IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 2 Ver. 1 (Mar -Apr. 2015), PP 13-19
www.iosrjournals.org

Chemical Composition and Biological Activity of the Essential Oils Obtained From Yellow and Red Carrot Fruits Cultivated In Egypt

Noha Khalil[1], Mohamed Ashour[2], Abdel Nasser Singab[2] and Osama Salama[1]
[1]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt* [2]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

Abstract: *The chemical composition of the essential oils from the fruits of Daucus carota var. sativus (yellow carrot) and var. boissieri (red carrot) was determined using GC/FID and GC/MS. Altogether; 29 and 32 compounds were identified accounting for 96.58 and 96.72 % of the total detected components in the hydrodistilled yellow and red carrot oils, respectively. Carotol constituted the major component in both oils (~66–68 %). The antioxidant activity was assessed using both diphenylpicrylhydrazyl (DPPH•) and 2-deoxyribose degradation (2-DR) assays. Both oils reduced DPPH• and inhibit 2-DR with IC_{50} values of 12.71, 14.15, mg/ml and 87.15, 88.75 µg/ml for the yellow and red carrot oils, respectively. Inhibition of both 5-lipoxygenase (5-LOX) enzyme and prostaglandin E_2 (PGE_2) production were used to assess the anti-inflammatory activity. Yellow carrot oil showed better activity towards inhibition of 5-LOX (IC_{50} of 37.53 µg/ml). The cytotoxicity of both oils was determined against both MCF-7and HepG-2 cancer cell lines using the sulforhodamine B (SRB) assay. The highest cytotoxic activity was observed against HepG-2 cell with IC_{50} values ranging from 163-172 µg/ml for both oils. These results indicate promising antioxidant and anti-inflammatory activity of both oils and provide a rationale for their uses in many skin diseases.*

Keywords: *Antioxidant, Anti-inflammatory, Apiaceae, Cytotoxicity, Daucus carota, Essential oil.*

I. Introduction

Man relied upon the plant kingdom for prevention and alleviation of various ailments. Undoubtedly, this could be attributed to a plethora of causes comprising their lower side effects, reasonable cost as well as comparable efficacy to synthetic ones [1]. Generally, the efficacy of herbal remedies resides in their richness with phytochemicals embracing essential oils, which are oily odoriferous principles with multiple pharmacological and biological activities. Nowadays, aromatherapy attains a great popularity because of its compatibility with our bodies. That improves both physiological and psychological process and helps in healing many disorders [2].

Additionally, the attention towards the use of medicinal herbs as anti-inflammatory agents has been revived due to the risk of serious side effects accompanied with the usage of synthetic drugs (NSAIDs) [3]. Moreover, plants contain diverse free radicals scavenging molecules, which are used to minimize the effect of emitted radicals associated with inflammation [4].

Family Apiaceae (Umbelliferae), which is also known by carrot, celery or parsley family comprises about 434 genera represented by more than 3,700 species [5]. The family is highly popular by the presence of aromatic plants exemplified by angelica, anise, caraway, celery, coriander, cumin, fennel and galbanum. The notable fragrant aroma of most of its members is mainly attributed to the existence of volatile oils as well as oleoresins among their various parts [6].

The genus *Daucus*, belonging to family Apiaceae, includes about 60 species distributed mostly in Africa, Europe, West Asia and only few are among Australia and North America [5]. The most popular and substantially economic species, *Daucus carota* L., exists almost everywhere. However, six wild species are grown in Egypt among which the two varieties *Daucus carota* var. *sativus* and var. *boissieri* are widely cultivated for their fleshy edible roots [7].

Traditionally, *Daucus carota* was employed by the Ancient Egyptians as a stimulant, carminative and diuretic, concurrently its decoction was used for curing infantile diarrhea and as an anthelmintic agent [6]. Recently, it was shown that *Daucus carota* encompass multiple active constituents such as flavonoids [8,9], essential oils [10], polyacetylenes [11,12] and phenylpropanoids [13]. Besides, many reports were found in the current literature regarding the biological and pharmacological activities of its essential oil, including hypotensive, cardiac and CNS depressant, antibacterial, antibilharzial and fungicidal activities [14], whereas, carrots exhibited a potent hepatoprotective activity in chloroform-induced hepatic damage in experimental animals [15, 16].

In this study, the essential oils of two familiar *Daucus carota* varieties grown in Egypt, namely *D. carota* var. *sativus* and var. *boissieri* were compared regarding their chemical composition, antioxidant, anti-inflammatory and cytotoxic activity in HepG2 and MCF-7 carcinoma cells in an effort to emphazise their medicinal importance to which many of their traditional uses could be assigned.

II. Materials And Methods

2.1 Plant Material: The fruits of *D. carota* var. *sativus* (yellow carrot) and *D. carota* var. *boissieri* (red carrot) were purchased by one of the authors (N.K.) from a local herbal market. The identity of the fruits was ascertained morphologically by Prof. Abdel Sallam Al-Newaihi, Department of Botany, Faculty of Science, Ain Shams University. Voucher specimens of the plant material are deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University under the number of P332 and P333.

2.2 Isolation of the essential oil: The essential oils of the two varieties were obtained by hydrodistillation of the fruits separately for 6 h using a Clevenger-type apparatus. Both oils were dried over anhydrous sodium sulfate and kept in separated sealed vials at 4 ºC for analysis.

2.3 Identification of the essential oil components using Gas chromatography

*2.3.1 GC/FID analysis***:** The GC analysis was carried out on a Focus GC ® (Thermo fisher scientific®, Milan, Italy) equipped with TR5-MS fused bonded column (30 m x 0.25 mm x 0.25 µm) (Thermo fisher scientific®, Florida, USA) and FID detector; carrier gas was nitrogen (1.5 ml/ min); the operating conditions were: initial temperature 40 ºC, 1 min. isothermal followed by linear temperature increase till 230 ºC at a rate of 4 ºC / min., then 5 min. isothermal. Detector and injector temperatures were 300 and 220 ºC, respectively. The split ratio was 1: 20. Chrom-card® chromatography data system ver. 2.3.3 (Thermo Electron Corp. ®, Florida, USA) was used for recording and integrating of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used for calculation the % composition of each component.

2.3.2 GC/MS analysis: The analysis was carried out on Focus GC ® (Thermo fisher scientific®, Milan, Italy) equipped with the same column and conditions mentioned in the GC/FID. The capillary column was directly coupled to a quadrupole mass spectrometer Polaris Q, (Thermo Electron Corp. ®, Milan, Italy). The injector temperature was 220 ºC. Helium carrier gas flow rate was 1.5 ml/min. All the mass spectra were recorded with the following condition: filament emission current, 100 mA; electron energy, 70 eV; ion source, 250 ˚C; diluted samples were injected with split mode (split ratio, 1:15). Compounds were identified by comparison of their spectral data and retention indices with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library (December 2005), our own laboratory database and the literature [17,18].

2.4 Antioxidant activity

2.4.1 Radical Scavenging activity: The radical scavenging activity of the essential oils were evaluated according to a standard procedure [19] using diphenyl picryl hydrazyl (DPPH•). Equal volumes of sample solutions containing 0.02–40 mg/ml of the oils and 0.2 mM methanolic solution of DPPH• were mixed and the absorbance was measured against a blank at 520 nm using a Jenway® 6800 UV/VIS spectrophotometer (Essex, UK) after incubation in the dark for 30 min. at room temperature compared to DPPH• control after background subtraction. Quercetin was used as a positive control. The percent inhibition was calculated from three different experiments using equation (1):

$$\text{Inhibition (\%)} = 100 \times [\text{A520 (control)} - \text{A520 (sample)}/\text{A520 (control)}] \quad (1)$$

*2.4.2 Deoxyribose degradation assay***:** The ability of the tested samples to prevent the degradation of the deoxyribose was determined according to a standard procedure [20,21]. A mixture of freshly prepared 28 mM 2-deoxy-2-ribose (2-DR) in phosphate buffer pH 7.1, 1.04 mM EDTA and 200 µM $FeCl_3$, 1.0 mM H_2O_2 and 1.0 mM ascorbic acid was mixed with an equal volume of various sample concentrations (0.002–6 mg/ml of each oils) and kept at 37 °C for 1 h. A mixture of thiobarbituric acid and 2.8% trichloroacetic acid, (1:1 v/v) was added to the reaction mixture and incubated at 100 °C for 20 min. Absorbance was measured at 540 nm against blank using a Jenway® 6800 UV/VIS spectrophotometer (Essex, UK). Quercetin was used as a positive control. Inhibition of deoxyribose degradation was measured in triplicate at 540 nm as above.

2.5 Anti-inflammatory activity

2.5.1 Prostaglandin E_2 inhibition assay: The effect of the tested samples on prostaglandin E_2 (PGE_2) production was assessed using Monoclonal EIA Kit®. Briefly, poorly differentiated pancreatic MIA PaCa-2 cancer cells were treated with 50 µl sample solutions (25 µg/ml for oil and 3.14 µg/ml for the positive control), incubated at

room temperature and 24 h later, cells were stimulated with 30 µM arachidonic acid and the culture supernatants were collected (after 15 min) then centrifuged to remove debris. Prostaglandin levels were determined in three independent experiments by a competitive enzyme immunoassay [22]. Inhibition of the prostaglandin E_2 level was calculated relative to the blank control and N-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398) which was used as a positive control.

2.5.2 5-Lipoxygenase inhibition assay: Inhibition of the soybean 5-lipoxygenase by the tested samples was determined spectrophotometrically [23]. Briefly, one milliliter of 0.1 M phosphate buffer pH 9.0 containing 10 µl enzyme (7.9 U/ml) and 20 µl of 10 different concentrations of the tested samples (10–350 µg/ml oils) were incubated at room temperature for 10 min. The reaction was initiated by adding 25 µl of 62.5 µM sodium linoleate and the reaction kinetics was monitored at 234 nm at 10 sec intervals using Jenway® 6800 UV/VIS spectrophotometer (Essex, UK). The initial reaction rates were determined from the slope of the straight line portion of the curve and inhibition of the enzyme activity was calculated from three independent experiments by comparison with the control (ethanol). Nordihydroguaiaretic acid (NDGA) was used as a positive control (IC_{50} 0.24 µg/ml).

2.6 Antiproliferative activity

2.6.1 Cell culture: HepG2 (hepatic cancer) and MCF-7 (breast cancer) cell lines were maintained in DMEM complete media (L-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ ml penicillin, and 100 U/ml streptomycin). Cells were grown at 37 ºC in a humidified atmosphere of 5% CO_2. All experiments were performed with cells in the logarithmic growth phase.

2.6.2 Cytotoxicity and SRB cell viability assay: Sensitivity of HepG2 (hepatic cancer) and MCF-7 (breast cancer) cell lines to tested samples was determined in triplicate using the SRB cell viability assay [24]. Exponentially growing HepG2 and MCF-7 cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates Greiner Labortechnik® (Frickenhausen, Germany) at 1000-2000 cells/well. The cells were cultivated for 24 h and then incubated with various concentrations of the serially diluted tested samples (stock solution 1 mg/ml) at 37 ºC for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 ºC. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and optical density intensity was measured at 545 nm using microplate reader Chromate® 4300, (Awareness Technology Inc. Florida, USA).

III. Results And Discussion

3.1 GC/FID and GC/MS analyses: Essential oils of *D. carota* var. *sativus* and var. *boissieri* are obtained in a woody, earthy sweet smell and are yellow or amber-colored to pale orange-brown in appearance. The yields ranged from 1% v/w and 1.2 % v/w respectively (ml/100 gm dried fruits). Twenty nine and thirty two compounds were unambiguously identified accounting for 96.58 % and 96.72 % of the total components in both yellow and red carrot oils, respectively (Fig. 1a, 1b). Carotol was the major components of both oils (Table 1).

Yellow carrot fruit essential oil had a lower percentage of monoterpenes representing 2.24% and containing mainly β-pinene (0.52%) and α-limonene (0.43%) and oxyegenated monterpenes represented 0.66% containing mainly linalool (0.34%). Red carrot fruit essential oil had higher percentage of monoterpenes representing 3.92% and containing mainly β-pinene (1.04%) and p-cymene (1.01%) and didn't contain any oxygenated monoterpenes. On the other hand, sesquiterpenes had a higher percentage in yellow carrot fruit essential oil representing 96.11% containing mainly carotol (66.7%), β-bisabolene (3.91%), trans-α-bergamotene (3.41%), Germacrene (2.34%) and α-curcumene (2.2%). Almost the same percentage and different pattern can be noticed for the red carrot fruit essential oil (carotol (67.71%), β-bisabolene (7.66%), trans-β-caryophyllene (4.79%) and trans-α-asarone (2.33%)). Only n-nonanal as a non terpene compound was present in yellow carrot fruit essential oil (0.05%). These results are in accordance with other earlier published data about the *Daucus* oils [25].

However, the geographical location of *D. carota* plays an important role in the pattern of the main components. In England, its main constituents are carotol (20.20%), sabinene (12.80%), β-caryophyllene (8.04%) and α-pinene (6.05%)[26]. In another study, the essential oils of *D. carota* from Tunisia were identified. The main volatile compounds identified were β-bisabolene (39.33%), sabinene (8.53%), geranyl acetate (7.12%), and elemicin (6.26%) [27]. The oils from Portuguese samples are predominantly composed of geranyl acetate (65.0%) and α-pinene (37.9%) [28]. Similar results could be noticed for Swiss samples [29]. Generally, the variations may be due to different climatological factors, the nutritional status of the plants, variety and other factors that can influence oil composition. As a result, differences in the physical properties of fruits having about the same size were probably due to environmental conditions in conjunction with the analytical methods used [30].

3.2 Antioxidant and anti-inflammatory activities: Generally, inflammation involves the formation of both prostaglandins and leukotrienes followed by the liberation of the neutrophil and production of the reactive oxygen species.

Both oils were able to reduce DPPH$^{\bullet}$ and to prevent the degradation of the deoxyribose sugar in a concentration dependent manner Figure (2a, 2b). Carrot oils showed promising scavenging activity of DPPH$^{\bullet}$ with an IC_{50} of 12.71 mg/ml and 14.15 mg/ml for the yellow and red carrot oils, respectively. Inhibition of deoxyribose degradation was much observed with an IC_{50} of 87.15 and 88.75µg/ml, respectively. The presence of a high percentage of oxygenated sesquiterpenes in both oils is relevant for the promising antioxidant activities, especially in the deoxyribose assay. However, the effect is much more pronounced in the deoxyribose assay experiment due to lack of phenolic species required to achieve a good activity in the DPPH$^{\bullet}$ assay.

The anti-inflammatory activity of the essential oils was clearly observed through their abilities to suppress soybean 5-LOX and inhibition of prostaglandin E_2 production (Fig. 3a, 3b). Inhibition of the lipoxygenase was observed with IC_{50} of 37.53 and 53.76 µg/ml for the yellow and red carrot oils, respectively. Inhibition of PGE_2 formation was seen in both oils with percentages of 14.02% and 26.03% for the yellow and red carrot oils, respectively. The inhibition of both 5-LOX and COX-II is used as a criterion for the excellent anti-inflammatory activity that could be used for the treatment of many inflammatory disorders.

3.3 Cytotoxic activity: The cytotoxicity of the essential oils was evaluated using two human cancer cell lines namely HepG-2 (liver hepatocellular carcinoma cells) and MCF-7 (breast adenocarcinoma cells) after 72 h incubation. The IC_{50} values are represented in fig. 4. The highest cytotoxic activity was also observed against HepG-2 cell with IC_{50} values of 172.07 and 163.83µg/ml for the yellow and red carrot oils, respectively. *Daucus carota* oil demonstrated a significant increase in cell death and decrease in cell proliferation on different cancerous cell lines [31]. The moderate cytotoxic activity of the oils may be attributed to the presence of carotol, which is the major component in both yellow and red carrot oils has proved its ability to inhibit the growth of myeloid leukemia cancer cell lines [32].

IV. Figures and Tables

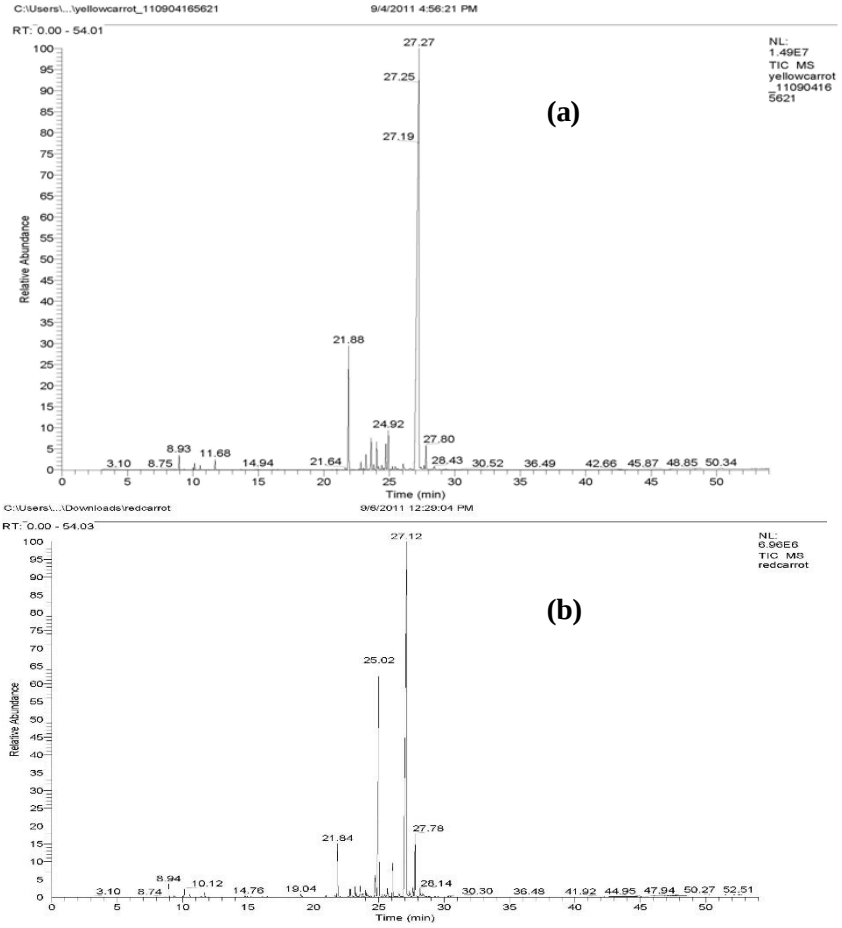

Figure 1: GC-profile of the essential oil of the fruits of *Daucus carota* var. *sativus* (yellow carrot) (a) and var. *boissieri* (red carrot) (b)

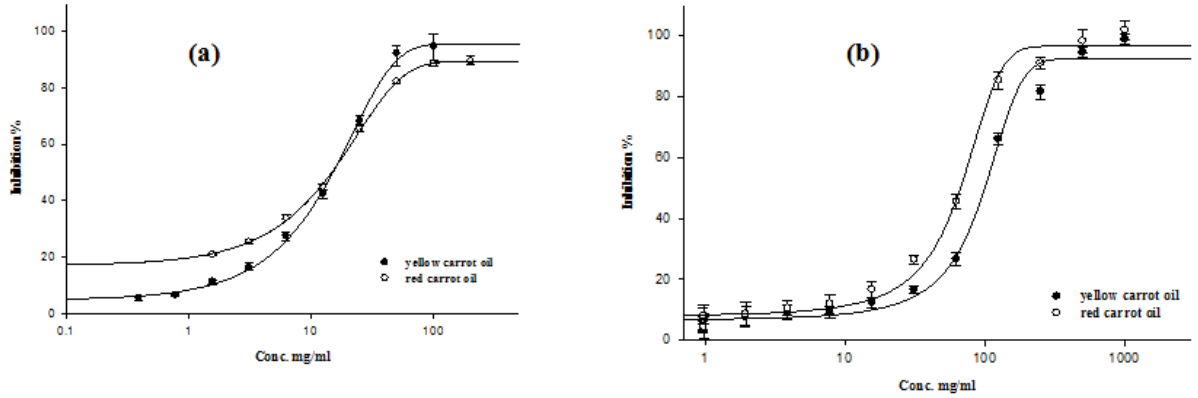

Figure 2: The inhibitory effects of the essential oil of the fruits of *Daucus carota* var. *sativus* (yellow carrot) and var. *boissieri* (red carrot) on DPPH• (a), and 2-deoxyribose (b).

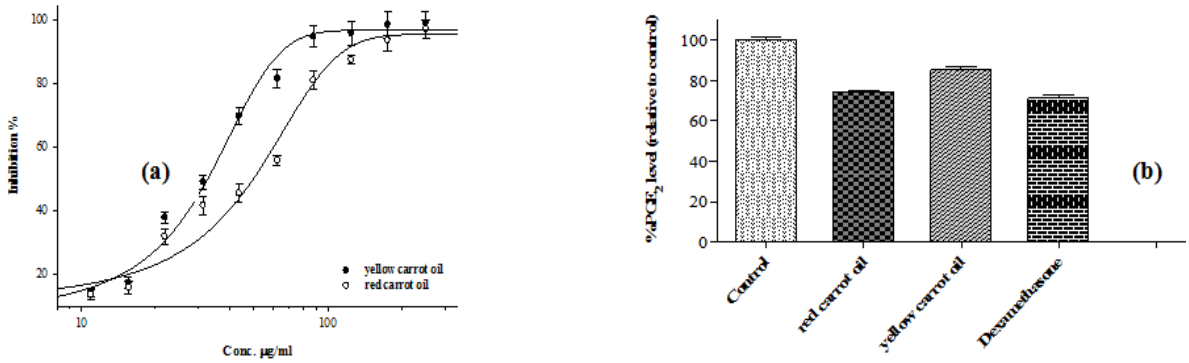

Figure 3: The inhibitory effects of the essential oil of the fruits of *Daucus carota* var. *sativus* (yellow carrot) and var. *boissieri* (red carrot) on 5-liboxygenase (a), and prostaglandin E_2 production (b).

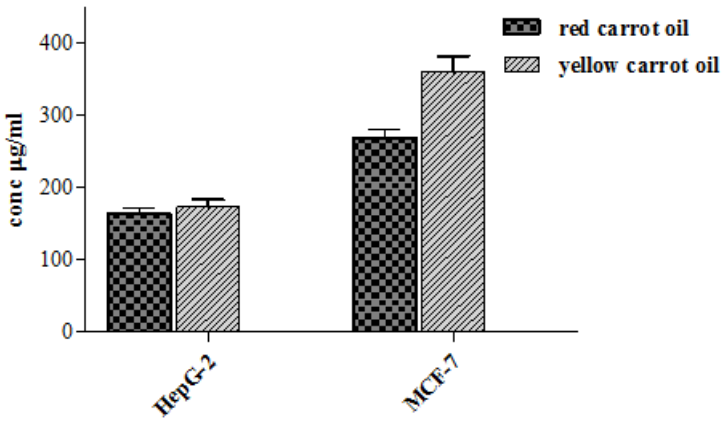

Figure 4: The antiproliferative activity of the essential oil of the fruits of *Daucus carota* var. *sativus* (yellow carrot) and var. *boissieri* (red carrot) on HepG2 and MCF-7 cell lines.

Table 1: Chemical composition of the essential oil of the fruits of *D. carota* var. *sativus* and *D. carota* var. *boissieri*

No.	Retention Time (min.)		Compounds	Kovat's index		Area %		Method of Identification
	var. *sativus*	var. *boissieri*		calculated (TR5-MS)	reported	var. *sativus*	var. *boissieri*	
1.	---	7.00	α- Thujene	912	914	---	0.07	MS, RI, AU
2.	7.21	7.18	α- Pinene	918	918	0.17	0.62	MS, RI, AU
3.	7.6	7.57	Camphene	931	930	0.04	0.01	MS, RI, AU
4.	8.47	8.44	Sabinene	956	958	0.10	0.01	MS, RI, AU
5.	8.57	8.94	β- Pinene	959	959	0.52	1.04	MS, RI, AU
6.	9.21	8.99	β- Myrecene	979	979	0.17	0.46	MS, RI, AU
7.	10.19	9.15	p- Cymene	1008	1008	0.07	1.01	MS, RI, AU

8.	10.51	9.48	α- Limonene	1017	1016	0.43	0.43	MS, RI, AU
9.	10.58	10.56	γ- Terpinene	1049	1052	0.08	0.27	MS, RI, AU
10.	10.79	---	Linalool	1058	1053	0.34	---	MS, RI, AU
11.	10.92	---	n- nonanal	1096	1092	0.05	---	MS, RI
12.	11.01	---	trans- pinocarveol	1121	1121	0.04	---	MS, RI
13.	11.35	---	trans- verbinol	1136		0.08	---	MS, RI, AU
14.	11.56	---	p- cymene- 8-ol	1154	1156	0.07	---	MS, RI
15.	11.77	---	α- terpineol	1175	1175	0.07	---	MS, RI, AU
16.	21.31	20.12	Daucene	1372		8.74	1.10	MS, RI
17.	---	21.43	Iso dauca- 4,7(14)-diene	1375		---	0.07	MS, RI
18.	21.39	25.35	α- Cedrene	1404	1405	---	0.29	MS, RI
19.	21.48	21.84	trans- β- Caryophyllene	1406	1406	1.1	4.79	MS, RI, AU
20.	21.88	22.07	trans- α- Bergamotene	1427	1422	3.41	1.23	MS, RI
21.	---	22.17	Aromadendrene	1431	1430	---	0.57	MS, RI
22.	---	22.49	α- Humulene	1440	1440	---	0.04	MS, RI
23.	---	22.63	(E)-β-Farnrsene	1445	1446	---	1.82	MS, RI
24.	22.66	---	Epi-β-Santalene	1448	1453	0.15	---	MS, RI
25.	22.78	---	(Z,Z)-α-Farnesene	1451		1.86	---	MS, RI
26.	22.96	---	Germacrene D	1453		2.34	---	MS, RI
27.	23.37	23.32	α- Curcumene	1468	1468	0.23	0.14	MS, RI
28.	23.50	23.49	β- Selinene	1472	1472	2.2	0.10	MS, RI
29.	---	23.52	δ- Selinene	1473	1478	---	0.06	MS, RI
30.	23.90	23.85	α- Selinene	1485	1485	0.89	0.22	MS, RI
31.	---	24.91	β- Himachalene	1492	1494	---	0.31	MS, RI
32.	24.53	---	Bicyclogermacrene	1501	1501	1.87	---	MS, RI
33.	24.92	25.02	β- Bisabolene	1507	1507	3.91	7.66	MS, RI
34.	---	25.76	γ- Cadinene	1513	1513	---	0.19	MS, RI
35.	26.4.	26.30	E-α-Bisabolene	1530	1531	1.90	1.14	MS, RI
36.	---	26.61	Germacrene B	1540	1540	---	0.56	MS, RI
37.	---	27.18	β- Caryophyllene oxide	1559	1561	---	0.33	MS, RI, AU
38.	27.27	27.12	Carotol	1583	1594	66.78	67.71	MS, RI
39.	---	27.22	Acora- 3,5 -diene- 11-ol	1592		---	0.29	MS, RI
40.	27.84	27.78	Daucol	1614	1624	0.45	1.61	MS, RI
41.	---	27.80	trans α-asarone	1646	1650	---	2.33	MS, RI
42.	---	28.14	Acorenone B	1673		---	0.20	MS, RI
			Total			**96.58**	**96.72**	

[a]) The composition (%) is an average of three analyses; tr: trace; [b]) MS, identification based on mass spectral data, [c]) RI, identification based on comparison of published Kovats retention indices, [d]) AU, identification based on co-chromatography with authentic ones.

V. Conclusion

The increasing interest of alternative and complementary medicine in the last decade shade light on essential oils, which represent a big class of plant secondary metabolites that possess diverse biological activity. Essential oils of Family Apiaceae generally have proved to have potential antioxidant and anti-inflammatory activity. Although, carrot juice proved to protect the cardiovascular system by increasing total antioxidant status [33], however, there is no much reports about the biological activity of its essential oil. The promising radical scavenging activity and significant inhibition of both prostaglandin E_2 and 5-lipoxygenase by both carrot oils validated the ethno pharmacological use of these oils in the treatment of many skin diseases associated with inflammation. Further studies on these oils are needed to employ them in practical applications.

References

[1]. M.J. Balunas and A.D. Kinghorn, Drug discovery from medicinal plants. Life Sci, 78(5), 2005, 431-41.
[2]. K.H.C. Baser and G. Buchbauer, Handbook of essential oils : science, technology, and applications (Boca Raton: CRC Press/Taylor & Francis, 2010).
[3]. M.L. Schmitz and S. Bacher, Novel molecular targets in the search for anti-inflammatory agents. Phyto Rev, 4(1), 2005, 19-25.
[4]. J.H. Doughari, I.S. Human, S. Bennade, and P.A. Ndakidemi, Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. J Med Pl Res, 3(11), 2009, 839-848.
[5]. D.J. Mabberley, Mabberley's plant-book : a portable dictionary of plants, their classification and uses (Cambridge, UK ; New York: Cambridge University Press, 2008).
[6]. B.E. Van Wyk and M. Wink, Medicinal plants of the world : an illustrated scientific guide to important medicinal plants and their uses (Portland, Or.: Timber Press, 2004).
[7]. L. Boulos, Flora of Egypt (Cairo, Egypt: Al Hadara Pub., 1999).

[8]. N.H. El-sayed, T.M. El-kubesy, and T.J. Mabry, Flavone glucosides and other constituents of the Egyptian variety of *Daucus carota* var. boissieri grown in Egypt. Bioch Syst Eco, 22(7), 1994, 762.
[9]. A.B. Singab, Y. Masuda, Y. Okada, G. Mahran, T. Khalifa, and T. Okuyama, Phenolic Constituents from Egyptian Carrot Fruits of *Daucus carota* var. *boissieri*. Nat Med, 49(1), 1995, 96.
[10]. V. Kilibarda, N. Nanusevic, N. Dogovic, R. Ivanic, and K. Savin, Content of the essential oil of the carrot and its antibacterial activity. Pharmazie, 51(10), 1996, 777-778.
[11]. E.D. Lund, Polyacetylenic carbonyl compounds in carrots. Phytoch., 31(10), 1992, 3621-3623.
[12]. T. Degen, H.-R. Buser, and E. Städler, Patterns of oviposition stimulants for carrot fly in leaves of various host plants. J ch ecol, 25(1), 1999, 67-87.
[13]. D. Douds, G. Nagahashi, and G. Abney, The differential effects of cell wall-associated phenolics, cell walls, and cytosolic phenolics of host and non-host roots on the growth of two species of AM fungi. New Phytol, 133(2), 1996, 289-294.
[14]. K. Dwivedi, S. Dwivedi, V.G. Pandey, and N. Dubey, Effect of essential oils of some higher plants on *Aspergillus flavus* Link. infesting stored seeds of guar (*Cyamopsis tetragonoloba* L.(Taub.)). Flav fr J, 6(4), 1991, 295-297.
[15]. A. Bishayee, A. Sarkar, and M. Chatterjee, Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. J ethnoph, 47(2), 1995, 69-74.
[16]. O.A. Eldahshan, N.A. Ayoub, A.-E.N.B. Singab, and M.M. El-Azizi, Carotamine, a Unique Aromatic Amide from *Daucus carota* L. var *biossieri* (Apiaceae). Mol, 7(6), 2002, 501-506.
[17]. M.L. Ashour, M. El-Readi, M. Youns, S. Mulyaningsih, F. Sporer, T. Efferth, and M. Wink, Chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum* (Apiaceae). J Pharm Pharmacol, 61(8), 2009, 1079-1087.
[18]. R.P. Adams, Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy (Illinois: Allured Pub Corp, 2004).
[19]. W. Brandwilliams, M.E. Cuvelier, and C. Berset, Use of a free-radical method to evaluate antioxidant activity. LWT-Food Sci Technol, 28(1), 1995, 25-30.
[20]. B. Halliwell, J.M.C. Gutteridge, and O.I. Aruoma, The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem, 165(1), 1987, 215-219.
[21]. P.J. Houghton, R. Zarka, B. de las Heras, and J.R.S. Hoult, Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med, 61(01), 1995, 33-36.
[22]. G.D. Basu, L.B. Pathangey, T.L. Tinder, S.J. Gendler, and P. Mukherjee, Mechanisms underlying the growth inhibitory effects of the cyclo-oxygenase-2 inhibitor celecoxib in human breast cancer cells. Breast Cancer Res, 7(4), 2005, 422-435.
[23]. S. Baylac and P. Racine, Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromatherapy, 13(2-3), 2003, 138-142.
[24]. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, and M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst, 82(13), 1990, 1107-12.
[25]. A.C. Tavares, M.J. Goncalves, C. Cavaleiro, M.T. Cruz, M.C. Lopes, J. Canhoto, and L.R. Salgueiro, Essential oil of *Daucus carota* subsp. *halophilus*: composition, antifungal activity and cytotoxicity. J Ethno, 119(1), 2008, 129-34.
[26]. T. Ma, J. Luo, C. Tian, X. Sun, M. Quan, C. Zheng, L. Kang, and J. Zhan, Influence of technical processing units on chemical composition and antimicrobial activity of carrot (*Daucus carota* L.) juice essential oil. Food Chem, 170, 2015, 394-400.
[27]. N. Rokbeni, Y. M'Rabet, S. Dziri, H. Chaabane, M. Jemli, X. Fernandez, and A. Boulila, Variation of the chemical composition and antimicrobial activity of the essential oils of natural populations of Tunisian *Daucus carota* L. (Apiaceae). Chem Biodivers, 10(12), 2013, 2278-2290.
[28]. A. Maxia, B. Marongiu, A. Piras, S. Porcedda, E. Tuveri, M.J. Goncalves, C. Cavaleiro, and L. Salgueiro, Chemical characterization and biological activity of essential oils from *Daucus carota* L. subsp. carota growing wild on the Mediterranean coast and on the Atlantic coast. Fitoterapia, 80(1), 2009, 57-61.
[29]. G. Flamini, E. Cosimi, P.L. Cioni, I. Molfetta, and A. Braca, Essential-oil composition of *Daucus carota* ssp. *major* (Pastinocello Carrot) and nine different commercial varieties of *Daucus carota* ssp. *sativus* fruits. Chem Biodivers, 11(7), 2014, 1022-1033.
[30]. J.L. Guil, J.J. Gimenez, and M.E. Torija, Mineral nutrient composition of edible wild plants. J F Compos Anal, 11, 1998, 322-326.
[31]. W.N. Shebaby, M. El-Sibai, K.B. Smith, M.C. Karam, M. Mroueh, and C.F. Daher, The antioxidant and anticancer effects of wild carrot oil extract. Phytother Res, 27(5), 2013, 737-44.
[32]. J.K. Radoslaw, Hydroindene-derived chiral synthons from carotol and their cytotoxicity. Phytoch, 23(14), 2012, 1038-1045.
[33]. A.S. Potter, S. Foroudi, A. Stamatikos, B.S. Patil, and F. Deyhim, Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. Nutr J, 10, 2011, 96.