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Chemical Composition of Volatile Oils from Algerian *Nigella sativa* L. seeds

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Abstract

The chemical composition of the volatiles obtained from the fixed oils of *Nigella sativa* L. seeds growing in Algeria was investigated by GC and GC/MS analysis. Two solvents were studied for extracting the oils, hexane and isopropanol.

The composition of *N. sativa* seeds varieties having four location origins in Algeria, were determined. Alcohols and ketones formed the main proportion using the two solvents, respectively (hexane: 27.4–36.1%, isopropanol: 40.2–59.0%) and (hexane: 39.9–44.3%, isopropanol: 17.5–50.7%), among which thymoquinone and thymohydroquinone were the predominant antioxidant compounds.

The monoterpene hydrocarbons constitute relatively the lower fraction compared to the precedent chemical families, particularly by using the isopropanol solvent.

Key Word Index

Nigella sativa, Ranunculaceae, p-Cymene, Thymoquinone, Thymohydroquinone, GC, GC/MS.

Introduction

Nigella L. is a genus of about 14 species of annual plants in the family Ranunculaceae, native to southern Europe, North Africa and southwest Asia, among which appears *Nigella sativa* L., the species known as black cumin which is the most cultivated and marketed worldwide (1).

Nigella sativa grows to a maximum height of about 60 cm, has finely divided foliage (1). In the natural forms, flowers are bluish, with a variable number of sepals, and characterized by the presence of nectaries. The gynoecium is composed of a variable number of multi-ovule carpels, developing into a follicle after pollinization, with single fruits partially connected to form a capsule-like structure (2).

That seed grows in the temperate areas as well as the arid region of the Algerian Sahara with low rainfall and high average temperatures. However, it has been proved that *N.sativa* cultivated in an irrigated garden in an arid location (Algeria) (3) produced a seed possessing a high yield of essential oil and the biological active compounds such as thymoquinone and p-cymene compared to that grown in temperate areas.

Considering the success of the culture of spices, and more particularly *N. sativa* seed in the arid areas (Ouargla, Timimoun and Adrar), it is useful to investigate the chemical composition of this seed and to make the comparison with those of the moderate climate area (Médéa).

N. sativa seed oil is considered to be among the important sources of edible oils, thanks to its important role in human nutrition and health. Notable pharmacological properties (4, 5) have been reported to possess antihistaminic (6), anti-inflammatory (7), anti-tumor (8), immunopotentiation (9), antidiabetic (10), antihypertensive (11), antimicrobial (12), antibacterial (13), antiviral (14) and anti-malarial (15) activities.

Many of these activities have been attributed to thymoquinone, thymohydroquinone, thymol, carvacrol and p-cymene

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Table I. Origin of Algerian *Nigella sativa* seeds

	Ouargla (O)	Timimoun (T)	Adrar (A)	Médéa (M)
Latitude	31.57°	29.25 °	27.9 °	2.75°
Longitude	5.19°	0.25 °	- 0.28 °	36.2°
Elevation (m)	150	323	276	880
Average annual Pluviometry (mm/year))	25-74	<24	<24	475-725
Average annual Temperature (°C)	20.5-30.0	30.5-35.0	30.5-35.0	10.5-15.0
Average salinity (% of NaCl /1000 g of water)	Negligible	2.0	4.5	Negligible
Environmental constraints	Dry areas	Dry areas	Dry areas	Erratic rainfall and cold stress risk
Water supply	Irrigated garden (foggaras)	Irrigated garden (drillings)	Irrigated garden (drillings)	Mainly by precipitation

(16-19). Preliminary experiments (20) showed that both the fixed oil and thymoquinone, the main and active compound of the essential oil, inhibit non-enzymatic lipid peroxidation in liposomes. *N. sativa*, therefore, appears to be a potential multi-purpose crop of possible interest.

This investigation was undertaken to obtain information about the chemical composition of *N. sativa* seeds produced from plants cultivated in arid and temperate areas of Algeria, and to determine the biologically active compounds of their fixed oil extracted by polar and non- polar solvent extraction. In addition, the aim of the present study was also to evaluate the solvent effect on the thymoquinone and thymohydroquinone amount isolated from the extracts, which were examined by GC and GC/MS analysis.

Experimental

Seed material: Four varieties of mature black cumin (*Nigella sativa*) seeds were collected from different towns of Algeria, Ouargla (O), Timimoun (T), Adrar (A) and Médéa (M) at the maturation stage, during the month of June, which conditions are reported in Table I.

The seeds were stored at 4°C until extraction. They were authenticated by the Botanic Department of the National Agronomic Institute, Algiers, Algeria. The initial moisture of these seeds was 9%.

Seed material (150 g) was milled in an electric heavy-duty grinder for 30 s to 180–250 µm average size (Ika Werke standard model Germany) at a speed of 20,000 rpm, and subjected immediately to oil extraction.

Extraction of volatile oil: Crushed seeds (80 g) were continuously extracted with organic solvent (hexane or isopropanol) using a Soxhlet apparatus for 3 h. The extraction procedure was repeated twice, and the solvent was removed under vacuum. The solvent residue in the oil was removed under a stream of N_2.

The volatile oil was isolated from fixed oil by steam distillation. Extraction of aqueous distillate with n-hexane and removal of the solvent yielded the volatile oil (21), which is transferred into glass scaled amber dark bottles, capped and stored until analyzed.

GC/MS analysis: GC analysis was performed on a HP 6890 standard model using the following conditions: fused-silica-capillary column with a non polar stationary phase HP5-MS (30 m, 0.25 mm, 0.25 µm film, 5% biphenyl, 95 % dimethylpolysiloxane), detector used FID, carrier gas He (0.03 MPa, flow rate 0.5 mL/min), injector and detector temperature are respectively regulated at 280°C and 300°C, respectively. The splitless injection mode was used; injection volume for all samples, 0.1 µL; the oven temperature was programmed at 60°C for 8 min, then progressed from 60°C to 250°C at 2°C/min and was held at 250°C for 30 min.

The oil samples were injected in a HP 6890 chromatograph connected to a HP 5973 mass-selective detector.

Oven temperature progression, column operating conditions, volume and injection mode, carrier gas conditions and injector temperature were similar to GC ones. The temperature interface of the mass spectrometer was fixed to 280°C; the solvent delay time was 4 min. The source temperature was 230°C and the quadrupole temperature 150°C. The instrument was operated in electron-impact (EI) mode, with an electron energy of 70 eV, and scanned in the 30–550 m/z range.

The homologous n-alkanes series injected in GC and GC/MS in the same conditions as the oils were used to calculate the retention indices. Peak area percentages were calculated by using the normalization method where the response factor for each component was assumed to be equal to one. The component identification used the comparison of the mass spectral fragmentation patterns with those stored in the database (NIST 2002, Wiley 7) and with the previously published spectra. The comparison of the retention indices (RI) of the oil constituents compared with those of the published index data (21, 3, 22) confirmed the identifications.

Analyses were performed at least three times, and the mean values were reported.

Results and Discussion

Extraction: Table II lists the yields according to the solvent extraction used. The hexane (H) solvent provides yields different to those obtained by means of isopropanol (I). The higher values were attributed to seeds from the Adrar area A_H (1.3%) and A_I (0.3%), respectively from the hexane and isopropanol extract followed by O_H (0.6%) and O_I (0.2%).The I solvent gives globally the lower yield percentages. However, since the isopropanol is widely used in the food industry, very available and of low cost, these yields can be considered fairly satisfactory.

Chemical composition: Analysis of the oils by GC and GC/MS revealed 61 compounds (60 identified) in the *N. sativa* seed oils using hexane and isopropanol, from O, T, A and M

Table II. Yield and chemical composition of the oils isolated from hexane and isopropanol extracts of *Nigella sativa* from different regions of Algeria

Compound	RI	Relative percentage (%)	O_H	O_I	T_H	T_I	A_H	A_I	M_H	M_I
α-thujene	928	0.3	0.1	0.1	-	tr	-	1.4	0.1	
camphene	956	-	-	-	-	-	-	-	0.1	
sabinene	972	0.1	0.2	0.1	-	-	-	0.1	-	
β-pinene	976	0.2	-	-	-	tr	-	0.7	-	
α-terpinene	1115	0.1	0.1	-	-	-	-	0.1	-	
p-cymene	1025	19.9	15.4	4.0	1.7	19.2	6.2	18.0	11.1	
γ-terpinene	1058	0.7	0.5	1.0	-	tr	-	0.9	0.4	
m-cymenene	1082	0.1	-	0.1	-	0	-	0.1	-	
terpinolene	1092	0.7	-	0.9	-	0.7	-	0.7	0.8	
linalool	1099	-	-	-	-	-	-	0.1	-	
nonanal	1103	-	-	0.2	-	-	-	-	-	
p-mentha-1,3,8-triene	1113	-	-	-	-	-	-	0.1	-	
Not identified[1]	1115	4.5	5.7	4.4	2.2	5.2	1.8	5.2	4.0	
karahanaenone[ti 2]	1116	0.4	0.4	0.5	0.3	0.4	-	0.4	0.4	
terpinen-4-ol	1174	1.1	1.1	1.9	1.2	1.3	0.4	1.2	0.9	
p-cymen-8-ol	1184	0.1	0.4	0.2	0.3	-	-	0.3	0.1	
myrtenal	1192	-	-	1.0	0.1	-	-	-	-	
myrtenol	1196	-	-	-	-	-	0.6	-	-	
trans-dihydrocarvone	1198	-	0.8	-	0.8	1.8	-	1.2	0.7	
cuminaldehyde	1239	-	-	0.4	-	-	-	-	-	
carvone	1243	-	0.1	0.6	0.3	-	4.0	0.4	1.0	
thymoquinone	1252	43.1	17.9	43.1	25.5	42.1	46.7	37.6	15.2	
trans-sabinene hydrate acetate	1256	-	-	-	-	-	-	0.1	-	
(E)-2-decenal	1264	-	0.1	-	-	-	-	-	0.2	
neothujyl acetate	1269	0.1	0.1	0.2	-	-	-	-	0.2	
decanol	1273	-	-	0.4	-	-	-	-	-	
(E)-anethole	1285	-	-	0.9	-	-	-	0.4	0.5	
bornyl acetate	1288	0.1	0.2	-	0.3	-	-	-	-	
p-cymen-7-ol	1290	-	0.2	-	-	-	-	0.1	0.5	
thymol	1292	0.1	0.2	0.4	0.2	tr	tr	-	-	
2-undecanone	1294	-	-	-	0.2	-	-	0.1	-	
(E,Z)-2,4-decadienal	1295	-		1.2	-	-	-	0.1	-	
carvacrol		1301	8.0	0.7	5.6	9.5	6.9	3.5	3.9	3.9
(E,E)-2,4-decadienal	1314	-	0.1	2.6	-	-	-	-	0.4	
β-longipinene	1352	0.1	0.8	0.4	0.8	0.5	0.5	0.3	0.4	
eugenol	1360	-	-	0.1	-	-	-	-	-	
γ-nonalactone	1363	-	-	-	-	-	-	0.1	-	
α-copaene	1376	-	-	0.8	-	-	-	-	-	
β-longipinene	1400	0.1	-	-	0.1	-	-	0.1	-	
longifolene	1403	0.6	5.9	-	3.5	2.0	-	2.2	1.8	
isocaryophyllene	1404	-	0.1	-	-	-	-	-	-	
β-caryophyllene	1420	-	-	-	-	-	-	0.1	0.1	
α-himachalene	1448	-	0.1	-	-	-	-	-	-	
(Z)-methyl isoeugenol	1454	-	0.2	-	-	-	-	-	-	
cis-muurola-4-(14), 5-diene	1457	-	0.9	-	-	-	-	-	-	
6-methyl-g-(E)-ionone ti 3	1478	0.1	0.1	-	0.9	-	-	0.1	0.2	
2-tridecanone	1494	0.1	0.1	-	0.9	-	-	-	-	
β-bisabolene	1504	0.1	0.1	-	-	-	-	-	-	
γ-cadinene	1514	-	0.3	-	-	-	-	-	-	
δ-cadinene	1521	0.1	0.1	-	0.9	-	-	-	-	
citronellyl butyrate	1528	0.1	0.1	-	0.1	-	-	-	-	
calacorene	1538	-	0.1	-	-	-	-	-	-	
thymohydroquinone	1558	18.2	46.1	27.5	44.7	19.7	35.7	22.3	53.6	
isoeugenol acetate*	1566	-	-	-	0.2	-	-	-	-	
longiborneol acetate	1678	0.2	0.2	-	0.3	-	-	-	-	
(E,Z)-farnesol	1741	-	0.2	-	0.9	-	-	-	-	
tetradecanoic acid	1780	0.1	0.2	0.6	2.4	-	-	0.9	1.7	
(Z,E)-farnesyl acetate	1821	0.2	-	-	-	-	-	-	-	
isopropyl myristate	1825	-	0.1		0.3	-	-	-	0.2	
methyl hexadecanoate	1927	0.1	-	0.2	0.7	-	-	0.3	1.2	
pimaradiene	1940	0.3	-	0.6	0.7	-	0.5	0.3	0.2	
Monoterpene hydrocarbons	22.1	16.3	6.2	1.7	19.9	6.2	22.1	12.5		

Table II. Continued

Compound	RI	Relative percentage (%)	O_H	O_I	T_H	T_I	A_H	A_I	M_H	M_I
Sesquiterpene hydrocarbons	1.0	8.4	1.2	5.3	2.5	0.5	2.7	2.3		
Diterpene hydrocarbons	0.3	-	0.6	0.7	-	0.5	0.3	0.2		
Alcohols	27.4	48.9	36.1	56.8	27,9	40.2	27.9	59.0		
Ketones	43.7	19.4	44.2	28.9	44.3	50.7	39.9	17.5		
Ethers	-	0.2	0.9	-	-	-	0.4	0.5		
Aldehydes	-	0.2	5.4	-	-	-	0.1	0.6		
Esters	0.8	0.7	0.4	1.9	-	-	0.4	1.6		
Acids	0.1	0.2	0.6	2.4	-	-	0.9	1.7		
Volatile oil yield of seed weight (%)	0.6	0.2	0.2	0.2	1,3	0.3	0.3	0.2		

RI: calculated retention indices in this work, OH: Volatile oil from Ouargla extracted by hexane, OI: Volatile oil from Ouargla extracted by isopropanol, TH: Volatile oil from Timimoun extracted by hexane, TI: Volatile oil from Timimoun extracted by isopropanol, AH: Volatile oil from Adrar extracted by hexane, AI: Volatile oil from Adrar extracted by isopropanol, MH: Volatile oil from Médéa extracted by hexane, MI: Volatile oil from Médéa extracted by isopropanol, tr = trace, less than 0.1%, ti: Tentitatively identified, 1 m/z (% rel.int.): 168(M)+(2), 153 (88), 136 (37), 125 (100), 121 (42), 93 (80), 85 (84), 81 (74), 72 (82), 2 m/z (% rel.int.): 152(M)+(3), 137 (2), 119 (4), 109 (70), 95 (15), 81 (85), 67 (100), 53 (20), 41 (60), 3 m/z (% rel.int.): 206[M]+(5), 150 (59), 135 (100), 123 (20), 107 (58), 91(38), 79 (10), 65 (5), 55 (6), 43 (30); * Correct isomer not identified

Table III. Concentration of major active components identified in *Nigella sativa* oils

Major active components	Concentration mg/g *							
	O_H[a]	O_I	T_H	T_I	A_H	A_I	M_H	M_I
p-cymene	1.3	0.3	0.1	< 0.1	2.5	0.2	0.5	0.2
thymoquinone	2.8	0.4	0.8	0.4	5.4	1.3	1.0	0.3
carvacrol	0.5	< 0.1	0.1	0.2	0.9	0.1	0.1	0.1
thymohydroquinone	1.2	1.0	0.5	0.8	2.5	1.0	0.7	1.1
terpinen-4-ol	0.1	< 0.1	<0.1	0	0.2	0	< 0.1	< 0.1
Total	5.9	1.8	1.6	1.4	11.5	2.6	2.4	1.7

* mg of component / g of ground seeds
[a] for origins see Table II

(Table II). In the H oils, 30 components were identified in O, 28 in T, 14 in A and 32 in M. In the same way, in the I oils, 36 components were identified in O, 27 in T, 10 in A and 26 in M. The ketone fraction was found to be the predominant chemical class in the H oils (39.9–44.3%), in which the thymoquinone (TQ) represented the major component (37.6–43.1%) and has also been previously reported as being the major constituent (21,3). Whereas, the I oils could be distinguished from the H oils by their richness in alcohols in which the amount varied from 40.20% to 59.0% and only 27.9% to 36.1% in the H oils. In this fraction, thymohydroquinone (THQ) was present in large amounts (35.7–53.6% in I oils and only 18.2–27.5 in the H oils).

Furthermore, it is interesting to note that the previously characterized dithymoquinone or nigellone (23) was not detected in all oils seen by the authors, as confirmed by Burits and Bucar (21).

The antioxidant and biocidal compounds such as carvacrol and terpinen-4-ol (21) (Table II) were present in large amounts in T_I (9.5% and 1.2% respectively), O_H (8.0%, 1.1%), A_H (6.9%, 1.3%), M_I (3.9%, 0.9%) oils and a lower percentage of thymol was noted in some oils as O and T (0.1–0.4%).

As shown in the Table II, the monoterpene hydrocarbons constituted an important fraction in the H oils (12.5–22.1%) but a lower percentage in the I oils (1.7–16.3%); in this fraction p-cymene was the major hydrocarbon (4.0–19.9% in the H oils and 1.7–15.4% in the I oils). Pharmacologically, previous results showed that the natural antimicrobial compounds, carvacrol added to p-cymene could potentially be used against both spoilage yeast and *Escherichia coli* O157:H7 (18).

Notable amounts of sesquiterpene hydrocarbons were identified in the H and I oils as O_I (8.4%) and T_I (5.3%) dominated mainly by longifolene and β-longipinene (24). Whereas, a moderate amount of the diterpene hydrocarbon pimaradiene was identified in many of the oils not exceeding 0.7%.

As reported in literature (1, 18, 25), it is important to note the contribution to the beneficial chemical effects, which have some active constituents, such as thymoquinone, thymohydroquinone, p-cymene, carvacrol and terpinen-4-ol (Table III). They represent a global amount of 11.5 mg/g of ground seed in A_H, 5.9 mg/g in O_H and 2.6 mg/g in A_I. As a result, it is clear that the H solvent yielded oils with higher amounts of more valuable constituents as compared to the I oils.

According to the data values (Table II), it must be pointed out that the solvent effect on the composition is very important. The solvent selectivity is closely related to their polarity.

A comparison between the four regions O, T, A and M for the two solvents shows that the A region (Adrar) yields an oil

of better quality in terms of yield and quantity of its pharmacologically active products.

Nigella sativa seeds cultivated under the same climatological environment conditions, in Timimoun (T) and Adrar (A) results in yields and chemical composition which are different. This is probably due to the many factors such as the heredity, age of the plant, harvesting time, fertilization (26) and irrigation regiments in these areas. Indeed, the Adrar soil is more fertile compared to that in the Timimoun gardens. It seems that at least a lower yield in active compounds in T compared to A is not due to the salinity variability (Table I) as previously reported in literature (27).

As indicated in previous reports on *N. sativa* oil isolated by microwave and hydrodistillation (3, 28), it appears clearly that thymohydroquinone was generated from thymoquinone during the heating that took place during the longer oil isolation time. This corroborates the fact that thymohydroquinone could not be considered as a biosynthetic compound. Moreover, it is necessary to underline that this bi-alcohol compound possesses a high antibacterial activity (25).

In conclusion it can be observed that the arid climate and the water-stress of the Sahara regions does not prevent the growth and the culture of *N. sativa* seeds with best quality in terms of yield and quantity of the antioxidants and active components such as thymoquinone, thymohydroquinone and carvacrol.

The variations in yield and chemical composition could be due to the nature of the soil, the amount of sunlight and temperature variations, and the occurrence of chemotypes. Thus, the authors believe that intrinsic and extrinsic factors could have affected the content and composition of the *N. sativa* oil.

The sample from Adrar region appears to be potential multi-purpose crops of great interest, as has been previously reported (3).

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