

CHEMICAL COMPOSITION OF THE TUNISIAN *NIGELLA SATIVA*. NOTE I. PROFILE ON ESSENTIAL OIL

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Abstract

Nigella sativa L., *Ranunculaceae* Family, commonly named blackseed, is a spontaneous plant. *Nigellae sativae semen* was used for a long time in the Arabian and Romanian ethnopharmacological field for its tonic effect. The scientific basis for using this plant was confirmed by phytochemical studies. These studies represent the scientific confirmation of the empirical use of this vegetal product. The main compounds from the lipophylic fraction are responsible for the pharmacological activity of the *Nigellae sativae semen*. The qualitative and quantitative analysis of the essential oil were performed using a gas-chromatography-mass spectrometry (GC-MS) method. The analysis of the *Nigella sativa* essential oils extracts allowed the identification of 30 compounds in considerable percentages, among them: alpha- pinene (13.75 %), limonene (2.55 %), *p*-cymene (43.58 %), carvacrol (2.53 %) and tymoquinone (1.65 %). The results show that the essential oils composition depends in a great extent on the used extraction technique, and this offers the possibility to choose the methods which could favour the isolation of certain compounds.

Rezumat

Nigella sativa L. (*Ranunculaceae*) denumită popular chimen negru sau negrilică este o plantă medicinală spontană, utilizată în medicina tradițională arabă și română datorită efectului tonic. Fundamentarea științifică a utilizării plantei a fost demonstrată prin studii fitochimice, care constituie confirmarea științifică a utilizării sale empirice. Compușii fracției lipofile se fac responsabili de activitatea farmacologică a produsului vegetal

Nigellae sativae semen. Analizele calitativă și cantitativă ale uleiului volatil au fost realizate prin gaz-cromatografie cuplată cu spectrometrie de masă (GC-SM). Analiza uleiului volatil extras din *Nigella sativa* a permis identificarea a 30 de compuși în cantități semnificative, printre care: alfa- pinen (13,75 %), limonen (2,55 %), *p*-cimen (43,58 %), carvacrol (2,53 %) și timochinonă (1,65 %). Compoziția uleiului volatil depinde în mare măsură de metodologia de extracție folosită, ceea ce oferă posibilitatea de a alege metoda care favorizează izolarea anumitor compuși de interes.

Keywords: *Nigellae sativae semen*, *Ranunculaceae*, seeds, essential oil, gas-chromatography-mass spectrometry (GC-MS).

Introduction

Nigella sativa, commonly known as blackseed is a dicotyledon of the *Ranunculaceae* family, native to south Europe, north Africa and southwest Asia [1]. This annual herbaceous plant grows about 20 – 40 cm in height, has finely divided leaves, and delicate pale-blue or white flowers. Its seeds represent the main useful product and are angular, small sized, dark grey or black coloured and contain saturated and unsaturated fatty acids, aminoacids, vitamins, minerals, and traces of isoquinoline and pyrazolic alkaloids [2].

Nigella sativa has been traditionally used for centuries in Asia, the Middle East, and Africa, both as a herb and pressed into oil, as a spice as well as for a variety of effects, related to respiratory, stomach and intestinal diseases, kidney and liver function, circulatory and immune system support and for the general well-being. As mentioned in literature, the seed oil of *Nigella sativa* has been reported to have antioxidant, cytostatic, antibacterial and anti-inflammatory activity.

The aim of the present work was to study the *Nigella sativa* species from the chemical point of view. For this purpose, we focused on the *Nigella sativa*'s essential oil extraction respectively on the analysis of these extracts by gas-chromatography-mass spectrometry (GC-MS).

Materials and methods

Chemicals

The hexane used in the experiments was of HPLC grade and was purchased from Merck. The other chemicals used were of analytical grade.

Plant material

Mature seeds of *Nigella sativa* were collected in April and May 2005 from cultivated plants from the Nabeul and Menzel Temime regions (Northeast of Tunisia). Samples of these seeds were brought to the Pharmacognosy Department of the Faculty of Pharmacy, "Vasile Goldis" West University of Arad. They were soaked in water, washed, air-dried and stored in hermetic bags and deep frozen until used.

Extraction and analysis of essential oil

Air-dried and finely ground *Nigella sativa* seeds samples (100 g) were hydrodistilled for four hours. The distillate was extracted with *n*-hexane. The organic layer was separated, concentrated under reduced pressure and dried over anhydrous sodium sulfate. The essential oil was analyzed by gas-chromatography-mass spectrometry (GC-MS) [6,7] using a Hewlett-Packard 5890/ 5972A system, with capillary column Innowax (30 m x 0.25 mm x 0.25 μ m) [3,6,8,9]. The carrier gas was helium with a flow rate of 1mL/min. The column temperature was programmed from 50 to 280°C with 5°C/min [8]. The constituents were identified by matching their mass spectra in the Wiley 275.L library, HP Chemstation mass spectra library, containing the standard compounds spectra and by comparison of their retention indices with literature values [8,9].

Results and discussion

After the hydrodistillation of the extract from the seeds of *Nigella sativa* a yellowish volatile oil was obtained, characterized by a spicy odor.

During the extraction by the hydrodistillation procedure, it was noticed that for one hour extraction time, the number of extracted constituents was maximum. This period of time allowed the obtaining of 30 peaks corresponding to 30 compounds, thus a 30 minutes period allowed the extraction of only 24 compounds. For this reason an optimization of the extraction time seemed necessary. The chromatogram is presented in figure 1.

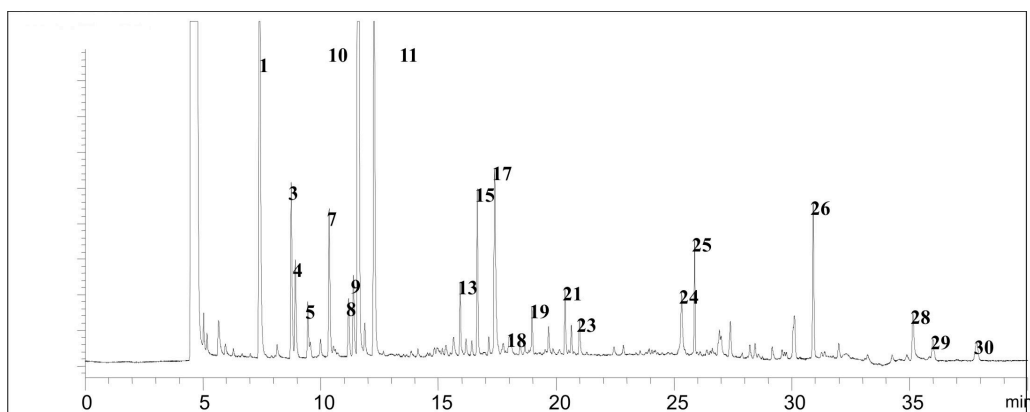


Figure 1

The chromatogram of the Tunisian *Nigella sativa* essential oil

The quantitative analysis of the *Nigella sativa* essential oil extracts allowed the identification in considerable percentages of: α -pinene (13.75 %), limonene (2.55 %), *p*-cymene (43.58 %), carvacrol (2.53 %) and thymoquinone (1.65 %). The main compounds of the *Nigella sativa* seeds essential oil extracts are presented in Table I.

Table I

The main compounds of the essential oil extracts of Tunisian *Nigella sativa* seeds, analysed by GC-MS

No.	Retention Time, [min]	Percentage [%]	Compounds	Chemical Family
1	7.401	13.75	α -pinene	TH
2	8.160	Tr	Camphene	TH
3	8.746	3.00	β -pinene	TH
4	8.924	1.66	Sabinene	TH
5	9.452	0.94	β -Myrcene	TH
6	9.992	Tr	α - terpinene	TH
7	10.358	2.55	Limonene	TH
8	11.179	0.96	Ethyl hexanoate	AG
9	11.387	1.40	γ - terpinène	TH
10	11.587	43.58	<i>p</i> - cymene	TH
11	12.260	9.08	Terpinolene	TH
12	15.644	Tr	Heptanal	ALDEHYDE
13	15.917	1.18	Ethyl heptanoate	AG
14	16.172	Tr	Ethyl octanoate	AG
15	16.646	2.41	Junipene	ST
16	17.132	Tr	Bornyl acetate	ESTER
17	17.381	4.25	Terpinene-4-ol	TA
18	18.009	0.95	α -longipinene	ST
19	18.969	0.91	Estragole	PME
20	19.675	0.62	α -amorphene	ST
21	20.368	1.06	Germacrene	ST
22	20.610	Tr	α -bisabolene	ST
23	20.983	0.79	L-carvenol	TA
24	25.315	2.12	Ethyl tetradecanoate	AG
25	25.870	1.67	Thymol	TP
26	30.904	2.53	Carvacrol	TP
27	31.992	Traces	Ethyl hexadecanoate	AG
28	35.140	1.65	Thymoquinone	TC
29	35.989	0.84	Ethyl octadecanoate	AG
30	37.847	0.64	Ethyl oleate	AG

* TH: Terpenic hydrocarbure, TA: Terpenic Alcohol; TP: Terpenic phenol ; TC: Terpenic cetone; ST: Sesquiterpene; AG: esterified fatty acid; PME: phenol-methyl ether

The results thus obtained were validated using the Kovats index calculation for the capillary gas-liquid chromatography, due to the fact that these indices are useful in the identification of substances, being an unique characteristic of a certain stationary phase. The calculation of the Kovats index is based on the retention times of the chromatograms of the essential oil and of the mixture of standard hydrocarbons (C10 to C32).

Among the 30 obtained compounds, 25 could be identified as basic monoterpenoidic constituents of the essential oil (92 % of the total constituents). These are mainly terpenoid hydrocarbons (e.g. α -pinene, camphene, β -pinene, sabinene, β -myrcene, α -terpinene, limonene, γ -terpinene, *p*-cymene, terpinolene. The *p*-cymene is the main component, being present in 43.58 % of the total constituents, followed by α -pinene 13.75% and terpinolene 9.08 %. The terpenoid alcohols represent 12 % of the total constituents. Among these, we can specify terpinene-4-ol as 4.25%, L-carvenol (0.79 %) and borneol (in traces). The terpenoid phenols (8 % of the total constituents) are 1.68 % thymol and 2.53 % carvacrol. The most important compound, responsible for the immunostimulating effect of the seeds, is one terpenoid ketone - the thymoquinone representing about 1.65 % of the total essential oil fraction. The other essential oil components are one terpenoid aldehyde (heptanal), five sesquiterpenoids (representing about 23 % of the total constituents: 0.95 % α - longipinene, 0.6 % α -amorphene, 1.06 % germacrene and traces of α -bisabolene) and 0.91 % estragole, a phenol-methyl ether. These results are presented in Table 1.

The total volatile constituents extracted by the organic solvents are characterized by the predominance of terpenoid hydrocarbones, with *p*-cymene as the main constituent (43.6%). These results are quite similar to those obtained by D'Antuono in the case of the hydrodistillation of *Nigella sativa* essential oil extracts originated from Morocco [4]. The low percentage of thymoquinone (1.7%) is quite close to the results obtained by Nickavar by the hydrodistillation of Iranian *Nigella sativa* seeds [5]. The results presented in this paper shown that the Tunisian and Romanian *Nigella sativa* seeds have the same main compound, the *p*-cymene. It can be seen from these results that the Romanian *Nigella sativa* contains more *p*-cymene (75 %) than the Tunisian one. The other main compounds found in these essential oils are different, respectively α -pinene (13.75 %) and terpinolene (9.08 %) in the Tunisian one and γ -terpinene (11.2 %) and α -thujene (4.6 %) in the Romanian one. The studied Tunisian *Nigella sativa* essential oil does not contain, neither in trace, α -thujene, a well known neurotoxic compound, but this compound was found in large amounts in the

Romanian one [10].

Regarding the obtained results concerning thymoquinone, they are very similar to those obtained in the literature [2,4,5]. These studies have also used another extraction method, which led to a richer content in thymoquinone (about 48 %) in the essential oil. This method involved the Soxhlet extraction of lipophilic substances, the evaporation under reduced pressure of the solvent (petroleum ether) and the steam distillation of the residue. However, the evaporation under reduced pressure could alter the final results due to the fact that it could favour a certain loss of the most volatile compounds at the solvent's evaporation temperature.

These results establish the rich content of *Nigella sativa*'s essential oil in some compounds exhibiting biological activity. Among these, *p*-cymene, limonene, α -pinene, borneol, linalol and carvacrol, that present bactericide properties, and antioxidant properties, assigned to thymoquinone. According to the studies it was demonstrated that thymol and carvacrol exhibit antimicrobial activities [7]. These results could justify and partially support the use of *Nigella sativa* in traditional medicine, for the treatment of different infections.

Conclusions

Nigella sativa essential oil extract obtained by hydrodistillation is characterized by the presence of two terpenic phenols, which constitute about 4.19 % of the essential oil. These are thymol (1.67 %) and carvacrol (2.52 %). The terpenic hydrocarbons are mainly represented in the essential oil (75.96 %), *p*-cymene being present in the highest percentage (43.58 % of the essential oil).

This composition is also characterized by an important percentage of terpenic alcohols (5.05 % of the total components).

The Tunisian *Nigella sativa* essential oil is also characterized by the presence of thymoquinone (1.65 % of the total compounds), a compound known for its antioxidant properties.

It seems that the volatile composition depends in a great extent on the used extraction technique, and this offers the possibility to choose the methods which could favour the isolation of certain compounds.

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Manuscript received: Januray 7th 2010