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Chemical composition and *in vitro* antioxidant activities of essential oil from *Nigella sativa* L. seeds cultivated in Syria

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Abstract: *Nigella sativa* is an aromatic herb used as medicinal plant and widely cultivated in Mediterranean countries. In this study, the essential oils obtained by hydrodistillation from *N. sativa* seeds cultivated in two different Syrian areas, Aleppo and Daraa, were analyzed by gas chromatography-mass spectrometry (GC-MS) and their total Phenol and antioxidant activities were assessed. The main constituents found in Aleppo and Daraa *N. sativa* oil were p-cymene (34.1%- 39.9%) followed by thymoquinone (17.2%- 25.9%) with minor amounts of β-pinene (1.8%- 4.5%) and α-Pinene (1.3%- 4.4%). The total phenol contents (gallic acid equivalents, mg GAE per g) in *N.sativa* essential oil from Aleppo and Daraa was calculated as 21.19± 0.31 and 30.27± 1.70, respectively, by Folin-Ciocalteau method. The antioxidant activity of essential oil was evaluated by 2.2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods. IC_{50} values related to scavenging DPPH radicals of *N. sativa* essential oil showed a significant difference ($P < 0.05$) between Aleppo and Daraa samples 0.607±0.001 mg/ml and 0.240±0.002 mg/ml, respectively. The total phenolic content of *N. sativa* essential oil revealed that essential oil has few amounts of total phenols. Syrian *N. sativa* essential oil has a prominent antioxidant activity. For authors' knowledge, this is the first report comparing *N. sativa* essential oil cultivated in two cities in Syria.
Keywords: *Nigella sativa*; Essential oil; GC–MS; Antioxidant activity; Total phenol.

1. Introduction:

Antioxidants are added to foods to preserve lipid components from quality deterioration. Synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) are commonly used. Owing to their suspected action as promoters of carcinogenesis, there is growing interest in natural antioxidants[1]. Although it is the first preservatives designed for widespread industrial use, there are some physical properties of BHA and BHT, such as their high volatility and instability at elevated temperatures, strict legislation on the use of synthetic food additives, and consumer preferences, have shifted the attention of manufacturers from synthetic to natural antioxidant[2].

Herbs, spices are one of the most important targets in the search for natural antioxidants from safety point of view. Man has used them not only for flavoring foods but also for antiseptic and medical purposes since the prehistoric era [3]. Many herbal spices are known as excellent sources of natural antioxidants, and the consumption of fresh herbs in diet may therefore contribute to the daily antioxidant intake. Phenolic compounds are the primary antioxidants present in spices and there is a linear relationship between the total phenolic content and the antioxidant properties of spices. Essential oils, oleoresins and even aqueous extracts of spices possess antioxidative properties[1].

Among various medicinal plants, *Nigella sativa* L. (Ranunculaceae family; commonly known as Black Cumin) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, and Saudi Arabia[4].

N. sativa seeds and their oil have been widely used for centuries in the treatment of various ailments throughout the world. Also it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda[5]. Both the seeds and oil are used as a nutritional supplement [6]. Nigella seed oil has protective and curative actions[7]. It is considered to be one of the newer sources of edible oils that have an important role in human nutrition and health[8]. The seed oil has been reported to possess antitumor activity[9], antioxidant activity [10,11], anti-inflammatory activity [12], antibacterial activity[13] and a stimulatory effect on the immune system [7]. seeds oil is also used to treat respiratory condition like bronchitis, asthma and emphysema[14]. In addition, it is used to support stomach and intestinal health as well as kidney and liver function[15]. Nigella is thought to have antihistamine-like properties that make it useful in treating congestion[16].

The aims of the present study were to assess the chemical composition by GC-MS, obtain information about the total polyphenol content and antioxidant activities of *N. sativa* essential oil from different samples cultivated in Syria.

2. Materials and Methods:

2.1. Chemicals:

Folin–Ciocalteu, gallic acid, 2.2 diphenyl-1-picrylhydrazyl (DPPH), Thymoquinon, α-Pinene, and β-Pinene were purchased from Sigma- Aldrich. Vitamin C and ethanol were purchased from Merck, n- hexane was purchased from SCP, n-alkanes C8–C20 was purchased from Fluka. All chemicals and solvents used were of analytical grade.

2.2. Plant material:

Two varieties of *N. sativa* seeds were used in this study; they were purchased from the local market of two cities Aleppo and Daraa, Syria.

2.3. Extraction of essential oil:

N. sativa seeds were milled in an electric grinder. 200 g were submitted to hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia and extracted with 1 L water for 2.5 h. The essential oil was collected, dried over anhydrous sodium sulphate and stored at +4 °C until used.

2.4. Chemical Characterization:

2.4.1. GC-MS.

Analysis of the essential oils was run on a Agilent 7890A GC-Ms system coupled to a quadruple mass spectrometer (model 5975C) with a capillary column of HP-5MS 5 % Phenyl Methyl Silox; 30 m × 250 µm × 0.25 µm. GC-MS interface, ion source, selective mass detector and injector temperatures were maintained at 280 °C, 230 °C, 150 °C, and 260 °C, respectively. Carrier gas used was helium with a flow rate of 1 ml/min. The oven temperature was programmed at 60 °C then increased from 60 to 200 °C at the rate of 4 °C/min and held at

the rate of 8 °C/min and held at 260 °C for 7.5 min. Diluted oil in n-hexane (0.5/100, v/v) of 1.0 µl were injected in the split ratio 1:10.

2.4.2. Identification of components.

The components were identified on the basis of comparison of their retention indices and mass spectra with published data [17,18], and computer matching was done with the NIST Mass spectral version 2.0 f (2008) and National Institute of Standards Technology libraries provided with the computer controlling GC-MS systems. The retention indices were calculated using a homologous series of n-alkanes C8–C20, which are reported in Tables 1.

2.5. Antioxidant Activity:

2.5.1. Determination of total polyphenol content:

Total phenolics of *N. sativa* essential oil were determined for each sample using the Folin–Ciocalteu reagent, according to the method described by Shaghaghi *et al.*[19]. An aliquot of 1 ml of ethanol diluted (1:1000) essential oil was added to 4.8 ml of double distilled water and 4 ml of 2 % sodium carbonate solution, then 0.2 ml of the Folin–Ciocalteu reagent was added. The mixture was mixed vigorously, and held for 60 min at room temperature. The absorbance of the solution was then measured at 760 nm using a spectrophotometer (Optizen 2120UV PLUS) against a blank. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of essential oil through the calibration curve of gallic acid. The sample was analyzed in three replications.

2.5.2. Scavenging effect on 2.2 diphenyl-1-picrylhydrazyl (DPPH):

The hydrogen atoms or electrons donation ability of the corresponding pure compounds were measured from the bleaching of purple colored methanol solution of DPPH. 1 ml of various concentrations (0.1–0.7 mg/ml) from each sample of the essential oil in ethanol and water was added to a 3 ml of 45 µg/ml DPPH radical solution in ethanol. The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Optizen 2120UV PLUS). The scavenging activity was estimated according to the percentage of inhibition of free radical DPPH and calculated as follow:

$$\text{Inhibition of percentage} = 100 \times \frac{\text{A Control} - \text{A Sample}}{\text{A}}\text{Control}$$

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. Vitamin C was used as a control.

3. Statistical Analysis

For each sample of the essential oil, three samples were prepared for every antioxidant attribute. The data was presented as mean ± standard deviation of three determinations. Statistical analyses were performed using SPSS (Version 20.0). A probability value of $P<0.05$ was considered to be significant.

4. Results and discussion:

4.1. Extraction:

Essential oil isolation by hydrodistillation gave 0.12 % of the total amount of Aleppo sample and 0.15 % of Daraa sample. Burits and Bucar [11] reported 0.18 % by using the same method and Clevenger-type apparatus.

4.2. Essential oil composition:

Two samples of *N. sativa* essential oil were investigated by GC-MS. Table 1 shows the identification of eight components in *N. sativa* essential oil obtained from Aleppo sample, representing 90.6 % of the total amount. The major component in essential oil was *p*-cymene (39.9 %) followed by *α*-thujene (18.2 %), thymoquinone (17.2 %), β-pinene (4.5 %), and α-Pinene (4.4 %). Whereas nine components were identified in

N. sativa essential oil obtained from Daraa sample, representing 85.9 % of the total amount. The major component in essential oil was *p*-cymene (34.1 %) followed by thymoquinone (25.9 %), 1-Dodecanol (12.7 %), and α-thujene (5.3 %). Environmental factors like climate, location and genetic makeup might be possible reasons explaining these variations. Various studies across the world gave some evidences about the composition of Nigella seeds essential oils. Burits and Bucar [11] characterized many components such as thymoquinone (27.8 to 57.0 %), *p*-cymene (7.1 to 15.5 %), 4-terpineol (2.0– 6.6 %), and (1.0–8.0 %) of longifolen. Singh, Marimuthu *et al* [20] also reported that the major component in *N. sativa* essential oil was *p*-cymene (36.2 %) followed by thymoquinone (11.27 %), α-thujene (10.03 %), longifolene (6.32 %), β-pinene (3.78 %), α-pinene (3.33 %) and carvacrol (2.12 %) Another study indicated *p*-cymene as a major component (49.48 %), however thymoquinone represented only (0.79 %) following α-Thujene (18.93 %), α-pinene (5.44 %), and β-pinene (4.31 %) [21]. Studies conducted at different regions show that *N. sativa* essential oil could be influenced by environmental factors.

Table 1: Chemical composition of *Nigella sativa* essential oil of two samples analyzed by GC-MS

	Compounds	%MS		RT	RI	Identification
		sample from Aleppo	sample from Daraa			
1	α-Thujene	18.2	5.3	4.848	925	MS, RI
2	α-Pinene	4.4	1.3	4.997	932	MS, RI, std
3	Sabinene	2.2	-	5.869	973	MS, RI
4	β-Pinene	4.5	1.8	5.965	978	MS, RI, std
5	*p*-Cymene	39.9	34.1	7.198	1027	MS, RI
6	D-Limonene	2.6	1.8	7.275	1030	MS, RI
7	γ-Terpinen	1.6	-	8.088	1059	MS, RI
8	Terpinen-4-ol	-	1.7	11.734	1179	MS, RI
9	Thymoquinon	17.2	25.9	14.136	1254	MS, RI, std
10	Longifolen	-	1.3	19.034	1408	MS, RI
11	1-Dodecanol	-	12.7	21.114	1477	MS, RI
	Total	90.6	85.9			

4.3. Antioxidant activity:

4.3.1. Total phenolic content:

The amount of total phenols was determined by Folin-Ciocalteu reagent. Gallic acid was used as standard compound. The absorbance of different gallic acid dilutions with Folin-Ciocalteu reagent and sodium carbonate was obtained. Standard curve equation was defined: $y = 0.004*C$, $R^2 = 0.997$ (Figure 1). The total phenol contents (gallic acid equivalents, mg GAE per g of essential oil) in *N. sativa* essential oil were calculated, values were (21.19± 0.31) and (30.27± 1.7) for Aleppo and Daraa samples, respectively (Table 2). It was revealed that *N. sativa* essential oil has less amount of total phenols as reported in some studies [22,23]. It was also observed that essential oil obtained from Aleppo sample had a lower phenolic content when compared to Darra one. These differences in total phenolic content might be due to many reasons, such as environmental conditions, genetic background, or agricultural applications [23].

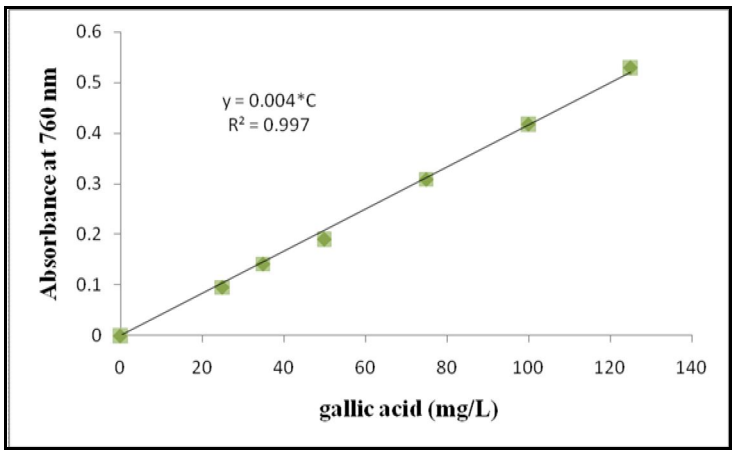

Figure 1: Calibration curve of gallic acid.

4.3.2. Scavenging Effect on DPPH Radical.

DPPH is usually used as a reagent to evaluate free radical. It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The disappearance of the DPPH radical absorption at 517 nm by the action of antioxidants was considered to be the antioxidant activity. The scavenging effects of *N. sativa* essential oil on DPPH radical linearly increased as concentration increased from 0.1 to 0.7 mg/ml (Figure 2). The IC_{50} values of scavenging DPPH radicals for *N. sativa* essential oil showed a significant difference ($P<0.05$) between Aleppo and Daraa samples for which means were 0.607±0.001 mg/ml and 0.240±0.002 mg/ml, respectively (Table 2). This result showed that essential oil from Daraa sample had more scavenging activity than essential oil from Aleppo one, this might be due to containing higher concentration of thymoquinone, which is already known as natural antioxidant [24]. However, the scavenging activity of vitamin C was more effective at lower concentration (IC_{50}= 29.4 µg /ml). Though, the antioxidant potential of *N. sativa* essential oil was found to be lower ($P<0.05$) than those of vitamin C. This study revealed that the essential oil, obtained from both Aleppo and Daraa samples, has prominent antioxidant activity. Phenolic compounds also exist in these two samples, probably due to the high antiradical properties of *N. sativa* essential oil.

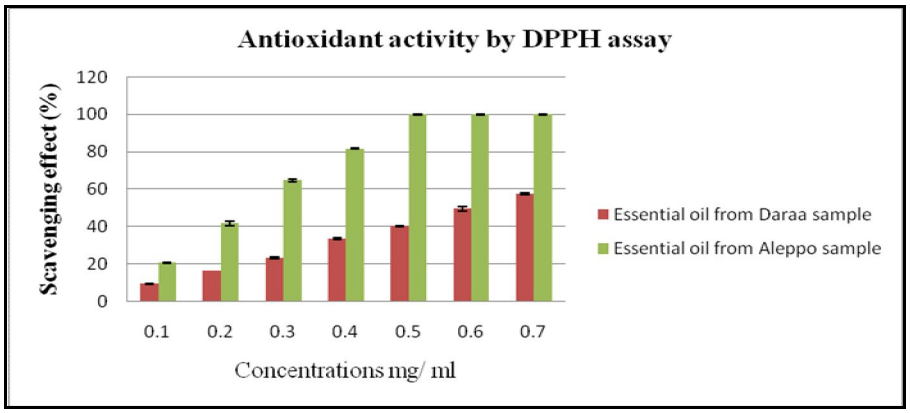

Figure 2: Antioxidant activities of *N. sativa* Essential oil tested by DPPH assay. Error bars represent standard deviations (n = 3).

Table 2: Antioxidant activity and total phenolic content in *N. sativa* essential oil.

Sample	DPPH IC_{50} (mg/ml)	Total Phenolic Content (mg GAE/g from essential oil)
Aleppo	0.607±0.001	21.19± 0.31
Daraa	0.240±0.002	30.27± 1.7
Vitamin C	0.0294	-

The antioxidant activity increased proportionally with the polyphenol content, also a positive correlation between IC_{50} values and total phenolic compounds was found [25]. Razali *et al*. [26] mentioned that most phenolics showed high levels of antioxidant activity.

Antioxidant activities of *N. sativa* essential oil could be related to the presence of some compounds such as terpenes and phenolic acids, which act in various ways like inhibition of peroxidation and scavenging the radicals. The main constituents of *N. sativa* essential oil were *p*-cymene (34.1 %- 39.9 %) and thymoquinone (17.2 %- 25.9 %) with minor amounts of β-pinene (1.8 %- 4.5 %) and α-Pinene (1.3 %- 4.4 %) which were responsible for the antioxidant activity of *N. sativa* essential oil [11,23,24,27,28].

Conclusions:

This study characterised *N. sativa* essential oil obtained from Aleppo and Daraa, samples by (GC-MS) analysis. Result revealed the major components; *p*-cymene followed by thymoquinone with minor amounts of β-pinene and α-Pinene. The total phenolic content of *N. sativa* essential oil from Aleppo and Daraa samples revealed that essential oil has few amounts of total phenols. Antioxidant activity of essential oil showed a significant difference ($P < 0.05$) between Aleppo and Daraa samples. Syrian *N. sativa* essential oil has a prominent antioxidant activity. Phenolic compounds found in these two samples could be attributable to the high antiradical properties of *N. sativa* essential oil.

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References:

1. Pokorný, J.; Yanishlieva, N.; Gordon, M.: Antioxidants in food: practical applications; Elsevier, 2001.
2. Zengin, G.; Aktumsek, A.; Guler, G. O.; Cakmak, Y. S.; Yildiztugay, E. Antioxidant properties of methanolic extract and fatty acid composition of Centaurea urvillei DC. subsp. hayekiana Wagenitz. Rec Nat Prod 2011, 5, 123-132.
3. Gardner, P. T.; McPhail, D. B.; Crozier, A.; Duthie, G. G. Electron spin resonance (ESR) spectroscopic assessment of the contribution of quercetin and other flavonols to the antioxidant capacity of red wines. Journal of the Science of Food and Agriculture 1999, 79, 1011-1014.
4. Kharelep, C.: Encyclopedia of Indian Medicinal Plants (Regional Western Therapy Ayurvedic and Other Traditional Usage, Botany). Springer-verlag, Berlin, Heidelberg, New York, 2004.
5. Goreja, W.: Black seed: nature's miracle remedy; Karger Publishers, 2003.
6. Ramadan, M. F. Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): an overview. International journal of food science & technology 2007, 42, 1208-1218.
7. Salem, M. L. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol 2005, 5, 1749-1770.
8. Cheikh-Rouhou, S.; Hentati, B.; Besbes, S.; Blecker, C.; Deroanne, C.; Attia, H. Chemical composition and lipid fraction characteristics of Aleppo pine (Pinus halepensis Mill.) seeds cultivated in Tunisia. Food science and technology international 2006, 12, 407-415.
9. Worthen, D. R.; Ghosheh, O. A.; Crooks, P. A. The in vitro anti-tumor activity of some crude and purified components of blackseed, Nigella sativa L. Anticancer Res 1998, 18, 1527-1532.
10. Salem, M. L.; Hossain, M. S. Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection. Int J Immunopharmacol 2000, 22, 729-740.
11. Burits, M.; Bucar, F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000, 14, 323-328.
12. Houghton, P. J.; Zarka, R.; de las Heras, B.; Hoult, J. R. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 1995, 61, 33-36.
13. Morsi, N. M. Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiol Pol 2000, 49, 63-74.

14. ATTA, M. B. Some characteristics of nigella (Nigella sativa L.) seed cultivated in Egypt and its lipid profile. Food chemistry 2003, 83, 63-68.
15. Salih, B.; Sipahi, T.; Donmez, E. O. Ancient nigella seeds from Boyali Hoyuk in north-central Turkey. J Ethnopharmacol 2009, 124, 416-420.
16. El Shenawy, N. S.; Soliman, M. F.; Reyad, S. I. The effect of antioxidant properties of aqueous garlic extract and Nigella sativa as anti-schistosomiasis agents in mice. Rev Inst Med Trop Sao Paulo 2008, 50, 29-36.
17. Adams, R. P.: Identification of essential oil components by gas chromatography/mass spectrometry; Allured publishing corporation, 2007.
18. Akloul, R.; Benkaci-Ali, F.; Zerrouki, M.; Eppe, G. Composition and biological activities of the essential oil of Nigella sativa seeds isolated by accelerated microwave steam distillation with cryogenic grinding. American Journal of Essential Oils and Natural Products 2014, 1, 23-33.
19. Shaghaghi, M.; Manzoori, J. L.; Jouyban, A. Determination of total phenols in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin–Ciocalteu spectrophotometric method. Food chemistry 2008, 108, 695-701.
20. Singh, G.; Marimuthu, P.; de Heluani, C. S.; Catalan, C. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of Nigella sativa seeds. Journal of the Science of Food and Agriculture 2005, 85, 2297-2306.
21. Harzallah, H. J.; Noumi, E.; Bekir, K.; Bakhrouf, A.; Mahjoub, T. Chemical composition, antibacterial and antifungal properties of Tunisian Nigella sativa fixed oil. African Journal of Microbiology Research 2012, 6, 4675-4679.
22. Goga, A.; Hasić, S.; Bećirović, Š.; Ćavar, S. Phenolic Compounds and Antioxidant Activity of Extracts of Nigella sativa L. 2012.
23. Singh, S.; Das, S. S.; Singh, G.; Schuff, C.; de Lampasona, M. P.; Catalan, C. A. Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (Nigella sativa L.). Biomed Res Int 2014, 2014, 918209.
24. Milos, M.; Makota, D. Investigation of antioxidant synergisms and antagonisms among thymol, carvacrol, thymoquinone and p-cymene in a model system using the Briggs–Rauscher oscillating reaction. Food chemistry 2012, 131, 296-299.
25. Malencic, D.; Maksimovic, Z.; Popovic, M.; Miladinovic, J. Polyphenol contents and antioxidant activity of soybean seed extracts. Bioresource technology 2008, 99, 6688-6691.
26. Razali, N.; Razab, R.; Junit, S. M.; Aziz, A. A. Radical scavenging and reducing properties of extracts of cashew shoots (Anacardium occidentale). Food chemistry 2008, 111, 38-44.
27. Hajhashemi, V.; Ghannadi, A.; Jafarabadi, H. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother Res 2004, 18, 195-199.
28. Hosseinzadeh, H.; Parvardeh, S.; Asl, M. N.; Sadeghnia, H. R.; Ziaee, T. Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 2007, 14, 621-627.

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