Measurements of essential oil extract and antioxidants in Syrian *Myrtus communis* L. leaves using photochemiluminescence assay

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S u m m a r y

The essential oil extracts and antioxidant measurements of Syrian *Myrtus communis* L. leaves as hydrophilic and hydrophobic existence species have been carried out. The plant leaves as a source of antioxidants was tested by the influence of its aqueous and essential oil extracts on the yield of PCL solution applying very sensitive and reliable method. By means of a PCL assay, it was possible to assess the total antioxidants capacity of hydrophilic and hydrophobic species existence in Syrian *Myrtus communis* L. leaves. It has been found that the integral antioxidant capacity value of Syrian *Myrtus communis* L. leaves was found in Kurdaha site which has a value of 465.67±1.18 TE/g DM. The following three main substances were found in the essential oil extracts: a-pinene, cineole and limonene.

Key words: *antioxidant capacity, ascorbic acid, Myrtus communis L., photochemiluminescence, PC*L

INTRODUCTION

Myrtus communis L. is an evergreen plant belonging to the *Myrtaceae* family. Leaves, berries, and flowers are widely used medicinally in Mediterranean area. The leaves are generally used in the treatment of many illnesses (common cold, common flu, stomachache, nausea, etc.). They grow in forests.

A great number of herbal and medicinal plants contain chemical species exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. myrtus, rosemary, sage, oregano, which resulted in a development of natural antioxidant formulations for food, cosmetics and other applications [1]. Chemical and biological diversity of aromatic and medicinal plants depends on various factors: cultivation area, climat conditions, vegetation phase, genetic modifications and others very important in studying flora of different growing sites, countries and geographical zones.

It is well known that oxidative stress is involved in the pathogenesis of numerous diseases. Nevertheless, no optimal natural antioxidant has been found for therapeutics. Therefore, polyphenol antioxidants have been searched in many medicinal plants. Myrtle (*Myrtus communis* L.) leaves are one of the sources. It is a plant that has been used in folk medicine as an anti-inflammatory drug which has very high concentration of polyphenol antioxidants [2-4]. The chemical composition of the essential oil of the plant has been reported by many research groups [5-10]. The oil consists mainly of two categories and each of them is divided into two subgroups according to the ratio of α-pinene to myrtenyl acetate or α-pinene to cineole. Therefore, the presence of phenolic compounds (phenolic acids, polyphenols and flavonoids) in herbs and spices, along with the essential oil have given a great attention due to their high antioxidants activity and flavoring properties [11-15].

The diversity and richness of Syrian flora is well known. It contains a large number of medicinal and aromatic species. Most of Syrian herbs and other medicinal plants are used by Syrians and is taken in the form of herbal tea to contradict cough, flue and cold.

A number of assays have been carried out for the detection of both general and specific antioxidants action of complex mixtures [16]. Some of following assays and many others were widely used: TEAC for long life radical anions [17], DPPH for measuring the antioxidants capacity in fruit and vegetable juices or extracts [18], TRAP for monitoring the antioxidant compounds interference as results of the reaction between peroxyl ($ROO^{\bullet}$) radicals and the target probe [19], ORAC for measuring the antioxidant capacity in botanical samples [20] and FRAP for the antioxidant efficiency of the sample as a result of reduction of ferric to ferrous which give intense blue color line at 595 nm with a reference of known Fe^{2+} concentration [21]. Finally, a new photochemiluminescence assay (PCL) which is the inertest of this work has been used. The principal of this assay including its advantages will be given later.

Muzzoli and co-workers reported the intra-specific biodiversity of Italian myrtle (*Myrtus communis* L.) through chemical markers profile and biological activities of leaf methanolic extracts. The methanolic extracts of *Myrtus communis* L. leaves were processed in order to determine the content of myrtenol, linalool and eucalyptol. The extracts were also tested for antioxidant, antibacterial and antifungal activities [22].

Following our previous work on the determination of integral antioxidants capacity in Syrian hawthorn fruits and flowers using PCL assay [23], the work is extended to determine the antioxidant components of Syrian *Myrtus communis* L.

leaves using PCL assay. The assessment of such properties remains an interesting and useful task, particularly for finding new sources of natural antioxidants available in Syria which is also very important and interesting for Mediterranean scientists. However, to the best of our knowledge, no reports in the literature have dealt with the antioxidant capacity of Syrian *Myrtus communis* L., using PCL assay.

MATERIALS AND METHODS

Reagents

It should be mentioned that all chemicals used in the extraction processes and analysis were of HPLC and GR grades and were purchased from Merck and used as received. Kits of chemicals for determination of ACW (Antioxidant Capacity of Water-Soluble Substances) and ACL (Antioxidant Capacity of Lipid-Soluble Substances) for PCL assay were purchased from Analytik Jena AG (Jena, Germany).

Samples collection

Four collections of *Myrtus communis* L. leaves were carried out in five different sites located in the coastal part of Syria (west of the country, close to the Mediterranean). These five sites were distributed in the mountains and valleys: Kurdaha, Shadeti, Salah Al-Dein, Kusmeen and Wat-Alkhan. The collections of the leaves were collected at the end of (a) June, (b) September, (c) April and (d) November in 2008 from all sites, corresponding to the end of following seasons: (a) spring, (b) summer, (c) winter and (d) autumn, respectively. Table 1 shows the annual average meteorological parameters data for the collected Syrian *Myrtus communis* L. leaves used in this study.

Table 1.

The annual average meteorological parameters for the sites in which the samples were collected

Plant	site	vegetative stage	annual average Temp. [°C]	annual average humidity [%]	annual average rainfall [mm]	altitude [m]
Myrtus communis L.	**Kurdaha**	leaves	20–25	50–90	1055	300
	Shadeti	leaves	20–25	80–90	1033	305
	Salah Al–Dein	leaves	15–20	60–85	1089	350
	Kusmeen	leaves	20–25	50–85	1054	120
	Wat–Alkhan	leaves	20–25	50–80	1135	120

Extraction process and identifications

The preparation of lipid extract (essential oil) for the measurement of phenolics profile and antioxidant capacity formed by lipid-soluble antioxidants has been carried out as follows: the collected leaves from each site were separated from the stems and mixed thoroughly to ensure good homogeneity. All four collected *Myrtus communis* L. leaves samples used in this study were cleaned twice using distilled water, dried and grinded prior to subjection to steam distillation. The amount of 75 grams of the dried leaves were placed in the distillation flask (one liter capacity) with about 700 ml of water and extracted for three hours using a Clevenger-type apparatus. This process was repeated until the entire collection was extracted. The isolated essential oil from each distillation was added to each other and dried over anhydrous sodium sulphate and stored in dark bottles in refrigerator in 3–5°C. The separated component fractions from the extracted oil were carried out using a preparative high performance liquid chromatograph HPLC instrument (JASCO- LC-1500) from Jasco equipped with UV/VIS detector and ODS C18 preparative column. The following operation conditions were applied: THF/acetonitrile /H_2O as a mobile phase, a flow rate of 1.3 ml/ min., an injected sample volume of 150 μl and the analysis time is about 95 min. The used wavelength was 205 nm and the retention times of some individual constituents were compared with those of available authentic samples in order to check the credibility of the determinations. The identification of all separated components fraction from four collection times in five different sites were carried out using GC-MS and the results will be given later. The GC-MS (Agilent, 6869) system was used in order to identify the presented species obtained by the preparative HPLC instrument in following conditions: column HP5-MS, injection temperature 280°C, source temperature 230–280°C, fragment energy 70 eV and the volume injection is 1 μl. The preparation of water extract for the measurement of the antioxidant capacity formed by water-soluble antioxidants has been carried out as follows: 25 g of dried grinded leaves were extracted with 50 ml distillated water using ultrasonic bath for 30 min. The product was filtered and subjected directly to antioxidant measurements.

The PCL method

Photochemiluminescence (PCL) assay is based on the methodology of Popov and Lewin [24] and was used to measure the antioxidant activity of leaves extracts against superoxide anion radicals ($O_2^{\bullet -}$) generated from luminol, a photosensitizer, when exposed to UV light. In the PCL assay the photochemical generation of free radicals is combined with the sensitive detection using chemiluminescence. This reaction is induced by optical excitation of a photosensitizer S which results in the generation of the superoxide radical ($O_2^{\bullet -}$) [24]:

$$S + h\nu + O_2 \rightarrow \{S^{*} O_2\} \rightarrow S^{\bullet +} + O_2^{\bullet -}$$

Free radicals are visualised with the chemiluminescent detection reagent luminol. It works as photosensitizer as well as oxygen radical detection reagent. This reaction takes place in the Photochem® device. The antioxidant capacity of leaves extracts was measured using both ACW and ACL analytical kits provided by Analytik Jena (Leipzig, Germany) designed to measure the antioxidant capacity formed by hydrophilic and lipophillic compounds, respectively. For ACW studies, the luminol reagent and Trolox working solution were prepared on the day in which they were needed according to the ACW protocol. The presence of Trolox (or any other antioxidants from the extracts) retarded luminescence for a period; hence, a lag time was noted earlier than a signal was measured. The duration of the lag, which was calculated by the computer software from the first derivative of the detector signal at its turning point and intersection with the x-axis, was plotted against the concentration of Trolox added to the assay medium. The concentration of the extract solution added was the same as that the generated luminescence fell within the limits of the standard curve. Therefore, lag time (s) for the ACW assay was used as a radical-scavenging activity and the antioxidant capacity calculated by comparison with a Trolox standard curve and then expressed as nmol Trolox/g DM. In ACL studies, the kinetic light emission curve which exhibits no lag phase, was monitored for 180 s and expressed as nmol Trolox/g DM. The areas under the curves were calculated using the PCLsoft® control and analysis software. As greater concentrations of Trolox working solutions were added to the assay medium, a marked reduction in the PCL signal and hence area calculated from the integral were observed. This inhibition was used as a parameter for quantification and related to the decrease in the integral of PCL intensities caused by varying concentrations of Trolox. The observed inhibition of the signal was plotted against the concentration of Trolox added to the assay medium. The concentration of the extract solution added was the same that the generated luminescence during the 180 s sampling interval fell within the limits of the standard curve. The hydrophilic extracts for ACW and lipophillic extracts for ACL measurements were centrifuged prior to analysis. Measurements were performed with a Photochem® apparatus (Analytik Jena, Leipzig, Germany). The total antioxidant capacity was calculated as a sum of the ACW and ACL values. Finally it can be said that PCL assay performs rapidly and has many advantages over the other assays. It does not require high temperatures to generate radicals and is more sensitive (nano molar range) in measuring within few minutes (≤3 min). Most of other methods (TEAC, TRAP, DPPH, ORAC and FRAP) determine the antioxidant activity in micromolar range requiring minutes or hours. In previously mentioned methods, the measuring of antioxidant activity involves the generation of radical species and the presence of antioxidant causing the disappearance of these radicals. The PCL assay has been applied by many research groups for its advantages [25-27].

The use of PCL, spectrophotometric methods (TEAC, FCR reducing capacity) and cyclic voltammetry for the measurement of the antioxidant capacity of roots obtained from dark- and light-grown buckwheat sprouts has been carried out [26].

It has been found that the Photochem® device, two spectrophotometric assays – Trolox equivalent antioxidant capacity (TEAC) and FCR reducing capacity as well as cyclic voltammetric experiments were fully applicable for the evaluation of the antioxidant capacity of roots separated from buckwheat sprouts [26]. The order of antioxidant activity of flavone C-glucosides provided by updated analytical strategies TEAC and PCL have been carried out [27].

RESULTS AND DISCUSSION

Fourteen fractions from the end of June collections (spring) were separated by HPLC-preparative and then identified using GC-MS and the results were summarized in table 2. The average accounting identified species concentration was about 50.52 ppm for the five investigated sites and the major accounting concentration was about 53.37 and 51.36 ppm in Salah Al-Dein and Wat-Alkhan sites having α-pinene as a first major concentration component at about 17.03 and 17.45 ppm , respectively. The second major chemical component concentration of Cineole in Shadeti and kurdaha sites was about 12.07 and 10.43 ppm, respectively. Table 2 shows the concentrations in ppm unit per gram of dry matter of leaves for each component in June collection of *Myrtus communis* L. leaves with their IUPAC nomenclature which can be shifted to tables 3, 4, and 5, respectively.

Table 2.

The concentration of dry matter per gram(ppm) in the leaves of June collection

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified in the end of the June (spring season)
17.45	16.47	17.07	15.60	16.03	**a-pinene** 4,7,7-trimethylbicyclo[3.1.1]hept-3-ene
1.14	0.87	0.92	0.76	0.87	myrcene 7-methyl-3-methylene-1,6-octadiene
5.65	5.00	5.33	3.64	4.73	**limonene** 1-methyl-4-prop-1-en-2-ylcyclohexene
6.63	8.86	10.00	12.07	10.43	**cineole** 4,7,7-trimethyl-8-oxabicyclo[2.2.2]octane
1.25	1.30	1.36	1.79	1.52	**δ-terpinene** 1-methyl-4-(1-methylehtylidene)cyclohexene
2.77	2.55	3.15	1.41	1.85	**linalool** 3,7-dimethylocta-1,6-dien-3-ol
1.58	1.20	1.47	1.03	1.14	**tujene** 1-isopropyl-4-methylbicyclo[3.1.0]hex-3-ene
1.47	1.20	1.25	1.14	0.98	**citronellal** 3,7-dimethyloct-6-en-1-al
1.68	1.25	1.41	1.30	1.03	**nerol** (2E)-3,7-dimethylocta-2,6-dien-1-ol

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified in the end of the June (spring season)
1.58	1.41	1.47	1.14	1.20	**bornylacetate** (1,7,7-trimethyl-6-bicyclo[2.2.1]heptanyl) acetate
2.07	1.96	1.85	1.3	1.68	**geranyl acetate** 3,7-dimethyl-2,6- octadiene acetate
2.23	2.12	2.01	1.9	1.79	**eugenol** 4-allyl-2-methoxyphenol
2.66	2.61	2.93	2.55	2.83	**farnesyl alcohole** (2E,6E)-3,7,11-trimethyldodeca-2,6,10
3.21	2.99	3.15	3.48	2.88	**pulegon** (R)-5-methyl-2-(1-methylethylidine)cyclohexanone
51.36	49.78	53.37	49.13	48.97	total

Similarly 14 fractions from the end of September (summer) collections were separated by HPLC-preparative and then identified using GC-MS. The results were summarized in table 3. The average accounting identified species concentration was about 50.37 ppm for five investigated sites and the first major concentration for α-pinene was at about 16.14 and 15.65 ppm in Salah Al-Dein and Wat-Alkhan sites, respectively. The second major chemical component concentration of Cineole in Shadeti and Kusmeen sites was about 9.57 and 10.76 ppm, respectively. Table 3 shows the concentrations in ppm unit per gram of dry matter of leaves for each component in September collection of *Myrtus communis* L. leaves.

Table 3.

The concentration of dry matter per gram(ppm) in the leaves of September collection

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified in the end of the September (summer season)
15.65	14.08	16.14	14.62	14.95	a-pinene
1.41	1.47	1.52	1.58	1.20	myrcene
3.86	2.23	2.61	2.83	3.53	limonene
8.48	10.76	8.91	9.57	8.21	cineole
1.20	1.14	1.14	1.25	1.47	δ-terpinene
2.28	2.01	3.75	1.58	1.90	linalool
1.68	1.20	1.58	1.52	1.96	tujene
1.36	1.85	1.14	1.47	1.58	citronellal
1.58	1.47	1.25	1.41	1.47	nerol
2.12	1.68	1.85	1.90	1.58	bornylacetate
1.58	1.47	1.41	1.58	1.74	geranyl acetate

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified in the end of the September (summer season)
3.15	3.42	3.59	3.21	3.37	eugenol
3.53	3.48	3.70	3.91	3.75	farnesyl alcohole
3.64	2.77	3.37	3.21	3.04	pulegon
51.52	49.02	51.96	49.62	49.73	total

Similarly 14 fractions from the end of April collections (winter) were separated by HPLC-preparative and then identified using GC-MS and the results were summarized in table 4. The average accounting identified component concentration was about 50.09 ppm for five investigated sites. The first major concentration of chemical component was at about 18.15 and 17.88 ppm for α-pinene in Salah Al-Dein and Wat-Alkhan sites, respectively. The second major chemical component concentration of cineole was found in Shadeti site at about 9.18 ppm with similar concentration for Wat-Alkhan and Kurdaha sites at about 9.08 ppm. Table 4 shows the concentrations in ppm unit per gram of dry matter of leaves for each component at the end of April collection of *Myrtus communis* L. leaves.

Table 4.

The concentration of dry matter per gram(ppm) in the leaves of April collection

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified at the end of the April (Winter)
17.88	16.96	18.15	16.79	17.28	a-pinene
2.07	1.79	2.28	1.74	1.90	myrcene
3.48	4.02	4.57	3.21	3.48	limonene
9.08	8.86	7.55	9.18	9.08	cineole
0.92	1.03	0.87	1.20	0.98	δ-terpinene
1.79	2.07	1.90	1.30	1.58	linalool
2.23	2.12	1.90	2.01	1.68	tujene
1.25	1.52	1.47	1.85	1.58	citronellal
1.03	1.41	1.25	1.47	1.25	nerol
1.25	1.52	1.47	1.79	1.58	bornylacetate
1.85	1.58	2.12	1.52	1.74	geranyl acetate
1.58	2.01	1.79	2.07	1.85	eugenol
2.61	2.77	3.04	2.83	3.21	farnesyl alcohole
2.66	2.88	2.93	2.5	2.34	pulegon
49.67	50.54	51.30	49.46	49.51	total

At the end of November collection (autumn) again fourteen fractions were separated by HPLC-preparative method and then identified using GC-MS. The results were summarized in table 5. The average accounting identified species concentration was about 50.05 ppm and the first major concentration of chemical component was again for α-pinene at about 18.7 and 19.84 ppm in Kurdaha and Wat-Alkhan sites, respectively. The second major chemical component concentration of cineole was found in Shadeti and Kusmeen sites at about 8.86 and 8.80 ppm, respectively. Table 5 shows the concentrations in ppm unit per gram of dry matter of leaves for each component at the end of November collection of *Myrtus comunius* L. leaves.

Table 5.

The concentration of dry matter per gram(ppm) in the leaves of November collection

Wat-Alkhan ppm	Kusmeen ppm	Salah Al-Dein ppm	Shadeti ppm	Kurdaha ppm	components separated and identified at the end of the November (Autumn)
19.84	15.16	16.36	15.71	18.70	a-pinene
1.14	0.92	1.25	1.03	1.20	myrcene
7.77	5.33	6.68	5.76	6.74	limonene
6.14	8.80	7.28	8.86	6.30	cineole
0.71	1.30	1.47	0.98	0.82	δ-terpinene
2.34	2.55	2.66	2.28	2.01	linalool
1.14	1.25	1.41	1.30	1.52	tujene
1.30	1.90	1.25	1.36	1.20	citronellal
0.87	0.92	1.30	1.14	1.58	nerol
1.79	2.83	2.12	2.61	2.01	bornylacetate
2.12	1.52	1.90	1.68	1.79	geranyl acetate
1.68	2.39	2.01	2.12	1.90	eugenol
2.61	3.21	2.28	2.50	2.28	farnesyl alcohole
2.34	1.58	1.68	1.85	1.90	pulegon
51.79	49.67	49.67	49.18	49.95	total

Finally, it should be mentioned here that the third major limonene chemical species concentration was found in Wat-Alkhan site. The limonene concentration was 3.86 and 7.77 ppm in the end of June and November collections, respectively. Although at the end of April and November collection the major limonene concentration was found in Salah Al-Dein site with a concentration of 4.57 and 3.15 ppm, respectively. The major average concentration of the three major chemical components in four collections varies from 19.84 to 2.23 ppm. Three major chemical components are: α-pinene, cineole and limonene. The antioxidant capacity measurements of *Myrtus comunius* L. leaves using PCL assay was carried out both

for water- and lipid-soluble extracts. The end of September (summer season) extracts were chosen for these measurements due to the expectation of increasing concentrated fractions at the end of this collection.

Table 6 shows the antioxidant measurements for water and lipid soluble with integral antioxidant of Syrian *Myrtus comunius* L. leaves at five different sites as nano mole Trolox equivalent per gram of dry matter of leaves, DM.

Table 6.

Water- and lipid soluble antioxidants including total integral antioxidants in investigated sites

Site	water-soluble antioxidant (ACW)	lipid-soluble antioxidant (ACL)	integral antioxidant capacity (ACW+ACL)
	Trolox equivalent per gram of dry matter of leaves [DM/nmol]*		
Kurdaha	53.12±0.77	412.55±0.90	465.67±1.18
Shadeti	20.72±0.19	365.52±0.76	386.24±0.78
Salah Al-Dein	63.21±0.41	272.26±0.75	335.47±0.85
Kusmeen	12.77±0.36	220.15±0.83	232.99±0.91
Wat-Alkhan	12.99±0.17	215.73±0.13	228.72±0.21

*The results are given as the means and the standard deviation (±SD) of three independent measurements.

It can be noticed from table 6 that the major concentration of water-soluble antioxidant is presented in Salah Al-Dein site with a value of 63.21±0.41 TE/g DM. Regarding the lipid-soluble antioxidant capacity, the major concentration of antioxidants is in Kurdaha site with a value of 412.55±0.90 TE/g DM. The total integral antioxidant capacity measurements value of Syrian *Myrtus communis* L. leaves was found in Kurdaha site which has a value of 465.67±1.18 TE/g DM. A typical calibration curve of Trolox for ACL calculation is shown in the figure 1.

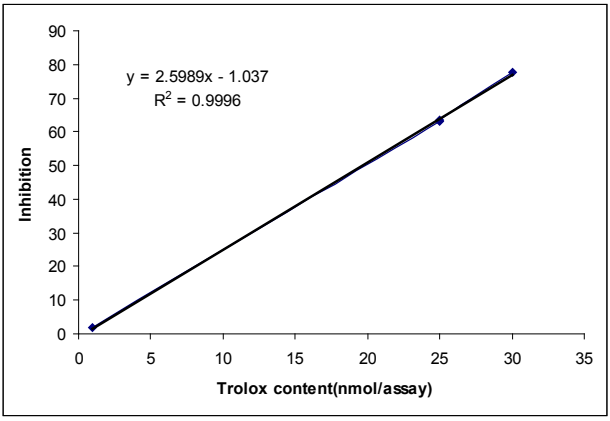

Figure 1.
A typical calibration curve of Trolox for ACL calculation

Before discussing the reported results, let us see the results of other groups assessing the compositions and antioxidant activities of *Myrtus communis* L. plant in different locations and environment. Hexane extracts obtained by percolation from the leaves of nine Mediterranean plants including *Myrtus communis* L. have been carried out. The extracts were examined for the presence of TLC, GC, HPLC and GC-MS [28]. Recently, Mimica-Dukić and co-worker reported the occurrence of the essential oil of *Myrtus communis* L. in two different locations in Serbia as a potential antioxidant and antimutagenic agents using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity array and identified the antimutagenic properties of the essential oil of myrtle (*Myrtus communis* L.). Significant differences between the samples were found in the ranges of α-pinene (14.7–35.9%) and myrtenyl acetate (5.4–21.6%). Both oils exhibited moderate DPPH scavenging activity, with IC50 values of 6.24 mg/ml and 5.99 mg/ml, respectively [29].

Exciting and interesting work was recently published by Komaitis and co-worker focusing on the seasonal variation of the essential oil composition, the antioxidant activity using DPPH and FRAPS assays and the total phenolic content of *Pistacia lentiscus* and *Myrtus communis* L, grown in the Greek Zakynthos island [30]. The essential oil was obtained by hydrodistillation and subsequently analyzed by GC-MS. The strongest antioxidant activity and the highest phenolic content were obtained in full flowering stage (August) [30].

The evaluation of antioxidant effect of different extracts of myrtle (*Myrtus communis* L*)* with different solvents using liquid-liquid extraction (LLE) has been carried out using TEAC assay [31]. All myrtle extracts were very rich in polyphenols. In particular, the hydroalcoholic extracts were contained galloyl-glucosides, ellagitannins, galloyl-quinic acids and flavonol glycosides. Ethyl acetate extract and aqueous residues after LLE contained flavonol glycosides and hydrolysable tannins (galloyl-glucosides, ellagitannins, galloyl-quinic acids), respectively. The antioxidant effect of three myrtle extracts decreased in the following order: hydroalcoholic extract, ethylacetate and aqueous residues after LLE. The extracts had the following IC50: 0.36, 2.27 and 2.88 μmol, when the sum of total phenolic compounds was considered after the correction of molecular weight based on pure compounds. The reported results suggest that the myrtle extracts have a potent antioxidant activity mainly due to the presence of galloyl derivatives. [31].

Stability and antioxidant activity of polyphenols of *Myrtus comunius* L. has been carried out. The antioxidant activity was measured using TEAC assay, and the free-radical scavenging activity was monitored during the stability evaluation. Anthocyanins were found to be the most instable compounds, but a considerable instability was also observed for flavonoids, suggesting the use of extracts not over 3 months from their preparation. The myrtle extract showed interesting free-radical scavenging activity and the antioxidant activity was preserved in 3 months only [32].

Then, all myrtle extracts were very rich in polyphenols and the 14 major found phenolic species in the essential oil extracts of Syrian *Myrtus communis* L leaves are: α-pinene, myrcene, limonene, cineole, δ-terpinen, linalool, tujene, citronellal, nerol, bornyl acetate, geranyl acetate, eugenol, farnesyl alcohol and pulegon. As it was stated earlier, the antioxidant measurements were made for essential oil extracts samples collected in the end of September (summer) due to the expectation of increasing concentrated fractions in the end of this collection. This decision was made according to work by Gardeli et al who stated that the strongest antioxidant activity and the highest phenolic content were obtained in full flowering stage (August) [30].

In our measurements it has been found that the major polyphenolic contents were almost high in Kurdaha site with lipid-soluble antioxidant extract value at 412.55±0.90 nmol. The average of integral antioxidant capacity in all five sites is about 330 nmol. Three main chemical species, responsible for antioxidant activities in Syrian *Myrtus comunius* L. leaves are: α-pinene, cineole and limonene which are consistent with literature [29]. It is well known that the myrtle extracts have a potent antioxidant activity mainly due to the presence of polyphenols and galloyl derivatives [23]. Significant differences between samples were found in the ranges of α-pinene (25.9–36.5%, 14.80–19.84 ppm), cineole (11.3–22.2%, 6.63–12.07 ppm) and limonene (14.3–4.1%, 2.23–7.77 ppm) in four samples collection. Our observed results for α-pinene (25.9–36.5%) are consistent with the range of previous results reported by Mimica-Dukić and co-worker (14.7–35.9%) [29]. The variation is due to seasonal, environmental effect and climatic conditions. In order to assure the main source of antioxidant fractions, the separated extracts of α-pinene, cineole and limonene using HPLC preparative instrument were subject to direct individual antioxidants measurements. It has been observed that between 70–80% of the measurements reported in table 6 are mainly due to three rich in α-pinene, cineole and limonene species in Syrian *Myrtus comunius* L. leaves grown in the coastal part in the West of the country close to the Mediterranean. Due to the technical problems, total phenolic content in water extract fraction using Folin-Ciocalteu's reagent (FCR) was not carried out.

CONCLUSION

It can be concluded that the reported results support the view that *Myrtus communis* L. leaves are a promising source of natural antioxidant properties and contains significant amounts of polyphenolic compounds. It has been found that the integral antioxidant capacity measurements value of Syrian *Myrtus comunius* L. leaves were found in Kurdaha site and obtained value was $465.67_{\pm}1.18$ TE/g DM. Three major chemical species were: α-pinene, cineole and limonene.

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OKREŚLENIE ZAWARTOŚCI OLEJKU ETERYCZNEGO I PRZECIWUTLENIACZY W MIRCIE ZWYCZAJNYM (*MYRTUS COMMUNIS L.*) ROSNĄCYM W SYRII ZA POMOCĄ METODY FOTOCHEMOLUMINESCENCYJNEJ

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Streszczenie

W mircie zwyczajnym (*Myrtus communis* L.), roślinie zawierającej związki hydrofilne i hydrofobowe występującej w Syrii, przeprowadzono badanie olejku eterycznego pod kątem obecności przeciwutleniaczy. Zawartość przeciwutleniaczy w liściach rośliny badano dla wyciągu wodnego i olejku eterycznego stosując czułą i godną zaufania metodę fotochemoluminescencji (PCL). Za pomocą PCL można było określić całkowitą zdolność przeciwutleniającą związków hydrofobowych i hydrofilnych liści mirtu zwyczajnego rosnącego w Syrii. Stwierdzono, że zmierzona aktywność przeciwutleniająca w liściach zebranych na stanowisku Kurdaha miała wartość 465.67$\pm$1.18 TE/g DM. W olejku eterycznym znaleziono trzy główne związki: α-pinen, cineol i limonen.

Słowa kluczowe: *aktywność przeciwutleniająca, kwas askorbowy, Myrtus communis L., fotochemoluminescencja, PCL*