and the antioxidant properties of *T. capitatus*, *J. oxycedrus*, *L. nobilis* and *E. vesicaria*, were determined.

## MATERIAL AND METHODS

### Chemicals

All the reagents and chemicals used in the experiments were of analytical grade. The chemicals DPPH (2,2-diphenyl-1-picrylhydrazyl radical), ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation), and Folin-Ciocalteu reagent, were obtained from Sigma Chemical Co., USA. Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane 2-carboxylic acid), quercetin were obtained from Aldrich Chemicals Co., U.S.A. All other chemicals used were obtained from the Spain suppliers.

### Plant material

*E. vesicaria*, *T. capitatus*, *J. oxycedrus* and *L. nobilis* were selected based on their beneficial use in traditional medicinal, their abundance in nature, and their potential for sustainable cultivation. The plants were collected in Laghouat, Algeria and air-dried at room temperature (20 - 25 °C) for one week, then stored in cloth bags.

### Extraction of essential oils

Essential oils were extracted by hydrodistillation of the dried plant material (100 g of sample in 500 mL of distilled water) for 4 h using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4 °C in amber glass vials until analysis.

### Chromatographic analysis

Chromatographic analyses of the plant extracts (isolation and identification of the volatile compounds) were performed on a gas chromatograph-mass spectrometer (GC-MS; GC-17A, Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer

detector (QP-5050A, Shimadzu). The GC–MS system was equipped with a TRACSIL Meta.X5 column (Teknokroma, Barcelona, Spain) packed with 95 % polydimethylsiloxane and 5 % polydiphenylsiloxane; 60 m × 0.25 mm, 0.25 µm film thickness. Analyses were carried out using helium as a carrier gas at a column flow rate of 0.3 mL·min<sup>-1</sup> and a split ratio of 1:11. The oven program was as follows: 80 °C, 0 min; a ramp of 3.0 °C·min<sup>-1</sup> from 80 to 210 °C and hold for 1 min; a ramp of 25 °C·min<sup>-1</sup> from 210 to 300 °C and hold for 6 min. The temperatures of the injector and detector were 230 and 300 °C, respectively.

Most compounds were identified by their retention indices, GC–MS retention times (authentic standards of all compounds were used for identification) and mass spectra (authentic chemicals and NIST05 spectral library collection; [29] NIST 2012). Analyses were run in triplicate. The concentration of each compound was expressed as a percentage of the total arbitrary area units, and it is referred to as the relative concentration.

### **Preparation of plant extracts**

Antioxidant compounds were extracted and analyzed by using the method reported by Chong *et al.* [30]. Briefly, dried plant material (0.5 g) was mixed with of MeOH/water (80:20 v/v) + 1 % HCl (10 mL), sonicated at 20 °C for 15 min, then left for 24 h at 4 °C. The extract was sonicated for 15 min, and then centrifuged at 15000 rpm for 10 min. The supernatant was collected and used to analyze total phenolic content and antioxidant capacity.

### **Total phenolic content**

Total phenolic content (TPC) was quantified by using the Folin-Ciocalteu colorimetric method described previously by Gao *et al.* [31]. Plant extract (0.1 mL) was mixed with Folin-Ciocalteu reagent (0.2 mL) and ultrapure water (2 mL), and then the mixture was incubated at room temperature for 3 min and 20 % sodium carbonate (1 mL) was added. TPC was determined after 1 h of incubation at room temperature. The absorbance of the resulting blue color was measured at 765 nm with a UV-Vis spectrophotometer (Uvikon XS, Bio-Tek Instruments, Saint Quentin Yvelines, France). TPC was quantified by using a gallic acid standard curve and the results were expressed as gallic acid equivalents (GAE), as milligrams of GAE per g dry weight.

### **Total flavonoids**

Flavonoid content was identified as described by Ahn *et al.* [32]. Briefly, 1 mL of 2 % AlCl<sub>3</sub> solution was added to 1 mL of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as standard for the construction of calibration curve.

### **Antioxidant capacity**

The antioxidant activity (AOC) was evaluated using the DPPH, ABTS, and ferric reducing antioxidant power (FRAP) methods. The DPPH radical method described by

Brand-Williams *et al.* [33] was used with a modified reaction time. Briefly, the supernatant (10  $\mu$ L) was mixed with MeOH (40  $\mu$ L) and added to DPPH solution (950  $\mu$ L). The mixture was shaken vigorously and incubated in the dark for 10 min. The decrease in absorbance was measured at 515 nm using the UV-Vis spectrophotometer. AOC was also evaluated by using the ABTS radical cation [34] and FRAP method [35]. Briefly, the supernatant (10  $\mu$ L) was mixed with ABTS or FRAP solution (990  $\mu$ L). After 10 min of reaction, the absorbance was measured at 734 nm for ABTS and 593 nm for FRAP by using the UV-Vis spectrophotometer. Calibration curve in the range 0.01 - 5.00 mmol Trolox/L was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as Trolox equivalent antioxidant capacity (TEAC); it is the concentration of Trolox required to give the same antioxidant capacity as 1 mM test substance) [36]

### Mineral analysis

Dried samples (0.5 g) were digested for 3 h at a temperature below 130 °C in 65 % HNO<sub>3</sub> (5 mL) by using a multi-sample digestion block (Block Digest 20, Selecta, Barcelona, Spain) [37]. Samples were cooled to room temperature, transferred to volumetric flasks, and then diluted with ultra-high-purity deionized water. Samples were stored at 4 °C until analysis. Ca, Mg, K, Cu, Fe, Mn, and Zn in previously mineralized samples were analyzed with an atomic absorption-emission spectrometer (Solaar 969, Unicam Ltd., Cambridge, UK). K and Na were analyzed by atomic emission and the other elements were analyzed by atomic absorption. The instrument was calibrated with certified standards. At least two reagent blanks were included in each batch of samples to assess the precision and accuracy of the chemical analysis. Calibration curves were used to quantify these elements and showed good linearity ( $R^2 \geq 0.997$ ). Analyses were run in triplicate.

### Statistical analysis

Experimental results are presented as the mean  $\pm$  standard error of three parallel measurements. Statistical analyses were performed by one-way ANOVA, followed by Tukey multiple comparisons test. A difference was considered to be statistically significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Chemical composition

The compounds in the essential oils were identified by comparing their GC-MS retention data with the retention indexes of ten *n*-alkanes used as internal standards, resulting in substantially improved identification of the compounds, and particularly those with similar fragmentation patterns [38]. Table 1 shows the chemical compounds, their relative percentage of the total chromatogram area, and their Kovats index of oil distilled from the aerial parts of *T. capitatus*. Thirty seven compounds were identified, of which the monoterpenes comprised the major fraction: thymol (82.79 %),  $\gamma$ -terpinene

(4.76 %), *p*-cymene (3.9 %), and sesquiterpenes ( $\beta$ -caryophyllene, 1.90 %). Other compounds were present at levels below 1 %.

**Table 1.** Volatile compounds in the essential oil extracted from the aerial parts of *T. capitatus* collected from Laghouat, Algeria

Compound <sup>†</sup>	Retention Time (min)	Retention Indexes		Concentration (% total area)
		Exp.	Lit.	
Methyl isovalerate	9.15	792	793	0.01
1-Hepten-3-ol	11.40	899	na <sup>‡</sup>	0.00
$\alpha$ -Thujene	12.55	954	935	0.27
$\alpha$ -Pinene	12.92	948	939	0.36
Camphene	13.58	954	954	0.06
$\beta$ -Myrcene	14.46	983	991	0.75
3-Octanol	14.91	997	996	0.03
$\alpha$ -Phellandrene	15.40	1007	1003	0.09
$\beta$ -Ocimene	15.52	1010	1023	0.02
$\alpha$ -Terpinene	15.81	1017	1017	0.72
<i>p</i> -Cymene	16.29	1029	1024	3.93
Limonene	16.36	1031	1029	0.17
$\beta$ -Phellandrene	16.52	1035	1030	0.11
1,8-Cineole	16.72	1040	1031	0.08
$\gamma$ -Terpinene	17.60	1062	1060	4.76
1-Octen-3-ol	18.30	1080	1072	0.06
Terpinolene	18.64	1088	1089	0.08
Linalool	19.28	1094	1097	1.30
Terpinen-4-ol	23.48	1186	1191	1.01
Thymyl methyl ether	25.68	1233	1232	0.09
Carvone	26.22	1244	1243	0.05
<i>trans</i> -Dihydrocarvone	26.81	1257	1242	0.04
Nerol	27.28	1267	1254	0.01
Neral	28.39	1290	1272	0.04
Thymol	29.00	1304	1293	82.79
Eugenol	32.09	1370	1370	0.06
Carvacrol	32.20	1373	1300	0.11
$\alpha$ -Gurjunene	34.10	1414	1413	0.06
$\beta$ -Caryophyllene	34.92	1432	1419	1.90
Aromadendrene	35.65	1448	1440	0.08
$\alpha$ -Humulene	36.46	1466	1462	0.08
Ledene	37.90	1500	1493	0.09
$\beta$ -Bisabolene	38.12	1505	1505	0.06
$\gamma$ -Cadinene	38.85	1522	1513	0.05
$\gamma$ -Bisabolene	39.47	1537	1543	0.03
Spathulenol	42.11	1599	1591	0.10
Caryophyllene oxide	42.44	1607	1594	0.55

<sup>†</sup>Compounds are listed in the order of their elution time from a TRACSIL Meta.X5 column

<sup>‡</sup>na = not available

A previous study of oil distilled from *T. capitatus* plants collected in Tunisia showed carvacrol as the major component [39], in contrast to our finding that thymol is the

major component. This discrepancy may be caused by differences in extraction procedure, time of harvest, location of growth, environmental factors, and differences in the plants such as genetic factors, age, sample part, and developmental stage. In addition, the plants differed in chemotype, which influences the plant biosynthetic pathways and thus the relative proportion of the principal compounds.

### Total phenolic content

In the present work, the extraction of dried plants was performed using hydro-alcoholic solvent and sonication as method of extraction, the Folin-Ciocalteu method was used to measure the total polyphenolic content (TPC) of the plant extracts and was expressed as milligrams of GAE per g dry weight.

The Folin-Ciocalteu method is an electron transfer based assay, and gives reducing capacity which has normally been expressed as phenolic contents. The TPC is an important indicator of AOC and can be used as a preliminary screen of natural sources of antioxidants for addition to functional foods [40].

The results are presented in Table 2. Among plants extracts, the highest total polyphenolic levels have been detected in *L. nobilis*, whereas the lowest levels in *E. vesicaria*. Phenolic contents of *L. nobilis*, *J. oxycedrus*, *T. capitatus* and *E. vesicaria* extracts were statistically similar ( $p > 0.05$ ) ( $19.11 \pm 0.22$  mg GAE·g<sup>-1</sup> dw,  $18.34 \pm 0.16$  mg GAE·g<sup>-1</sup> dw,  $17.99 \pm 0.31$  mg GAE·g<sup>-1</sup> dw,  $16.41 \pm 0.56$  mg GAE·g<sup>-1</sup> dw respectively).

The total phenolic content in the plants tested in this study was different than that reported by Ouchikh *et al.* [41], Chaouche *et al.* [42] and El Ouariachi *et al.* [43].

**Table 2.** Total phenolics and flavonoids content in the medicinal plant extracts

Plants	Part used	TPC (mg GAE·g <sup>-1</sup> dw)	FT (mg QE·g <sup>-1</sup> dw)
<i>E. vesicaria</i>	Aerial parts	$16.41 \pm 0.56^a$	$1.23 \pm 0.03^d$
<i>J. oxycedrus</i>	Needles, branches	$18.34 \pm 0.16^a$	$1.68 \pm 0.01^c$
<i>T. capitatus</i>	Aerial parts	$17.99 \pm 0.31^a$	$2.97 \pm 0.02^b$
<i>L. nobilis</i>	Leaves	$19.11 \pm 0.22^a$	$4.47 \pm 0.12^a$

<sup>a</sup>Each value in the table is represented as mean  $\pm$  SE (n = 3). GAE, gallic acid equivalents; QE, quercetin equivalents; dw, dry weight. Means followed by the same letter are not different according to ANOVA (analysis of variance) ( $p < 0.05$ ). The results are sorted in decreasing order: a > b > c > d

Variation in the amounts of phenolic compounds could be attributed to several reasons. The solubility of phenolic compounds is actually governed by the type of solvent used, the degree of polymerization of phenolics, as well as by the interaction of phenolics with other food constituents and formation of insoluble complex, genotypic factors that control accumulation of these compounds in the plant [44] and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples and determination methods [45, 46]. Moreover, other studies suggested that the biotic conditions (species, organ and physiological stage) and abiotic stresses (salinity, luminosity, water deficit and edaphic factors) widely present in the arid zone may enhance the phenolic metabolism as a response to oxidative stress [47].

Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups [48]. It is known that polyphenolic compounds have inhibitory

effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables [49]. Phenolic compounds from plants are known to be good natural anti-oxidants. However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants [50].

### **Flavonoids content**

Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics, which include the following classes: flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones. The role of flavonoids in plants is ubiquitous, including strong antioxidant and radical scavenging potential, as well as pollinator attraction in the case of pigments [51, 52]. Flavonoids are distributed in all plant organs. Flavonoid composition and quantity vary during ontogenesis and is believed to be dependent on both genetic and environmental factors [53]. Together with phenol acids, the flavonoids are important secondary metabolites for maintaining plant biochemical communication processes in disturbing ecosystems [54]. Flavonoids are known to have various functions in growth, development, and also in diverse stress responses in plants. It was reported that flavonoids pathway genes expression and accumulation of flavonoids compounds may be closely related to drought tolerance [55].

The results are presented in Table 2 and were expressed as mg of quercetin equivalent per milligram of dry weight. The total flavonoid contents in the medicinal plant methanolic extracts ranged from  $1.23 \pm 0.03$  to  $4.47 \pm 0.12$  mg QE·g<sup>-1</sup>. *L. nobilis* had the highest total flavonoid content ( $4.47 \pm 0.12$  mg QE·g<sup>-1</sup>), followed by *T. capitatus* ( $2.97 \pm 0.02$  mg QE·g<sup>-1</sup>) and *J. oxycedrus* ( $1.68 \pm 0.01$  mg QE·g<sup>-1</sup>), whereas the lowest content was in *E. vesicaria* ( $1.23 \pm 0.03$  mg QE·g<sup>-1</sup>) in their methanolic extracts. The total flavonoids content of the plants that we studied were lower to the literature values [41 – 43].

Differences in concentration of flavonoids in studied species are linked to different phylogeny, morphology, as well as physiological and molecular determinants in responses to environmental stresses, including capacity for their synthesis and accumulation which was already reported for a number of plants exposed to conditions out of the ecological optimum [54], including salinity [47] and drought [56].

### **Antioxidant activity**

Antioxidants in plants, and particularly free radical scavenging such as polyphenols, flavonoids and phenolic compounds, avoid the deleterious effects of oxidative stress. Free radicals play an important role in several diseases and the aging process. Antioxidants either counteract these free radicals or protect the body's antioxidant defense mechanisms, and thus help maintain the health of the organism. In addition to their therapeutic properties, medicinal plants are a source of a range of antioxidants that could meet the increasing demand for raw materials containing natural, potent antioxidants such as polyphenols. The antioxidant activity of polyphenols arises from their molecular structure, which comprises aromatic rings bearing several hydroxyl groups, allowing the breakdown of cellular oxidative and nitrosative cascades.

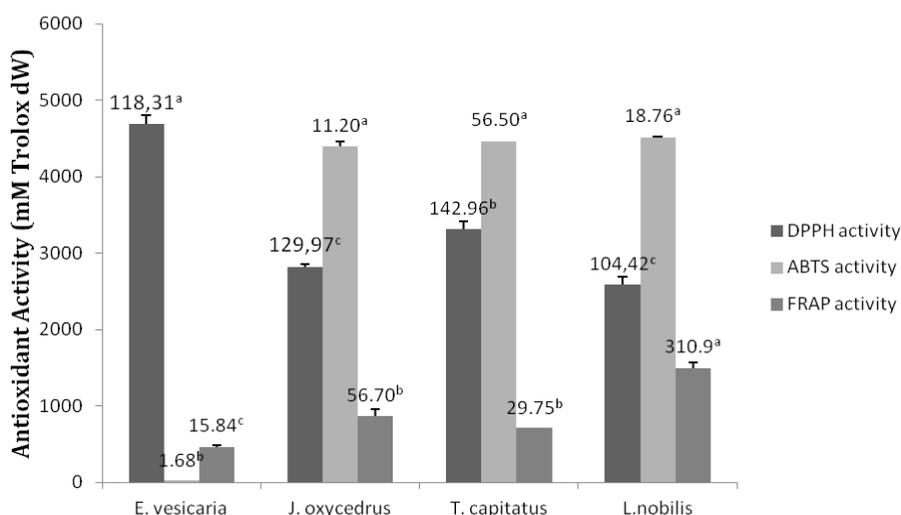
Polyphenols interact with and modulate enzymatic activities, thereby regulating the signal pathways controlling cell survival and death [57].

The antioxidant capacities of extracts of *E. vesicaria*, *J. oxycedrus*, *T. capitatus* and *L. nobilis* were determined by measuring reducing power and scavenging capacity against DPPH and ABTS radicals. The results, expressed as mM Trolox dry weight, are shown in Table 3. All the plant extracts showed a propensity to quench the free radicals, some greater extent than in others, however the three maximum values found in the DPPH, FRAP, and ABTS tests were for extracts *E. vesicaria*, *L. nobilis* respectively indicating high antioxidant potency of these extracts (Figure 1).

**Table 3.** Antioxidant activity of the four plant extracts, expressed as TEAC (mM Trolox dw) and determined by DPPH, ABTS and FRAP assays

Plants	DPPH (mM Trolox dw)	ABTS (mM Trolox dw)	FRAP (mM Trolox dw)
<i>E. vesicaria</i>	4687.43 ± 118.3 <sup>a</sup>	23.62 ± 1.68 <sup>b</sup>	467.42 ± 15.84 <sup>c</sup>
<i>J. oxycedrus</i>	2823.70 ± 129.97 <sup>c</sup>	4397.71 ± 11.20 <sup>a</sup>	870.17 ± 56.70 <sup>b</sup>
<i>T. capitatus</i>	3314.77 ± 142.96 <sup>b</sup>	4463.88 ± 56.50 <sup>a</sup>	711.43 ± 29.75 <sup>b</sup>
<i>L. nobilis</i>	2593.15 ± 104.42 <sup>c</sup>	4514.03 ± 18.76 <sup>a</sup>	1498.05 ± 310.9 <sup>a</sup>

<sup>a</sup>Each value in the table is represented as mean ± SE (n = 3). <sup>‡</sup> Values in the same column followed by a different letter are significantly different (p < 0.05). TEAC: Trolox equivalent antioxidant capacity; dw: dry weight



**Figure 1.** Antioxidant activity of the different plants extracts (DPPH, ABTS, FRAP). Values are means of triplicate determinations ± standard error. Mean values followed by different superscript in the same column are significantly (P < 0.05) different

The DPPH assay is a quick, reliable, reproducible parameter to determine the in vitro general antioxidant activity of pure compounds and plant extracts [58, 59]. DPPH is a protonated radical exhibiting a characteristic absorption maxim at 517 nm, which decreases as the proton radicals are scavenged by antioxidants, for example natural plant extracts. All four plant extracts showed high scavenging activity against DPPH in the order *E. vesicaria* (4687.43 ± 118.31 mM Trolox dw) > *T. capitatus* (3314.77 ± 142.96 mM Trolox dw) > *J. oxycedrus* (2823.70 ± 129.97 mM Trolox dw) > *L. nobilis* (2593.15 ± 104.42 mM Trolox dw). There were no significant differences (p > 0.05)

between these values (Table 3). The antioxidant capacity of plant extract may be due to the hydrogen donating ability of phenols and flavonoids present in it.

ABTS is one of the radicals generally used for testing the preliminary radical scavenging activity of a compound or plant extract. The  $ABTS^{\bullet+}$ , generated from oxidation of ABTS by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxy radicals) [60]. The results were expressed as mM Trolox dm dry weight of plant material. *L. nobilis*, *T. capitatus* and *J. oxycedrus* exhibited the highest antioxidant capacity in the ABTS assay ( $4514.03 \pm 18.76$  mM Trolox dw,  $4463.88 \pm 56.50$  mM Trolox dw and  $4397.71 \pm 11.20$  mM Trolox dw, respectively) and they were not significantly different ( $p > 0.05$ ), whereas *E. vesicaria* exhibited significantly lower activity ( $23.62 \pm 1.68$  mM Trolox dw) (Table 3).

We investigated the reducing capacity of medicinal plants by measuring  $Fe^{3+}$ - $Fe^{2+}$  conversion [61]. The antioxidant activities of putative antioxidants have been attributed to various mechanisms, such as the prevention of chain initiation, transition metal ion catalyst binding, peroxides decomposition, prevention of continued proton abstraction, and radical scavenging [62]. The reducing capacity of compounds can indicate their antioxidant potential [61], and a high absorbance at a high concentration of compound indicates strong reducing activity. The reducing abilities of the extracts as determined with the FRAP assay followed the order *E. vesicaria* ( $467.42 \pm 15.84$  mM Trolox dw)  $<$  *T. capitatus* ( $711.43 \pm 29.75$  mM Trolox dw)  $<$  *J. oxycedrus* ( $870.17 \pm 56.70$  mM Trolox dw)  $<$  *L. nobilis* ( $1498.05 \pm 310.69$  mM Trolox dw) (Table 3).

### Mineral composition

Table 4 shows the mineral elements into macroelements (K, Mg and Ca) and microelements (Mn, Cu, Fe and Zn) and show that the four extracts contained different amounts of them, caused by differences between the plant species, the mineral composition of the soil in which the plants grew and the element uptake preference of the plants.

The highest levels of Ca, K, Mg, Zn and Mn were found in *E. vesicaria*, whereas the levels of Fe and Cu were highest in *J. oxycedrus*. The level of Ca in *E. vesicaria* ( $23811.84 \pm 486.95$  mg·kg<sup>-1</sup>) was higher than in *T. capitatus* ( $15154.95 \pm 951.09$  mg·kg<sup>-1</sup>) and much higher than in *L. nobilis* ( $7958.92 \pm 143.14$  mg·kg<sup>-1</sup>) and *J. oxycedrus* ( $4117.42 \pm 143.15$  mg·kg<sup>-1</sup>) which were significantly different ( $p < 0.05$ ).

Potassium content of *E. vesicaria* extracts ( $19013.67 \pm 854.58$  mg·kg<sup>-1</sup>) was higher than that of *T. capitatus* ( $6981.58 \pm 731.50$  mg·kg<sup>-1</sup>), *L. nobilis* ( $6665.92 \pm 286.00$  mg·kg<sup>-1</sup>) and much higher than in *J. oxycedrus* ( $1374.03 \pm 165.03$  mg·kg<sup>-1</sup>).

The magnesium levels in the extracts followed the order *E. vesicaria* ( $3861.42 \pm 106.23$  mg·kg<sup>-1</sup>)  $>$  *T. capitatus* ( $2850.43 \pm 144.72$  mg·kg<sup>-1</sup>)  $>$  *J. oxycedrus* ( $2018.56 \pm 22.16$  mg·kg<sup>-1</sup>)  $>$  *L. nobilis* ( $1605.97 \pm 19.45$  mg·kg<sup>-1</sup>) which were significantly different ( $p < 0.05$ ).

**Table 4.** Mineral content of four plant extracts: *E. vesicaria*, *J. oxycedrus*, *T. capitatus* and *L. nobilis*

Macroelements (mg·kg <sup>-1</sup> dw)				
	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	
<i>E. vesicaria</i>	23811.84 <sup>†</sup> ± 486.95 <sup>‡a</sup>	3861.42 ± 106.23 <sup>a</sup>	19013.67 ± 854.58 <sup>a</sup>	
<i>J. oxycedrus</i>	4117.42 ± 143.15 <sup>d</sup>	2018.56 ± 22.16 <sup>c</sup>	1374.03 ± 165.03 <sup>c</sup>	
<i>T. capitatus</i>	15154.95 ± 951.09 <sup>b</sup>	2850.43 ± 144.72 <sup>b</sup>	6981.58 ± 731.50 <sup>b</sup>	
<i>L. nobilis</i>	7958.92 ± 143.14 <sup>c</sup>	1605.97 ± 19.45 <sup>d</sup>	6665.92 ± 286.00 <sup>b</sup>	
Micro-elements (mg·kg <sup>-1</sup> dw)				
	Iron (Fe)	Zinc (Zn)	Copper (Cu)	Manganese (Mn)
<i>E. vesicaria</i>	151.00 ± 14.12 <sup>c</sup>	56.46 ± 0.70 <sup>a</sup>	23.09 ± 0.95 <sup>b</sup>	32.84 ± 0.35 <sup>a</sup>
<i>J. oxycedrus</i>	454.59 ± 13.85 <sup>a</sup>	38.99 ± 1.26 <sup>b</sup>	38.27 ± 1.35 <sup>a</sup>	18.96 ± 0.36 <sup>c</sup>
<i>T. capitatus</i>	394.19 ± 16.82 <sup>b</sup>	39.33 ± 0.84 <sup>b</sup>	9.96 ± 0.45 <sup>c</sup>	21.49 ± 0.56 <sup>b</sup>
<i>L. nobilis</i>	162.12 ± 9.9 <sup>c</sup>	32.88 ± 1.04 <sup>c</sup>	20.36 ± 1.06 <sup>b</sup>	10.94 ± 0.30 <sup>d</sup>

<sup>†</sup>Values are presented as mean ± standard error of three replicates. <sup>‡</sup>Values followed by the same letter within a column are not statistically different according to Tukey's multiple range test

The concentrations of microelements (heavy metals), including Fe, Mn, Cu and Zn, were too low to be determined in the samples. The Fe content in the four extracts followed the order *J. oxycedrus* (454.59 ± 13.85 mg·kg<sup>-1</sup>) > *T. capitatus* (394.19 ± 16.82 mg·kg<sup>-1</sup>) > *L. nobilis* (162.12 ± 9.92 mg·kg<sup>-1</sup>) > *E. vesicaria* (151.00 ± 14.13 mg·kg<sup>-1</sup>).

Zinc content of *E. vesicaria* extracts (56.46 ± 0.70 mg·kg<sup>-1</sup>) was higher than that of *T. capitatus* (39.80 ± 0.97 mg·kg<sup>-1</sup>), *J. oxycedrus* (38.99 ± 1.26 mg·kg<sup>-1</sup>) and *L. nobilis* (32.88 ± 1.04 mg·kg<sup>-1</sup>).

The levels of copper in the extracts followed the order *J. oxycedrus* (38.27 ± 1.35 mg·kg<sup>-1</sup>) > *E. vesicaria* (23.09 ± 0.95 mg·kg<sup>-1</sup>) > *L. nobilis* (20.36 ± 1.06 mg·kg<sup>-1</sup>) > *T. capitatus* (9.96 ± 0.45 mg·kg<sup>-1</sup>).

The amount of manganese in the plants extracts was significantly different (p < 0.05): *E. vesicaria* has the highest manganese content (32.84 ± 0.35 mg·kg<sup>-1</sup>), followed by *T. capitatus* (21.49 ± 0.56 mg·kg<sup>-1</sup>), *J. oxycedrus* (18.96 ± 0.36 mg·kg<sup>-1</sup>) and *L. nobilis* (10.94 ± 0.30 mg·kg<sup>-1</sup>).

Our results of mineral contents of *E. vesicaria* extracts show minor differences when compared with literature [63] and higher than the results published by Nilufer O. *et al.* [64]; Ereifej *et al.* [65]; Ujowundu *et al.* [66] of *J. oxycedrus* extracts, *T. capitatus* and *L. nobilis* respectively. These differences might be due to growth conditions, genetic factors, geographical variations and analytical procedures [67, 68].

## CONCLUSION

To assess the antioxidant capacity of medicinal plant extracts, a variety of methods based on different mechanistic principles must be used in parallel, because different methods often give different results. The best amounts of total phenolics and total flavonoids were for *L. nobilis* extract and maximum values of TEAC found in DPPH, FRAP, ABTS tests were for *L. nobilis*, *E. vesicaria*, *T. capitatus*.

The appreciable concentrations of minerals such as potassium, calcium and magnesium obtained in these plants are interesting. It showed that these plants hold tremendous promise in providing the variable secondary metabolites and mineral supply that could enhance the curative process of ill health.

The obtained results are useful to further research such as the identification of specific polyphenolic compounds responsible for the antioxidant activities and to allow studying their structure-function interactions and enabling the development of functional products.

## REFERENCES

1. Zheng, W., Wang, S.Y.: Antioxidant activity and phenolic compounds in selected herbs, *Journal of Agricultural and Food Chemistry*, **2001**, 49, 5165-5170;
2. Astley, S.B.: Dietary antioxidants past, present and future, *Trends Food Scientific Technoology*, **2003**, 14, 93-98;
3. Atoui, A.K., Mansouri, A., Boskou, G., Kefalas, P.: Tea and herbal infusions: their antioxidant activity and phenolic profile, *Food Chemistry*, **2005**, 89 (1), 27-36;
4. Alasalvar, C., Shahidi, F.: *Tree Nuts: Composition, Phytochemicals and Health Effects*, CRC Press, Boca Raton, **2008**;
5. Hras, A.R., Hadolin, M., Knez, Z., Bauman, D.: Comparison of antioxidative and synergistic effects of rosemary extract with alpha-tocopherol, ascorbylpalmitate and citric acid in sunflower oil, *Food Chemistry*, **2000**, 71, 229-233;
6. Chen, C., Pearson, A.M., Gray, J.I.: Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds, *Food Chemistry*, **1992**, 43, 177-183;
7. Ramamoorthy, P.K.: Antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processes, *Journal of Engineering Science and Technology*, **2007**, 2 (1), 70-80;
8. Selvam, K., Arunprakash, S., Selvankumar, T., Govarathanan, M., Sengottaiyan, A.: Antioxidant prospective and secondary metabolites in abutilon indicum at different environment, *International Journal of Pharmaceutical Sciences and Research*, **2012**, 3 (7), 2011-2017;
9. Nanditha, B., Prabhasankar, P.: Antioxidants in bakery products: A review, *Critical Reviews in Food Science and Nutrition*, **2009**, 49, 1-27;
10. Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Salgueiro, L., Miguel, M.G., Faleiro, M.L.: Portuguese Thymbraand Thymus species volatiles: chemical composition and biological activities, *Current Pharmaceutical Design*, **2008**, 14, 3120-3140;
11. Miceli, A., Negro, C., Tommasi, L.: Essential oil variability in *Thymbracapitata* (L.) Cav. growing wild in Southern Apulia (Italy), *Biochemical Systematics and Ecology*, **2006**, 34, 528-535;
12. Miura, K., Nakatani, N.: Antioxidative activity of flavonoids from thyme (*Thymus vulgaris* L), *Agricultural and Biological Chemistry*, **1989**, 53, 3043-3045;
13. Bouraoui, O.: *Traditional medicine in the governorate of Sousse*, Medicine Thesis, Sousse, Tunisia, Faculty of Medicine of Sousse, **2000**;
14. Stahl-Biskup, E., Saez, F.: *Thyme: The Genus Thymus*, Taylor & Francis, London, **2002**;
15. Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, I.G.: Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff et Link, *Food Chemistry*, **2007**, 105, 146-155;
16. Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., Bruni, R.: Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods, *Food Chemistry*, **2005**, 91, 621-632;
17. Loizzo, M.R., Rosa, T., Conforti, F., Saab, A.M., Statti, G.A., Menichini, F.: Comparative chemical composition, antioxidant and hypoglycaemic activities of *Juniperus oxycedrus* ssp. *oxycedrus* L. berry and wood oils from Lebanon, *Food Chemistry*, **2007**, 105, 572-578;

18. Leung, A.Y., Foster, S.: *Encyclopedia of common natural ingredients*, John Wiley & Sons, Inc., New York, **1996**;
19. Sanchez de Medina, F., Gamez, M. J., Jimenez, I., Jimenez, J., Osuna, J. I., Zarzuelo, A.: Hypoglycemic activity of juniper berries, *Planta Medica*, **1994**, 60, 197-200;
20. Pignone, D.: Present status of rocket genetic resources and conservation activities in: *Rocket, a Mediterranean crop for the world* (Editors: Padulosi S., Pignone D.), International Plant Genetic Research Institute, Rome, **1997**, 2-12 ;
21. Hungard, B.L., Goldstein, D.B., Villegas, F., Cooper, T.: The ganglioside GM 1 reduces ethanol induced phospholipase activity in synaptosomal preparation from mice, *Neurochemistry International*, **1988**, 25, 321-325;
22. Bukhsh, E., Malik, S.A., Ahmad, S.S.: Estimation of nutritional value and trace element contents of *Carthamusoxycantha*, *Eruca sativa* and *Plantagoovata*, *Pakistan Journal of Botany*, **2007**, 39 (4), 1181-1187;
23. Alqasoumi, S., Al-Sohaibani, M., Al-Howiriny, T., Al-Yahya, M., Rafatullah, S.: Rocket (*Eruca sativa*): A salad herb with potential gastric anti-ulcer activity, *World Journal of Gastroenterology*, **2009**, 15 (16), 1958-1965;
24. Ekaterini, R.: Performance of arugula (*Eruca sativa*) as a green manure and trap crop for fungal pathogens and parasitic nematode suppression in potato, *American Phytopathological Society*, **2006**, (abstract) 96S (9), 1-2;
25. Dall'Acqua, S., Viola, G., Giorgetti, M., Loi, M.C., Innocenti, G.: Two new sesquiterpene lactones from the leaves of *Laurus nobilis*, *Chemical and Pharmaceutical Bulletin*, **2006**, 54, 1187-1189;
26. Loi, M.C., Poli, F., Sacchetti, G., Seleno, M.B, Ballero, M.: Ethnopharmacology of ogliastra (Villagrande Strisaili, Sardinia, Italy), *Fitoterapia*, **2004**, 75, 277-295;
27. Bruni, A., Ballero, M., Poli, F.: Quantitative ethnopharmacological study of the Campidano valley and Urzulei district, Sardinia, Italy, *Journal of Ethnopharmacol*, **1997**, 57, 97-124;
28. Elmastas, M., Gülcin, I., Isildak, O., Küfrevioğlu, Ö.İ., İbaoglu, K., Aboul-Enein, H.Y.: Radical scavenging activity and antioxidant capacity of bay leaf extracts, *Journal of the Iranian Chemical Society*, **2006**, 3, 258-266;
29. <http://webbook.nist.gov/chemistry/name-ser.html>, NIST (National Institute of Standards and Technology), accessed January 7, **2012**;
30. Chong, C.H., Law, C.L., Figiel, A., Wojdyło, A., Oziembłowski, M.: Colour, phenolic content and antioxidant capacity of some fruits dehydrated by a combination of different methods, *Food Chemistry*, **2013**, 141, 3889-3896;
31. Gao, X., Ohlander, M., Jeppsson, N., Bjork, L., Trajkovski, V.: Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation, *Journal of Agricultural and Food Chemistry*, **2000**, 48, 1485-1490;
32. Ahn, M.R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., Nakayama, T.: Antioxidant activity and constituents of propolis collected in various areas of China, *Food Chemistry*, **2007**, 101, 1383-1392;
33. Brand-Williams, W., Cuvelier, M.E., Berset, C.: Use of free radical method to evaluate antioxidant activity, *Lebensmittel-Wissenschaft und Technology*, **1995**, 28, 25-30;
34. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biology and Medicine*, **1999**, 26, 1231-1237;
35. Benzie, I.F.F., Strain, J.: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Analytical Biochemistry*, **1996**, 239, 70-76;
36. Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M., Kader, A.A.: Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing, *Journal of Agricultural and Food Chemistry*, **2000**, 48 (10), 4581-4589;
37. Carbonell-Barrachina, A. A., Garcia, E., Sanchez-Soriano, J., Aracil, P., Burlo F.: Effects of raw materials, ingredients and production lines on arsenic and copper concentrations in confectionery products, *Journal of Agricultural and Food Chemistry*, **2002**, 50, 3738-3742;
38. De Lima, S.G., Neto, J.M.M., Cito, A.M.G.L., Da Costa, J.G.M., Reis, F.A.M.: Monoterpenes, sesquiterpenes and fatty acids from *Julocroton triquetra* (Euphorbiaceae) from Ceara - Brazil, *Journal of the Chilean Chemical Society*, **2009**, 53, 1718-1720;

39. Lassaad, H., Mehrez, R., Henri, P., Manef, A.: Towards gas chromatography–mass spectrometry coupling protocols for both identifying and quantification essential oils of *Thymus capitatus* Hoff et Link, *Journal of Chromatography A*, **2005**, 1064, 129-134;
40. Viuda-Martos, M., Ruiz-Navajas, Y., Sánchez-Zapata, E., Fernández-López, J., Pérez-Alvarez, J.A.: Antioxidant activity of essential oils of five spice plants widely used in Mediterranean diet, *Flavour and Fragrance Journal*, **2010**, 25, 13-19;
41. Ouchikh, O., Chahed, T., Ksouri, R., Ben Taarit, M., Faleh, H., Abdelly, Ch., Kchouk, M.E., Marzouk, B.: The effects of extraction method on the measured tocopherol level and antioxidant activity of *L. nobilis* vegetative organs, *Journal of Food Composition and Analysis*, **2011**, 24, 103-110;
42. Chaouche, T.M., Haddouchia, F., Atik-Bekaraa, F., Ksouri, R., Azzi, R., Boucherit, O.Z., Choukri, T., Romain, L.: Antioxidant, haemolytic activities and HPLC–DAD–ESI–MS characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp, *Industrial Crops Products*, **2015**, 64, 182-187;
43. El Ouariachi, E.M, Paolini, J. , Bouyanzer, A., Tomi, P., Hammouti, B., Salghi, R., Majidi, L., Costa, J.: Chemical composition and antioxidant activity of essential oils and solvent extracts of *Thymus capitatus* (L.) Hoffmanns and link from Morocco, *Journal of Medicinal Plants Research*, **2011**, 5 (24), 5773-5778;
44. El-Waziry, A.M.: Nutritive Value Assessment of Ensiling or Mixing Acacia and Atriplex Using in vitro Gas Production Technique, *Research Journal of Biological Sciences*, **2007**, 3(6), 605-614;
45. Kim, D.O., Lee, C.Y.: Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship, *Critical Reviews in Food Science and Nutrition*, **2004**, 44 (4), 253-273;
46. Shan, B., Cai, Y.Z., Sun, M., Corke, H.: Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents, *Journal of Agricultural and Food Chemistry*, **2005**, 53, 7749-7759;
47. Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., Smaoui, A., Abdelly, C.: Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes, *Comptes Rendus Biologies*, **2008**, 331 (11), 865-873;
48. Hatano, T., Edamatsu, R., Hiramatsu, M., Mori, A., Fujita, Y.: Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical and on 1,1-diphenyl-2- picrylhydrazyl radical, *Chemical and Pharmaceutical Bulletin*, **1989**, 37, 2016-2021;
49. Tanaka, Y., Tsuda, S., Kusumi, T.: Metabolic Engineering to Modify Flower Color, *Plant and Cell Physiology*, **1989**, 39 (11), 1119-1126;
50. Ningappa, M.B., Ramadas, D., Leela, S.: Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts, *Food Chemistry*, **2008**, 106, 720-728;
51. Tanaka, Y., Sasaki, N., Ohmiya, A.: Biosintesis of plant pigments: anthocyanins, betalains and carotenoids, *Plant Journal*, **2008**, 54, 733-749;
52. Quideau, S., Deffieux, D., Douat-Casassus, C., Pouysegou, L.: Plant polyphenols :chemical properties, biological activities, and synthesis, *Angewandte Chemie International Edition*, **2011**, 50, 586-621;
53. Luengas-Caicedo, E.P., Braga, C.F., Brandao, C.G., de Oliveira, B.A.: Seasonal and intraspecific variation of flavonoids and proanthocyanidins in *Cecropia glaziovii* Sneth, leaves from native and cultivated specimens, *Z. Naturforsch*, **2007**, 62 c,701-709;
54. Treutter, D.: Significance of flavonoid in plant resistance: a review, *Environmental Chemistry Letters*, **2006**, 4, 147-157;
55. Ma, D., Sun, D., Wang, C., Li, Y., Guo, T.: Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress, *Plant Physiology and Biochemistry*, **2014**, 80, 60-66;
56. Wei, H., Zhou, B., Zhang, F., Tu, Y., Hu, Y., Zhang, B., Zhai, Q.: Profiling and identification of small rDNA-derived RNAs and their potential biological functions, *PLoS ONE*, **2013**, 8 (2), e56842, doi:10.1371/journal.pone.0056842;
57. Laranjinha, J.: Translation of chemical properties of polyphenols into biological activity with impact on human health in: *Recent advances in polyphenol research*, Vol. 2 (Editors: Santos-Buelga, C., Escribano-Bailon, M.T., Lattanzio, V.), Wiley-Blackwell Publishing, Oxford, **2011**;

58. Koleva, I.I., Van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N.: Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, *Phytochemical Analysis*, **2002**, 13, 8-17;
59. Gonçalves, C., Dinis, T., Batista, M.T.: Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity, *Phytochemistry*, **2005**, 66, 89-98;
60. Leong, L.P., Shui, G.: An investigation of the antioxidant capacity of fruits in Singapore markets, *Food Chemistry*, **2002**, 76, 69-75;
61. Meir S., Kanner J., Akiri B., Hada S. P.: Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves, *Journal of Agricultural and Food Chemistry*, **1995**, 43, 1813-1819;
62. Diplock, A.T.: Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease, *Free radical research*, **1997**, 27, 511-532;
63. Villatoro-Pulido, M., Moreno Rojas, R., Muñoz-Serrano, A., Cardeñosa, V., Amaro López, M.Á., Font, R., Del Río-Celestino, M.: Characterization and prediction by near-infrared reflectance of mineral composition of rocket (*Eruca vesicaria* subsp. *sativa* and *Eruca vesicaria* subsp. *vesicaria*), *Journal of Agricultural and Food Chemistry*, **2012**, 92 (7), 1331-40;
64. Nilufer, O., Erdogan, O.I., Ergun, F.: Insights into cholinesterase inhibitory and antioxidant activities of five *Juniperus* species, *Food and Chemical Toxicology*, **2011**, 49, 2305-2312;
65. Ereifej, Kh., Esoh, R., Rababah, T., Almajwal, A. M., Muhammad, H. Alu'datt: Minerals, proximate composition and their correlations of medicinal plants from Jordan, *Journal of Medicinal Plants Research*, **2012**, 6 (47), 5757-5762;
66. Ujowundu, C.O., Kalu, F.N., Nwosunjoku, E.C., Nwaoguikpe, R.N., Okechukwu, R.I., Igwe, K.O.: Iodine and inorganic mineral contents of some vegetables, spices and grains consumed in Southeastern Nigeria, *African Journal of Biochemistry Research*, **2011**, 5(2), 57-64;
67. Guil, J.L., Martinez, J.J.G., Isasa, M.E.: Mineral nutrient composition of edible wild plants, *Journal of food composition and analysis*, **1998**, 11, 322-328;
68. Ozcan, M., Akgul, A.: Influence of species, harvest date and size on composition of capers (*Capparis* spp.) flower buds, *Nahrung*, **1998**, 42, 102-105.