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Chemical composition and antioxidant activity of the essential oil and the methanol extract of Algerian wild carrot *Daucus carota* L. ssp. *carota*. (L.) Thell.

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Abstract:
In this study we identified the chemical composition and antioxidants effects of essential oils and methanolic extracts from (seeds and leaves) of wild carrot. A Total of 48 and 47 volatile compounds from the essential oils of leaves and seeds in *D. carota* L. subsp. *carota*, were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). These compounds were mainly α-pinene (27.44%), sabinene (25.34 %), germacrene D (16.33 %) in leaves essential oil and geranyl acétate (52.45 %), sedrone S (14.04 %), azarone E (11.39 %) in seeds essential oil. The total phenol contents of leaves extract was higher (13.83 mg GAE/g) than seeds extract (7.08 mg GAE/g). The antioxidant activity was estimated by two methods (radical scavenging activity was determined by DPPH and 2-thiobarbituric acid (TBA) method for the measurement of lipid oxidation). Thus antioxidant activity of methanolic extracts (leaves and seeds) relevant to *D. carota* present an antioxidant activity more significant than the essential oils. According to the results of this experience, the essential oils of *D. carota* L. subsp. *carota* may be considered as an interesting source of natural antioxidant.

Keywords: Daucus carota ssp *carota*, essential oil, methanolic extract, antioxidant activity.

1. Introduction

The antioxidants are an increasingly important ingredient in food processing. Their traditional role involves, as their name suggests, inhibiting the development of oxidative rancidity in fat-based foods, particularly meat and dairy products, and fried foods. The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability, as well as due to a suspected action as promoters of carcinogenesis [1].
Consequently, there has been considerable interest in the use of antioxidants compounds from natural sources for food preservation and increased stability of fats and oils [2]. In this regard, there was an increase in research conducted on many plant species to find new natural bioactive compounds.
Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [3], and these phytochemicals have been known to exhibit different biological properties [4].
These plant essential oils and extracts have been used for many thousands of years [5], in food preservation, alternative medicine and natural therapies [6], many researchers evaluated the antioxidant activities of essential oils, plant extracts [7-11], etc. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare.
For a long time, plants from the *Apiaceae* family have been used as spices or drugs, particularly due to their essential oils. A dozen important herbal medicinal products from this botanic family are described in some Pharmacopoeias, having antiseptic, expectorant, diuretic, carminative, vasodilator, or spasmolytic actions [12]. *Daucus* L. (Apiaceae) includes about 60 species (1972) growing in Europe, Africa, West Asia, and also a few of them in Australia and North America [13]. The wild ancestors of the carrot are likely to have come from

Afghanistan, which remains the center of diversity of *Daucus carota* L., the wild carrot, sometimes called Queen Anne's lace [14].
Daucus carota L. is an aromatic plant used since old times in traditional medicine, due to recognized therapeutic properties, namely the antibacterial and antifungal activity of their essential oils (carrot oil). Although this plant has been subject to investigations [15 – 21] some scientific reports do not refer to the subspecies, a crucial aspect of this polymorphic species presenting 11 interrelated subspecies [22].
Extracts of wild *Daucus carota* L., were known to be antioxidative [23, 24] and iron-chelative [23]. The composition of the D. carota L. essential oil was variable according to the area of harvest, the part of the plant, and the stage of development [25, 17]. However, it could be summarized from literature data that leaf, stem, and blooming umbel oils are dominated by monoterpenes [25, 17] or sesquiterpenes [15]. Conversely, oils isolated from umbels in nest or seeds were dominated by *β*-bisabolene and *β*-asarone [15] or by (*E*)-methylisoeugenol accompanied by α-pinene and elemicin such as in commercial oils from Corsica, isolated from aerial parts harvested at the end of the flowering stage [25].
To the best of our knowledge there is no information on the antioxidant properties of Algerian wild carrot in the literature. This study have two main objectives: (I) determination of chemical compositions of its leaves and seeds essential oils by GC and GC–MS, (II) evaluation of antioxidant capacity of essential oil and methanolic extracts of leaves and seeds *D. carota*.

2. Materials and methods

2.1. Plant material

The aerial parts (leaves & seeds) of *Daucus carota* L.ssp *carota*. (L.) Thell. (apiaceae) were collected from Bouira (north of Algeria) in june 2009. The aerial parts (leaves & seeds) were dried in the shade (at room temperature) and finally ground to a fine powder.

2.2. Essential oil extraction

Essential oil was extracted from each of the plant parts (leaves/seeds) by steam distillation. The essential oil was separated by decantation and it was dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4°C until the time of experiment.

2.3. Preparation of methanol extract

One gram of the dried plant material is extracted for 48 h with 10 ml of 70% (v/v) aqueous methanol at room temperature. This procedure was repeated successively three times with fresh solvent each time, followed by filtration. Filtred extracts were mixed and concentrated under reduced pressure at 40° using vaccum rotary evaporator to dryness and the residue obtained was redissolved in methanol. All extracts obtained were kept in the dark at +4° C prior to use.

2.4. Analysis of the essential oils

2.4.1. Gas chromatography

GC analysis was performed on a Shimadzu GC17A chromatograph using fused silica capillary column with stationary phase DB-5. The various parameters fixed for the column are: 30 m x 0.32 mm, 0.25 µm film thickness. The temperature program was 60°C for 3 min then 3°C/min to 240°C for 3 min; injector 250°C; detector 250°C; N_2 was used as carrier gas at a flow rate 1 mL/min in the split mode 1:50, with an injection volume of 0.2 µL. Quantitative data was obtained from electronic integration of area percentages without the use of correction factors.
In order to determine retentions indices (RI), a series of n-alkane (C_5-C_{28}) mixtures were analyzed under the same operative conditions on DB-5 column; the samples indices were calculated following Van den dool & Kratz (1963) [26].

2.4.2. Gas chromatography and mass spectrometry

The GC/MS analysis was performed on a Trace Ultra GC linked to DSQII mass spectrometer using a DB-5 capillary column (30 m x 0.32 mm, 0.25 µm film thickness). It was programmed from 60°C (3min) to 240°C (3min) at 3°C/min with He carrier gas at a flow rate of 1 mL/min and injector heater 250°C. The MS conditions were: EI source, electron energy 70 eV and source temperature 250°C. Acquisition mass range, m/z = 40-450.

2.4.3. Component identification

Identification of components was made on the basis of their retentions indices and by computerized matching of the acquired mass spectra with those stored in the spectrometer data base using the Nist 2.0 and wiley 8.0 mass spectral library and the literature [27, 28].

2.5. Determination of total phenolic content

Total phenol content was determined by the method adapted from [29, 30] with some modifications using the Folin-Ciocalteu reagent. An aqueous aliquot (0.025 mL) of the extract was added to 3.975 mL of distilled water in a test tube, followed by 0.25 mL of Folin-Ciocalteu reagent. After 3 min, 0.75 mL of 20% sodium carbonate was added. Tube contents were vortexed and heated at 40 °C for 40 min. The blue coloration was read at 685 nm against a blank standard. Results were expressed in mg of gallic acid $g^{-1}$of extract. The standard curve equation is, A (absorbance) = 0.1035 gallic acid (µg/ml) +0.1046. (R^2=0.98).

2.6. Determination of total flavonoid content

The $AlCl_3$ method [31, 30] was used for the determination of the total flavonoid content of the methanolic extract. 1.5 mL of 2% $AlCl_3.6H_2O$ dissolved in methanol was added to equal volumes of the extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature. Flavonoid contents were expressed in mg quercetin equivalent $g^{-1}$of extract. The standard curve equation is, A(absorbance)= 0.2829 quercitin (µg/ml) – 0.1155 (R^2=0,99).

2.7. Antioxidant properties

2.7.1. DPPH assay

The hydrogen atom-or-electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2-diphenylpicrylhydrazyl (DPPH), as a reagent [32]. A 1.5 mL of methanolic solution of DPPH (0.004%) was mixed with equivalent aliquot of different concentration of sample in a tube. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm.
Inhibition of free radical DPPH in percent (*I*%) was calculated as follows:

$$I\% = (A_{control} - A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. Ascorbic acid was used as reference compound. All tests were carried out in triplicate.

2.7.2. Thiobarbituric acid reactive spices test (TBARS)

In this experiment, egg yolk homogenates were used as lipid-rich media, obtained as described by [33, 34] i.e., an aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v). The yolk was then homogenized for 30 s, followed by ultrasonication for a further 5 min. For the TBARS assay, 500 µl of 10% (w/v) homogenate and 100 µl of sample, solubilized in methanol, were added to a test tube and made up to 1 ml with distilled water, followed by addition of 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulphate (SDS). This mixture was stirred in a vortex, and heated at 95 °C for 60 min. After cooling, at room temperature, 5 ml butan-1-ol was added to each tube, stirred and centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer Schimadzu 160-UV. All the values were expressed as antioxidant index (AI%), where by the control is completely peroxidized and each extract and tested substance demonstrated a comparative percentage of antioxidant protection. The AI% was calculated using the formula: (1−t/c) ×100, c being the absorbance value of the fully oxidized control and t, the absorbance of the test sample.

2.8. Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by a Student's test. The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor. Differences were considered significant at $p<0.05$.

3. Results and discussion

3.1. Chemical composition of essential oils

D. carota essential oil yields 3% for seeds and 2.1% for leaves. The oils were analyzed by GC and GC-MS and the qualitative and quantitative composition are presented in (table 1), where compounds are listed in order of their elution on DB-5 column. A total of 48 compounds were identified in *D. carota* essential oil of leaves, 46 in seeds essential oil. The essential oil from seeds is predominantly composed of oxygenated monoterpens (66.08 %) and oxygenated sesquiterpens (16.41%). The main components are geranyl acétate (52.45%), cedrone S and asarone in different amounts (14.04%, 11.39% respectively). Differently the oil from leaves is mainly composed of hydrocarbon monoterpenes (64.59%) and hydrocarbon sesquiterpenes (22.18%), α-pinene (27.44%), sabinene (25.34%), germacrene D (16.33%).

Table 1: Chemical composition of *Daucus carota* ssp *carota* leaves and seeds essential oils (EO).

RT (mn)	RI	Compounds[a]	EOL(%)	EOS(%)
7.45	927	α-thujene	0.29	0.01
7.73	938	α-pinene	27.44	0.99
8.34	949	Camphene	0.89	0.07
9.13	977	Sabinene	25.34	0.32
9.33	979	β-pinene	1.25	0.24
9.64	990	β-myrcene	2.52	0.07
10.80	1010	α-terpinene	0.87	0.05
11.18	1016	P-cymene	0.46	0.07
11.31	1024	Limonene	2.24	0.10
11.43	1028	β-phellandrene	0.57	-
11.51	1031	Ocimene<B-z>	0.12	-
11.95	1035	Ocimene<B-z>	0.32	-
12.53	1056	γ-terpinene	1.79	0.10
13.68	1087	Terpinolene	0.49	0.03
14.34	1098	Linalol	0.21	0.32
16..55	1144	Verbenol	-	0.12
18.11	1175	Terpinen	0.68	0.16
21.18	1268	Geraniol	-	1.40
22.65	1280	Acetate bornyle	0.51	0.24
24.67	1330	γ-elemene	0.35	-
25.39	1343	α-longipinene	0.04	0.82
26.48	1369	α-capaene	0.21	0.04
26.70-26.87	1377	Geranyl acetate	1.41	52.45
26.85	1385	β-bourbonène	0.18	-
27.08	1399	β-ilemene	0.32	-
28.40	1405	β-caryophyllene	0.62	-
28.85	1413	α-transbergamotene	0.26	0.19
29.22	1438	2-epi-beta funebrene	0.34	0.08
29.55	1444	β-damascone	-	0.19
29.68	1447	Farnesene(Z ,B)	0.25	0.10
29.95	1449	α-humulene(E ,E)	0.21	0.05
30.77	1471	γ-muurolen	0.20	0.06
31.04	1475	Germacrene	16.33	-
31.15	1478	β-himachalene	0.57	0.53
31.37	1482	Isoshybunone	-	0.04
31.60	1502	Bicyclogermacrene	0.45	-
31.78	1504	NI	0.11	0.72
32.05	1508	β-bisobolene	0.71	4.83

32.29	1512	4,5,9,10dihydroisolongifolene	1.83	0.12
32.48	1516	γ-cadinene	0.46	0.01
32.91	1531	Himachalene	-	0.16
33.25	1534	Ar-himachalene	-	3.54
33.54	1541	α-calacorone	-	0.05
34.18	1555	Germacrene β	0.68	-
36.12	1575	Spathulenol	-	0.30
36.3	1585	Caryophyllène oxyde	0.28	0.06
37.22	1627	Cubenol 1-epi	-	0.26
37.8-37.88	1635	Cederone S	0.86	14.04
38.32	1645	15-Nor-funebran-3-6	0.67	0.02
38.87	1656	Asorone(Z)	0.28	-
38.91	1675	Asorone €	-	11.39
39.05	1677	NI	0.21	0.25
39.16	1680	α-bisobolol	0.56	0.11
39.48	1682	NI	0.14	-
39.67	1686	Selin-11-en-4alpha-ol	0.11	0.73
42.88	1693	NI	-	0.18
44.51	1695	Cedrenol	0.22	0.08
45.78	1749	Curcumenol	0.08	0.83
50.29	2000	Manoyloxide	0.05	0.09
		Monoterpenes hydrocarbons	64.59%	2.05%
		Oxygenated monoterpenes	3.09%	66.08%
		Sesquiterpenes hydrocarbons	22.18%	10.46%
		Oxygenated sesquiterpenes	2.11%	16.41%
		Oxygenated diterpene	0.05%	0.09%
		Other	2.96%	1.52%
		Total identified	94.98%	96.61%

RT: Retention time; RI: retention indices on the DB-5 column relative to C_8-C_{24} n-alkanes; a: compounds listed in order to their elution on the DB-5 column; EOL: essential oil leaves; EOS: essential oil seeds; NI: non identified compounds.

Chemical composition of essential oil extracted from leaves of our species is similar to that of wild carrot of Serbia which is characterized by the presence of α-pinene, sabinene, the β-myrcene, limonene and germacrene D [35]. In reported reference [18] showed that oils obtained from different parts of wild carrot plant consist mainly of monoterpene hydrocarbons (71.9 – 83.8%). They have not confirmed the presence of carotol, daucol and daucene; the sesquiterpenes that are specific for a chemical composition of the oils obtained from the cultivated breeds of carrot (*D carota* ssp. *sativus*) [36]. In previous work [25] they found that the Corsican oil of *Daucus carota* L. contained E-methylisoeugenol (33.0%), α-pinene (24.9%) and elemicin (11.4%) as its major components. As was reported previously [16], trans-dauca-8,11-diene,duaca-5,8-diene, acora-4,9-diene, acora-4,10-diene, (E)-β-10,11-dihydro-10,11-epoxyfarneseneand (E)-methylisoeugenol were identified as the major components of *Daucus carota* seed oil.

3.2. Antioxidant properties

The following chart indicate, the rate of DPPH scavenging activity versus the different concentrations of *D. carota* ssp *carota* essential oils and extracts.

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical-scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form 1,1-diphenyl-2-picrylhydrazine (non radical) with the loss of this violet color [37]. DPPH scavenging activity is usually presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Therefore, extract concentrations providing 50% inhibition (IC_{50}) were calculated using the data plotted in Fig. 1, and were presented in Table 2. Lower IC_{50} value reflects better DPPH radical-scavenging activity.

A B

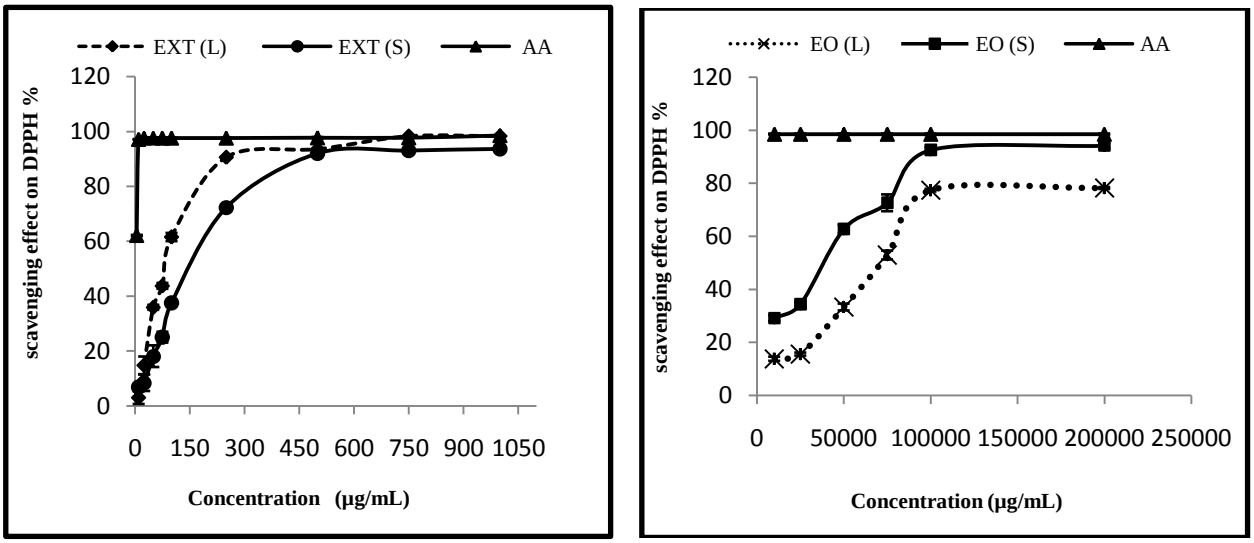

Figure1: DPPH radical-scavenging activities of *Daucus carota* (a) methanolic extracts, (b) essential oil and standard (ascorbic acid) measured at different concentrations.EXT (L): leaves extract, EXT(S): seeds extract, EO(L): leaves essential oil, EO (S): seeds essential oil, AA: ascorbic acid.

Table 2: antioxidant activities of *D. carota* ssp *carota* essential oils, methanol extracts, total phenolic and flavonoid content of methanol extracts

Sample	**DPPH IC_{50} (µg/mL)**	**TBARS assay IC_{50} (µg/mL)**	**Total phenol contents (mg GAE/g)**	**Total flavonoid contents (mg QE/g)**
Leaves methanolic extract	83±1	66.5±7.05	13.83±0.85	1.98±0.01
Seeds methanolic extract	136±3.21	21.17±1.26	7.08±0.41	1.51±0.03
Leaves essential oil	$76.33\times10^3\pm208$	$1.29.\ 10^3\pm150$	Nd	Nd
Seeds essential oil	$38.67\times10^3\pm153$	$0.82.\ 10^3\pm300$	Nd	Nd
Ascorbic acid	3.73±0.06	Nd	Nd	Nd
α-tocopherol	Nd	22.67±1.26	Nd	Nd

Nd : Not determined.

The leaves methanolic extract of *D. carota* provided the highest radical-scavenging activity with the lowest IC_{50} value of 83±1µg/ml than the seeds methanolic extract with IC_{50} of 136±3.21µg/ml and essential oil with IC_{50} value of 76.33±2.08mg/ml, 38.67±1.53mg/ml for leaves and seeds respectively. Furthermore, DPPH scavenging abilities of the methanolic extract and the oil were lower than that of synthetic antioxidant ascorbic acid. DPPH scavenging activity of extracts increased in the order of ascorbic acid > leaves methanolic extract > seeds methanolic extract>seeds essential oil> leaves essential oil.

The antioxidant activities of the plant extracts and the oils were also evaluated by TBARS assay. In the test of the 2-thiobarbituric acid (TBARS) a molecule of malonaldehyde (MA) reacts with two molecules of thiobarbituric acid (TBARS) to form a pink complex absorbing at 532 nm. All the essential oils and extracts showed some antioxidant capacity, increasing over the concentration range tested.

The calculated percentage of inhibition of the extract and the oil are given in fig 2. The IC_{50} values of lipid peroxydation were estimated as 66.5±7.05, 21.17±1.26, $1.29\times 10^3\pm150$ and $0.82\times10^3\pm300$ µg/ml in the presence of leaves, seeds of methanolic extracts and the essential oils, respectively.

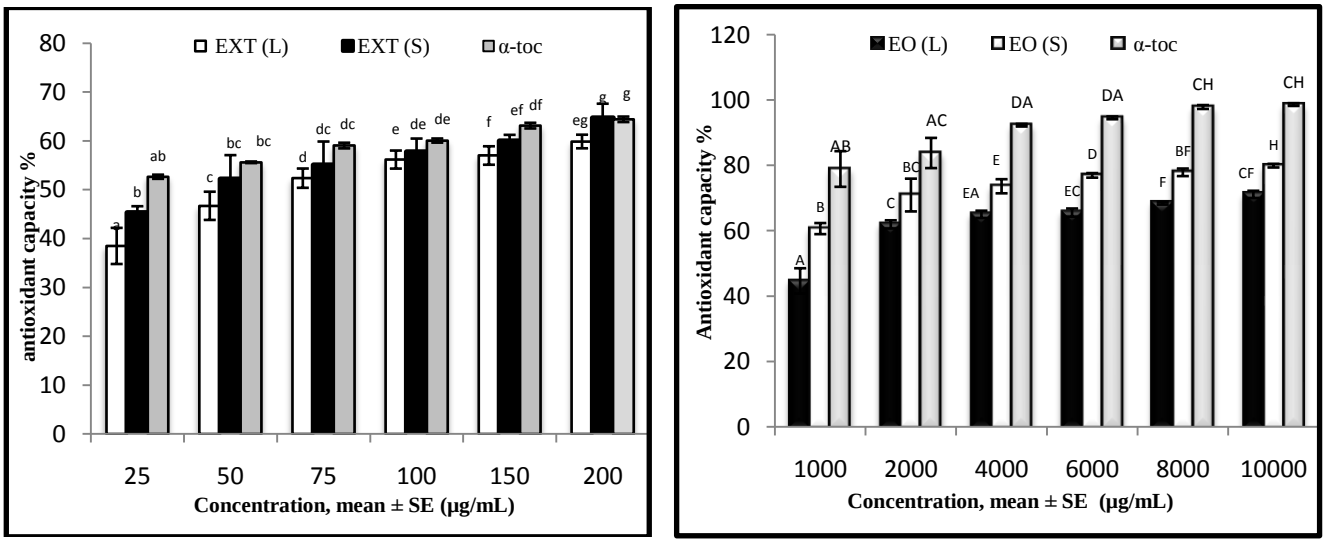

Figure2: Antioxidant capacity (%) of the essential oils and methanol extracts of *D. carota*s sp *carota* compared to α-tocopherol, in different concentrations, using TBARS assay SE, standard error. Means with different superscript letters are significantly different (P<0.05).

The seeds methanolic extract showed the highest antioxidant index, comparable to α-tocopherol . The essential oils of seeds and leaves showed much lower antioxidant indices than that of methanol extracts. The decrease in concentration of the oils and extracts produced a drastic reduction in their activity, and at the lowest concentration the oils and extracts were scarcely active.

The amounts of total phenolics in the extracts were determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents. Gallic acid is a water soluble polyhydroxy phenolic compound which can be found in various natural plants [38]. The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated.

The $AlCl_3$ method was used for the determination of the total flavonoid content of the methanolic extracts (leaves, seeds). Flavonoid contents were expressed in mg quercetin equivalent g^{-1}dry weight. The amounts of total phenols and flavonoids found in the plant methanolic extracts are shown in Table 2. The total phenolic contents of leaves and seeds methanolic extracts of *D. carota* are 13.83±0.85, 7.08±0.41mg gallic acid equivalent/g extract respectively. The results indicated that the leaves methanolic extract has higher total phenolic compounds than seeds methanolic extract. Also, according to these results, there is a relationship between total phenol contents and radical scavenging activity. Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups [9]. But no significant correlation between phenolic content of tested methanolic extract and TBARS assay was observed. These results are in agreement with other reports in the literature [39, 40]. The high antioxidant capacity of the seeds essential oil is probably due to its richness in oxygenated monoterpene compounds (66.08%), (table 1). The stronger antioxidant activity exhibited by investigated essential oil obtained from *Achillia pannonica*, in both the DPPH-test and TBA-assay, confirms results which showing that some of the oxygenated monoterpenes are mostly responsible for protective effects [41]. The antioxidant activity of essential oils were variable; this variability is mainly related to its molecular composition.

Conclusion

According to the results of this study, the essential oils or the methanolic extracts of *D. carota* subsp *carota* may be suggested as a potential source of natural antioxidant. The methanolic extract and the oil were found to be effective antioxidants in several in vitro assays including DPPH radical scavenging and TBARS assay which can be proposed as a natural additive in food industry to increase the shelf life of food stuffs and in pharmaceutical industries. There was a good correlation between total phenol content and DPPH scavenging capacity of the extracts. The best antioxidant activity of both extracts was registered against lipid peroxidation.

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