Determination of essential oil composition by GC-MS and integral antioxidant capacity using photochemiluminescence assay of two *Thymus* leaves: *Thymus syriacus* and *Thymus cilicicus* from different Syrian locations

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S u m m a r y

The Syrian *Thymus cilicicus* (*Th. cilicicus*) Boiss & Bal. and *Thymus syriacus* (*Th. syriacus*) Boiss chemical components were identified using GC-MS spectrometry. Sixteen constituents representing an average of 93.85% of the essential oil from Syrian *Thymus cilicicus* were characterized. The major average components in four collection rounds of *Thymus cilicicus* around the year were: thymol, carvacrol, γ-terpinene, carvyl acetate, dihydrocarvone and anis aldehyde. Eighteen components representing an average of 93.46% of the essential oil of *Thymus syriacus* were characterized. The major average components in four collection rounds of *Thymus syriacus* around the year were: carvacrol, dihydrocarvone, β-caryophyllene, *p*-cymene, farnesol, limonine, menthol, myrecene, γ-terpinene, terpinene-4-ol and thymol. The integral antioxidant capacity of aqueous and essential oils extracts of both *Thymus* species: *Th. syriacus* and *Th. cilicicus* have been determined by means of a photochemiluminescence assay (PCL). The highest integral antioxidant capacity has been found for *Th. syriacus* and *Th. cilicicus* in Fahel mountain and Salah Aldin locations which have a value of total nmol equivalent per gram of dry material at 374.6 ± 0.94 and 475.80 ± 1.20 nmol TE/g DM, respectively.

Key words: *antioxidant, Th. syriacus, Th. cilicicus, photochemiluminescence, essential oil*

INTRODUCTION

Thymus plant (local name Za'atar) is commonly used as herbal tea, flavoring agents, condiments and spices as well as medicinal plants. Syrian flora is well known for its diversity and richness. It consists of numerous species of medicinal use. Among plants grown in Syria, *Th. syriacus* and *Th. cilicicus* are widely used in those abovementioned purposes. In addition, *Th. syriacus* leaves are used also for cough and ulcer treatments.

In general, *Thymus* plant belongs to *Labiatae* family. It is considered as one of the most important family members of Syrian flora. This family contains more than two hundreds species of herbaceous perennial and sub shrub plants. The Mediterranean region can be described as the center of genus *Thymus* and numerous species for medical characters and some of these floras such as *Th. syriacus* and *Th. cilicicus* [1].The *Th. syriacus* is normally found in all east Mediterranean countries while *Th. cilicicus* is only found in the north of Syria and south of Turkey [2, 3].

It is well known that a great number of many herbal and medicinal plants contain chemical species of antioxidant properties. Numerous studies were carried out on some of these plants, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications [4, 5]. Other important useful applications of these herbal and medicinal plants, in particular of *Thymus* genus is due to their antibacterial, antimicrobial and antifungal activities effects in their essential oils [6-8]. Chemical activity and biological diversity applications of aromatic and medicinal plants depend on various factors: cultivation area, climatic conditions, vegetation phase, genetic modifications and others present in different growing locations, countries and geographical zones [9]. The characterization and antioxidant activity of the volatile oils of *Th. syriacus*, *var syriacus* and *Thymbra spicata* L. grown wild in Kurdistan-Iraq have been carried out using the Cu^{2+} mediated low density lipoprotein (LDL) oxidation method in order to assay their antioxidative properties [10]. Tepe *et al*. were compared the antioxidant potential of two *Thymus* species on the basis of the chemical compositions of their essential oils obtained by hydrodistillation method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid assays [11].

Bruni and co-workers reported a comparative evaluation of eleven essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods [12]. Their antioxidant and radical-scavenging properties including *Thymus x citriodorus* (*Lamiaceae*) were tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, *β*-carotene bleaching test and luminol-photochemiluminescence (PCL) assay. In the DPPH assay, *C. odorata*, *C. citratus*, *R. officinalis* and *C. longa* showed major effectiveness, with a radical inhibition ranging from 59.6±0.42 to 64.3±0.45%. In the *β*-carotene bleaching test, *C. odorata* (75.5±0.53%), *R. officinalis* (81.1±0.57%) and *C. longa* (72.4±0.51%) gave the best inhibition results. Similar results were obtained for the same essential oils in the PCL assay [12]. A number of assays has been developed for the detection of both general and specific antioxidants action of complex mixtures [13]. The following assays and many others were widely used: TEAC for long life radical anions [14], DPPH for measuring the antioxidants capacity in fruit and vegetable juices or extracts [15], TRAP for monitoring the antioxidant compounds interference as a results of the reaction between peroxyl ($ROO^{\bullet}$) radicals and the target probe [16], ORAC assay for measuring the antioxidant capacity in botanical samples [17] and FRAP for antioxidant efficiency of the sample as a result of reduction of ferric to ferrous which give an intense blue color line at 595 nm with a reference of known Fe^{2+} concentration [18]. However, to the best of our knowledge, no reports in the literature have dealt with the antioxidant capacity of Syrian *Thymus*: *Th. syriacus* and *Th. cilicicus* using photochemiluminescence (PCL) assay. The PCL is the interest of this work. The principal of this assay including its advantages will be given later. Following our two previous reports focusing on the determination of integral antioxidants capacity in Syrian hawthorn fruits and flowers and *Myrtus comminus* L. leaves using photochemiluminescence assay [19, 20], therefore the aim of the present paper is to extend our previous works and to determine the total antioxidant contents of *Th. syriacus* and *Th. cilicicus* using photochemiluminescence, PCL assay including the comparison of antioxidant potential of these two *Thymus* species on the basis of chemical compositions of obtained essential oils with their identifications using GC/MS spectrometry. 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 and worldwide interested scientists.

MATERIALS AND METHODS

Reagents

All chemicals used in the extraction processes and analysis were of HPLC and GR grades, 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 photochemiluminescence (PCL) assay were purchased from Analytik Jena AG (Jena, Germany).

Samples collection

Seven locations were selected for samples collection between 1st of January 2010 and 31st of December 2011. These seven locations were distributed in the mountains and valleys at Syrian coast (tab. 1) including some general important annual average meteorological parameters data for the collected Syrian *Thymus* locations used in this study. The annual average humidity in all locations ranged from 50 to 85%.

Table 1.

Presents some general meteorological parameters for collection locations

Location	Longitude	Latitude	Altitude [m]	Average rainfall [mm/year]	Highest monthly average temp. [ºC]	Lowest monthly average temp. [ºC]
Watta Al-Khan	36° 03’	35° 41’	110	1135	28.6	7.8
Kesmin	35° 59’	35° 38’	120	870	28.9	9.2
Salah Aldin	36° 04’	35° 36’	350	1089	25.2	9.8
Kasab	35° 56’	35° 56’	750	1521	22.8	6.5
Meshtayeh	36° 16’	34° 36’	400	300	32.4	9.5
Moukles	36° 22’	34° 48’	850	450	31.6	9.3
Fahel Mountain	36°25’	34° 51’	1000	650	29.4	6.3

The plants were collected from seven locations in four rounds over one year. The rounds performed as follows: April (Round 1), end of May (Round 2), beginning of September (Round 3), and middle of November (Round 4), respectively. Each round in 2010 was added to the same round in 2011 and was considered as one lot. The reason for choosing these timings is the beginning of vegetative growth, pre-blooming, end of a long dry season, and the fruiting periods of both Syrian species: *Th. syriacus* and *Th. cilicicus*, respectively.

The process of extraction and identification

Two separate extractions with use of different methods were carried out for both water and lipid soluble fractions due to the fact that two kits protocols, ACW and ACL are available for antioxidant measurements and the total accurate antioxidant measurements can be obtained. The preparation of lipid extracts (essential oil) for chemical compositions identification and the measurement of phenolics profile and antioxidant capacity formed by lipid-soluble antioxidants have been carried out as described hereafter.

Every sample of leaves in each round were taken and used only in this study. They were cleaned twice using distilled water and dried prior to subjection to steam distillation in clevenger apparatus. In each collected round the leaves from one location were separated from the stems and mixed thoroughly to ensure good homogeneity. An amount of 75 g of the dried leaves was placed in the distillation flask (1 l capacity) with about 700 ml of water and extracted for 3 h. This process was repeated until entire collection was obtained. The oil isolated from each distillation was added to each other similarly, dried over anhydrous sodium sulphate and stored in dark bottles in a fridge at 3 to 5°C. The analyses were carried out using a JASCO- LC-1500 preparative high performance liquid chromatography (HPLC) equipped with UV/VIS detector and ODS C18 preparative column. Few trial runs were performed in order to determine the conditions leading to the finest isolation of oil constituents. It was decided to use the following operation conditions: THF/ACN/H_2O as a mobile phase, a flow rate of 1.3 ml per minute, an injected sample volume of 150 μl, and an analysis time of 95 min. The wavelength used was 205 nm. Retention times of some individual constituents were compared with those of available authentic samples in order to check the credibility of determinations.

The identification of all separated components fractions from four collection rounds in seven different locations 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 obtained species by 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 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/methanol (ratio 30 ml/70 ml) using ultrasonic bath for 30 min. The product was filtered and subjected directly to antioxidant measurements.

It should be pointed out here that each individual component was identified with the comparison of both mass spectra and their GC retention index using reference standard materials (α-pinine, O-cymol, carvacrol, γ-terpinene) obtained from Aldrich.

Photochemiluminescence method

The photochemiluminescence (PCL) assay principle is based on the methodology of Popov and Lewin [19-21, 25]. Very briefly, the assay was used to measure the antioxidant activity of plant 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 sensitive detection using chemiluminescence. This reaction is induced by optical excitation of a photosensitizer S that results in the generation of superoxide radical ($O_2^{\bullet-}$) [21]:

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

Free radicals are visualised with the chemiluminescent detection reagent luminol. It works as photosensitizer as well as oxygen radical detection reagent. The PCL assay has been applied by many research groups for its advantages in comparison with different assays [22-25]. The integral antioxidative capacity of water-soluble substances (ACW) and lipid-soluble substances (ACL) protocols has been described in details [19-20].

RESULTS AND DISCUSSION

Table 2 shows the extracted essential oil percentage in four collected rounds from the seven different locations with an estimated standard deviation SD±0.01.

Table 2.

Total essential oil percentage in the four collected rounds

Location	Species	Round 1 (%)	Round 2 (%)	Round 3 (%)	Round 4 (%)	verage yield (%)
Watta Al-Khan (W)	*Th. cilicicus*	1.65	1.89	1.14	1.95	1.66
Kesmin (K)	*Th. cilicicus*	1.49	1.53	1.12	1.73	1.47
Salah Aldin (S)	*Th. cilicicus*	1.84	1.97	1.32	2.13	1.82
Kasab (Ka)	*Th. cilicicus*	1.95	1.99	1.37	2.34	1.94
Meshtayeh (M)	*Th. syriacus*	1.87	1.91	1.31	2.11	1.77
Moukles (Mo)	*Th. syriacus*	1.73	1.77	1.22	1.99	1.71
Fahel Mountain (F)	*Th. syriacus*	1.99	1.97	1.34	2.12	1.85

In order to simplify the interpretation and results analysis, an abbreviation to the location's name has been considered and that pointed out in table 2 summarizing the total essential oil percentage obtained from the leaves of two *Thymus* species in four collected rounds. Regarding *Th.cilicicus*, the lowest essential oil percentage throughout the year in comparison with other four locations has been observed in Kesmin (K) location. At this location, the high percentage with a value of 1.73 begins to increase in round 4 in the period of fruiting (middle November) while the low percentage with a value of 1.12 decreases in round 3 at the end of summer period after long dry season (beginning of September). Similar trend was seen in Kasab (Ka) location in the period of fruiting and at the end of summer period with a percentage values of 2.34 and 1.37, respectively. Regarding the *Th. syriacus*, the lowest essential oil percentage throughout the year in comparison with other three locations has been observed in Moukles (Mo) location. At this location, the high percentage with a value of 1.99 begins to increase in round 4 in the period of fruiting (middle November) while the low percentage with a value of 1.22 decreases in round 3 at the end of summer period after long dry season (beginning of September). Similar trend was seen at Fahel Mountain (F) location in the period of fruiting and at the end of summer period with a percentage values of 2.12 and 1.34, respectively. In general, the percentage of two *Thymus* species begins to build up and assumes high values in the season of pre-blooming (April and May rounds, 1 and 2) and then starts to decline in summer. Then, it reaches minimum values in the beginning of September (round 3), and begins to increase again in the period of fruiting in round 4.

The aim of the reported work was not to investigate the impact of metrological conditions on the yield of the obtained essential oil but to have an idea about our samples nature and their antioxidant contents since they are collected from different locations. It is known that the total average essential oil percentage is due to climatic and geographic conditions. The essential oil yield increases with high altitude, high average rainfall and at milder temperature. On the other hand, the essential oil yield decreases with the lower average rainfall and higher temperature as well as to the poorer soils as in the Moukles (Mo) and Meshtayeh (M). Total average percentage of *Th. cilicicus* from each location was separated by HPLC-preparative and then identified using GC-MS and the results were summarized in table 3. It should be pointed out here that, the component fraction percentage unit was chosen in order to match the literatures and to discuss the reported results. Sixteen component identification fractions from *Th. cilicicus* in four different W, K, S and Ka locations have been summarized in table 3.

Table 3.

Summary of component fraction (%) of *Th. cilicicus* in all locations from total percentage

Component's fraction	Round 1 **locations** (%)				Round 2 **locations** (%)				Round 3 **locations** (%)				Round 4 **locations** (%)			
	W	K	S	Ka	W	K	S	Ka	W	K	S	Ka	W	K	S	Ka
Anis aldehyde	3.1	5.3	5.2	4.3	2.9	2.9	3.2	5.6	6.4	9.9	7.9	7.3	5.5	7.2	6.4	4.8
Camphene	1.8	2.2	2.6	2.9	1.9	2.9	1.8	3.3	2.7	1.6	1.4	2.7	2.9	3.2	2.8	2.8
Carvacrol	28.1	27.7	29.4	28.4	27.3	24.7	31.2	26.7	22.3	21.3	27.3	23.6	24.9	22.8	28.9	25.3
Carvyl acetate	5.5	7.3	6.9	7.1	5.3	8.4	5.2	6.7	12.3	14.7	11.3	9.8	8.3	12.2	8.7	8.3
β-Caryophyllene	6.3	4.7	5.3	5.9	4.9	4.9	4.9	3.2	3.8	2.2	3.7	3.1	4.8	2.7	4.2	4.2
Cineol	2.2	2.4	1.3	1.2	1.8	1.8	1.2	1.9	3.2	3.7	2.1	2.6	2.6	2.9	1.9	2.2
Citronellal	2.9	2.4	2.4	2.1	2.4	1.9	1.7	2.3	1.7	1.6	1.2	1.9	2.2	1.7	1.8	2.8
Dihydrocarvon	4.3	7.2	5.3	4.9	4.1	6.4	4.2	6.3	10.4	12.6	6.7	9.2	7.2	10.4	8.2	6.1
Geraneol	1.7	3.2	2.6	2.3	4.7	5.6	3.8	3.5	1.6	2.9	2.3	1.1	1.9	4.8	2.9	1.9
Limonine	2.1	3.1	0.9	2.1	2.2	2.4	1.2	2.4	1.4	1.9	1.9	1.9	1.8	3.4	2.1	1.7
Linalool	1.8	1.2	2.4	1.4	1.4	1.2	1.6	1.4	1.2	0.7	2.1	1.1	2.1	1.6	2.7	1.7
Menthyl acetate	2.7	3.1	3.4	2.9	1.8	2.2	2.1	1.2	1.3	1.9	1.7	2.5	2.2	3.4	2.6	3.2
Nerole	1.7	2.1	2.3	2.1	2.1	2.3	2.2	2.4	1.8	1.8	1.9	1.8	2.8	2.6	3.2	2.6
α-Pinine	2.7	2.3	1.9	2.7	2.4	1.8	2.2	2.1	1.2	1.1	1.7	2.6	2.1	2.1	1.7	3.1
γ-Terpinine	11.1	6.2	9.7	10.3	10.5	7.3	10.7	10.6	8.2	3.3	8.3	9.2	9.4	5.1	8.8	9.9
Thymol	17.3	17.2	11.8	16.8	14.4	14.8	13.6	14.6	12.2	12.5	9.6	9.8	13.1	12.1	10.2	14.9
Total	**95.3**	**97.6**	**93.4**	**97.4**	**90.1**	**91.5**	**90.8**	**94.2**	**91.7**	**93.7**	**91.1**	**90.2**	**93.8**	**98.2**	**97.1**	**95.5**

The average accounting identified species percentage was about 95.92, 91.65, 91.67 and 96.15 for four investigated W, K, S and Ka locations, respectively. Note that according to the expectation, the high accounting percentage was observed in rounds 3 and 4 in the summer period after long dry season (September) and fruiting round (November), respectively. Sixteen constituents representing an average of 93.85% of the essential oil from Syrian *Th. cilicicus* were characterized. The major average components in four collected rounds from *Th. cilicicus* around the year were thymol (13.43%), carvacrol (26.20 %), γ-terpinene (8.60 %), carvyl acetate (8.61%), dihydrocarvone (7.00 %) and anis aldehyde (5.43%). The following three major fractions: carvacrol, thymol and γ-terpinene have been found in the first two rounds. Six major fractions: carvacrol, thymol , γ-terpinene, carvyl acetate, dihydrocarvone and anis aldehyde have been found in the last two rounds. Our reported identification fractions are in consistent with pervious two reports [26-27].

Total average percentage from each location of *Th. syriacus* was separated by HPLC-preparative and then identified using GC-MS. The results were summarized in table 4. Eighteen component identification fractions from *Th. syriacus* in three different M, Mo and F locations were also summarized in table 4.

Table 4.

Summary of component fraction (%) of *Th. syriacus* in all locations from total existence percentage

Component's fraction	Round 1 **locations**			Round 2 **locations**			Round 3 **locations**			Round 4 **locations**		
	M	Mo (%)	F	M	Mo (%)	F	M	Mo (%)	F	M	Mo (%)	F
Camphene	3.9	2.1	7.2	2.6	2.2	4.4	3.7	4.3	6.9	4.2	4.1	8.3
3-Carene	0.4	0.7	1.2	0.9	1.1	1.5	-	-	-	-	-	-
Carvacrol	34.3	35.9	38.3	33.4	37.3	39.5	29.8	33.2	31.4	32.1	34.8	33.9
β-Caryophyllene	7.2	4.6	5.4	6.3	5.3	6.9	5.2	4.2	4.7	5.4	4.1	4.5
p-Cymene	4.7	5.8	4.3	5.9	4.7	3.9	4.3	3.9	2.5	6.2	4.8	2.4
Cineole	3.6	2.4	2.2	4.6	2.6	2.4	3.3	4.9	3.8	3.1	5.3	4.3
Dihydrocarvon	3.3	6.4	3.9	3.4	4.9	4.2	6.7	7.2	6.7	4.2	6.9	4.3
Farnesol	6.6	4.9	5.4	5.9	6.5	5.8	3.8	4.2	4.9	5.7	4.4	5.1
Limonine	2.8	2.8	2.9	2.4	2.4	1.2	3.6	1.6	3.1	3.9	3.2	2.3
Linalool	3.6	2.2	3.1	3.3	1.9	2.9	2.9	2.6	4.3	2.4	0.9	4.1
ρ-Menth 4(8)-en-3on	-	-	0.9	-	-	-	-	-	-	-	-	-
Menthol	3.8	3.6	3.7	4.1	3.2	2.9	3.1	2.8	4.3	3.6	2.5	4.9
Myrecene	5.3	4.5	2.6	3.1	3.1	2.9	4.8	4.2	3.8	3.1	4.4	2.4
3-Octanol	0.6	0.6	1.4	0.7	0.8	1.7	-	-	-	-	-	-
α-Pinine	3.2	2.2	2.5	2.2	2.3	2.7	3.8	2.1	2.1	2.9	1.9	1.9
γ-Terpinine	8.1	8.8	6.4	9.7	9.4	7.8	7.3	8.2	5.7	6.9	6.2	4.8
Terpinene-4-ol	2.6	2.9	2.4	2.8	3.7	1.2	5.7	3.7	3.3	2.3	3.8	3.9
Thymol	4.5	4.1	3.4	4.1	3.6	4.3	2.8	3.3	3.1	4.2	4.6	3.8
Total	**98.5**	**94.5**	**97.2**	**95.4**	**95.0**	**96.2**	**90.8**	**90.4**	**90.6**	**90.2**	**91.9**	**90.9**

The average accounting identified species percentage was about 96.73, 95.53, 90.60 and 91.0 for the three investigated M, Mo and F locations, respectively. Note that high accounting percentage was observed in rounds 1 and 2 at the beginning of vegetative period growth and the pre-blooming stage, respectively. This reported result is attributed to the soil nature and low average rainfall at M, Mo and F locations.

Eighteen constituents representing an average of 93.46% of the essential oil from Syrian *Th. syriacus* were characterized. The major average components in four collected rounds from *Th. syriacus* around the year were: carvacrol (34.49%), dihydrocarvone (5.17%), β-caryophyllene (5.32%), *p*-cymene (4.45%), farnesol (5.26%), limonine(2.68%), menthol (3.55%), myrecene (3.68%), γ-terpinene (7.44 %), terpinene-4-ol (3.20%) and thymol (3.82%).These reported identification fractions are consistent with two previous reports [10, 28].

The antioxidant capacity measurements of Syrian *Th. cilicicus* and *Th. syriacus* leaves using PCL assay were carried out for both water- and lipid-soluble extracts.

In spite of low water soluble antioxidant concentration both in *Th. cilicicus* and *Th. syriacus* species, is worth to point out to these measurements. Although, the main antioxidant results were due to the essential oil of two *thymus* species.

Table 5 shows the antioxidant measurements for water and lipid-soluble extracts with integral antioxidant of *Th. cilicicus* leaves at four different locations as total nmol equivalent per gram of dry material (nmol TE/g DM).

Table 5.

Antioxidants measurements of *Th. cilicicus* leaves

Location	Water-soluble antioxidant (ACW)	Lipid-soluble antioxidant (ACL)	Integral antioxidant capacity (ACW +ACL)
	Total equivalent per gram of dry material, nmol TE/g DM		
Watta Al-Khan (W)	18.7±0.27	365.5±0.79	384.20±0.97
Kesmin (K)	23.8±0.34	354.9±0.77	378.70±0.95
Salah Aldin (S)	55.6±0.80	420.2±0.92	475.80±1.20
Kasab (Ka)	32.5±0.47	374.5±0.82	407.00±1.03

*The results are given as the means and the standard deviation,±SD of three independent measurements. The number of three measurement data is *n* and the mean of data is given by the following equation: $\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$, while the standard deviation of data is given by the following equation: $\sigma = \sqrt{\frac{(x_i - \bar{x})^2}{n-1}}$, the values of data given are written as $\bar{x} \pm \sigma$.

It can be noticed from table 5 that the major concentration of water-soluble antioxidant is presented at Salah Al-Dein location with a value of 55.6±0.80 nmol TE/g DM. Regarding the lipid-soluble antioxidant capacity, the major concentration of antioxidants is also found at Salah Al-Dein location with a value of 420.20±0.92 nmol TE/g DM.

Hence, the total integral antioxidant capacity measurements value of *Th. cilicicus* leaves is at Salah Al-Dein location with a total value of 475.80±1.20 nmol TE/g DM. In general, the average integral antioxidant capacity concentration is about 410 nmol TE/g DM per year.

Table 6 shows the antioxidant measurements for water and lipid soluble with integral antioxidant of *Th. syriacus* leaves at three different locations as total equivalent per gram of dry material (nmol TE/g DM). It can be noticed from table 6 that the major concentration of water-soluble antioxidant is presented at Meshtayeh location with a value of 44.2±0.64 nmol TE/g DM. Regarding the lipid-soluble antioxidant capacity, the major concentration of antioxidants is found at Fahel mountain location with a value of 337.80±0.78 nmol TE/g DM. Hence, total integral antioxidant capacity measurements value of *Th. syriacus* leaves is at Fahel mountain location with a total value of 374.60±0.94 nmol TE/g DM. In general, the average integral antioxidant capacity concentration is about 330 nmol TE/g DM per year. A typical calibration curve of Trolox for ACL measurements is shown in figure 1.

Table 6.

Antioxidants measurements of Th. syriacus leaves

Location	Water-soluble antioxidant (ACW)	Lipid-soluble antioxidant (ACL)	Integral antioxidant capacity (ACW +ACL)
	Total equivalent per gram of dry material [nmol TE/g DM]		
Meshtayeh (M)	44.2±0.64	265.4±0.57	309.60±0.78
Moukles (Mo)	28.9±0.42	279.2±0.60	308.10±0.77
Fahel mountain (F)	36.8±0.53	337.8±0.73	374.6±0.94

*The results are given as the means and the standard deviation (±SD) of three independent measurements. The calculation details are given in footnote of table 5.

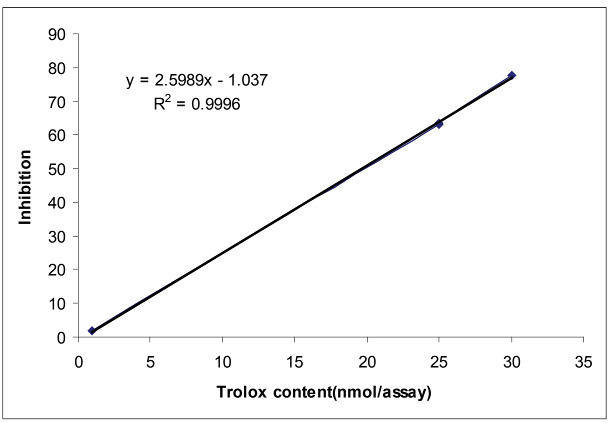

Figure 1.

Typical calibration curve of Trolox for ACL measurements

Before discussing the reported results, let us see the results of other groups concerning the antioxidant activities of *Th. cilicicus* and *Th. syriacus* and other *Thymus* families in different locations and environment. Demirtas and co-workers determined the antioxidant activities of various extracts and essential oil compositions of *Thymus* praecox subsp. *skorpilii* var. *skorpilii,* TPS [29]. Their antioxidant activities were evaluated by reduction of Mo(VI) to Mo(V), reducing power, superoxide scavenging activity, free radical-scavenging activity, metal chelating activity, linoleic acid peroxidation, hydrogen peroxide scavenging activity and peroxide scavenging activity. Essential oils were characterized in total to be 41 components, whereas 9 components were isolated by column chromatography for antioxidant activity. The TPS essential oil was found to contain thymol (40.31%) and *o*-cymene (13.66%) as major components. Ethanol, methanol and water extract exerted significant free radical-scavenging activity. Methanol and water extracts displayed the highest superoxide scavenging activity. Water extract has the highest total phenolics (6.211 mg gallic acid (GAE)/g DW) and flavonoids (0.809 mg quercetin/g DW) [29]. Grigore and co-workers determined the chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. Both by phosphomolybdenum reduction and DPPH assays they found that the *Thymus vulgaris* belongs to thymol chemotype [30]. The component fractions determined and listed both in tables 3 and 4 belong to following monoterpene function group: hydrocarbons, esters alcohols ketones as well as two other terpenoidic phenols and sesquiterpene hydrocarbons groups. These results are consistent with Grigore and co-workers report [29]. The PCL assay proves that both Syrian *Th. cilicicus* and *Th. syriacus* are *thymol* chemotypes and the total antioxidant content belongs mainly to lipid soluble substances. Major chemical contents both in Syrian *Th. cilicicus* and *Th. syriacus* contain carvacrol with an average percentage value of 26.20% and 34.49%, respectively. Therefore, it can be attributed that the antioxidant activity is due to terpenoidic phenols since the major fraction content is carvacrol.

We ensured that the carvacrol fraction was separated according to its retention time using HPLC preparative instrument, then subjected to direct antioxidant measurement. It has been found that almost 75–85% of the reported measurements both in tables 5 and 6 concern mainly carvacrol fraction.

It can be seen both in table 3 and 4 that major fraction percentage comes from carvacrol in both *Th. cilicicus* and *Th. syriacus*. By combining the lipid-soluble antioxidant (ACL) obtained concentrations with carvacrol major fraction percentage, it can be deduced that the ACL concentration is mainly due to carvacrol fraction both in *Th. cilicicus* and *Th. syriacus* at Salah Al-Dein and Fahel mountain locations with an average value of 29.2% and 35.77% in both locations, respectively.

A representative comparison mass spectrum of carvacrol as a reference material obtained from Aldrich (spectrum A) and the extracted carvacrol sample from investigated species (spectrum B) is shown in Figure 2. Note that the spectra are identical one to another.

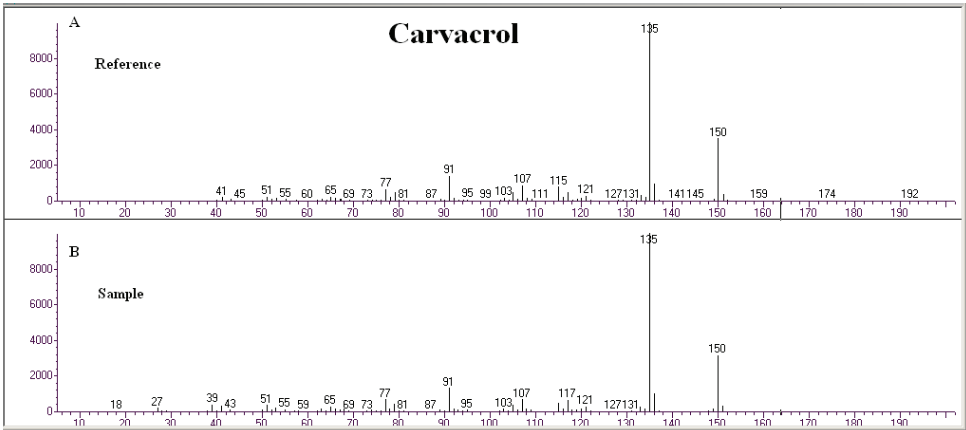

Figure 2.

The mass spectrum of carvacrol in the reference and extracted sample

Figure 3 shows a correlation diagram between the main carvacrol components in essential oil with ACL value. In left group (*Th. syriacus*), both carvacrol and ACL content are the highest in Fahel mountain in comparison with the other three locations. Similar conclusion was reached in the right group (*Th. cilicicus*): both carvacrol and ACL content are the highest in Salah Al-Dein in comparison with the other four locations. This confirms that the antioxidant activity is due to terpenoidic phenols because in both investigated species the major fraction content is carvacrol.

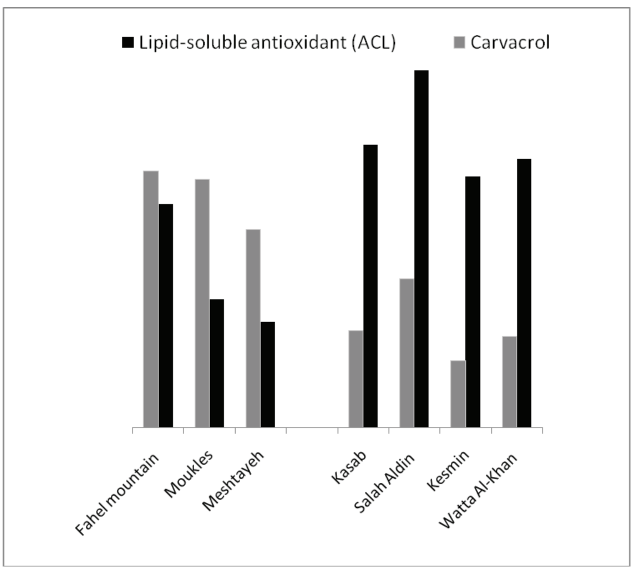

Figure 3.

The diagram of correlation between ACL and carvacrol

CONCLUSION

It can be concluded that the reported results support the view that Syrian *Th. cilicicus* and *Th. syriacus* leaves are a promising source of natural antioxidant for its potent antioxidant properties and contain significant amounts of terpenoidic phenols. The total integral antioxidant capacity measurements value of *Th. cilicicus* leaves is at Salah Al-Dein location with a total value of 475.80±1.20 nmol TE/g DM. Total integral antioxidant capacity measurements value of *Th. syriacus* leaves occurrs at Fahel mountain location with a total value of 374.60±0.94 nmol TE/g DM. The antioxidant activity is due to terpenoidic phenols because in both investigated species the major fraction content is carvacrol.

The ACL concentration is mainly due to carvacrol fraction both in *Th. cilicicus* and *Th. syriacus* at Salah Al-Dein and Fahel mountain locations with an average value of 29.2% and 35.77%, respectively.

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OKREŚLENIE SKŁADU OLEJKU ETERYCZNEGO ZA POMOCĄ GC-MS ORAZ ŁĄCZNEJ ZDOLNOŚCI ANTYOKSYDACYJNEJ METODĄ FOTOCHEMOLUMINESCENCYJNĄ W LIŚCIACH *THYMUS SYRIACUS* I *THYMUS CILICICUS* ROSNĄCYCH W RÓŻNYCH MIEJSCACH W SYRII

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S t r e s z c z e n i e

Składniki chemiczne *Thymus cilicicus* (*Th. cilicicus*) Boiss & Bal. and *Thymus syriacus* (*Th. syriacus*) Boiss rosnących w Syrii określono za pomocą spektrometrii GC-MS. Znaleziono i opisano 16 składników stanowiących średnio 93,85% olejku eterycznego *Thymus cilicicus*. Głównymi składnikami stwierdzonymi w materiale zebranym czterokrotnie w ciągu roku były: tymol, karwakrol, γ-terpinen, octan karwylu, dihydrokarwon i aldehyd anyżowy. Scharakteryzowano 18 składników stanowiących średnio 93,46% olejku eterycznego *Thymus syriacus*. Ważniejszymi składnikami stwierdzonymi w materiale zebranym czterokrotnie w ciągu roku były: karwakrol, dihydrokarwon, β-kariofilen, *p*-cymen, farnezol, limonin, mentol, myrecen, γ-terpinen, terpinen-4-ol i tymol. Najwyższa łączna zdolność antyoksydacyjna została znaleziona w *Th. syriacus* i *Th. cilicicus* zebranych w górach Fahal i w Salah Aldin. Wartość ta wyrażona jako łączny ekwiwalent antyoksydacyjny w nanomolach na gram suchego materiału wynosiła odpowiednio 374,6±0,94 i 47,80±1,20 nmola TE/g DM.

Słowa kluczowe: *antyoksydanty, Thymus cilicicus, Thymus syriacus, fotochemoluminescencja, olejek eteryczny*