Chemical Composition of the Fixed and Volatile Oils of *Nigella sativa* L. from Iran

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The chemical composition of the extracted fixed oil (total fatty acid composition) and volatile oil of *Nigella sativa* L. seeds grown in Iran were determined by GC and GC/MS. Eight fatty acids (99.5 %) and thirty- two compounds (86.7 %) have been identified in the fixed and volatile oils, respectively. The main fatty acids of the fixed oil were linoleic acid (55.6 %), oleic acid (23.4 %), and palmitic acid (12.5 %). The major compounds of the volatile oil were *trans*-anethole (38.3 %), *p*-cymene (14.8 %), limonene (4.3 %), and carvone (4.0 %).

Key words: Nigella sativa L., Fixed Oil Composition, Volatile Oil Composition

Introduction

The genus *Nigella* belongs to the Ranunculaceae family and comprises about eight species in Iran (Mozaffarian, 1998). *Nigella sativa* L. is one of these species, which is naturally distributed in different parts of the country. In addition, it is extensively cultivated in various regions of Iran (Mozaffarian, 1998; Zargari, 1990). Its seeds "Cyah-daneh in Persian" have been widely used in Iranian traditional medicine as a natural remedy for a long time. The seeds are believed to have galactagogue, carminative, laxative and antiparasitic properties (Amin, 1991; Zargari, 1990).

The seeds of *N. sativa* have been subjected to a range of pharmacological investigations in recent years. These studies have showed a wide spectrum of activities such as antibacterial (Ferdous *et al.*, 1992; Hanafy and Hatem, 1991; Rathee *et al.*, 1982), antitumor (David *et al.*, 1998), anti-inflammatory (Houghton *et al.*, 1995; Mutabagani and El-Mahdy, 1997), CNS depressant and analgesic (Khanna *et al.*, 1993), hypoglycemic (Al-Hader *et al.*, 1993), smooth muscles relaxant (Aqel, 1993; Aqel, 1995; Aqel and Shaheen, 1996) cytotoxic and immunostimulant (Swamy and Tan, 2000). Some of these activities have been predominantly attributed to the volatile and fixed oils. To the best of our knowledge chemical composition of the fixed and volatile oils obtained from the seeds of *N. sativa* has not been the subject of much study (Houghton *et al.*, 1995; Mozaffari *et al.*, 2000).

The aim of this study is to describe the detailed chemical composition of fixed oil (total fatty acid composition) and volatile oil of *N. sativa* seeds from Iran in order to complete their chemical characterization. This investigation will be useful to identify the bioactive compounds of the oils, which may be responsible for the therapeutic properties of the seeds.

Material and Methods

Plant material

Seeds of *N. sativa* were purchased from the local market in Tehran, Iran; authenticated by Dr. F. Mojab and stored in the Herbarium of Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Extraction and analysis of fixed oil

25 g seeds were crushed and extracted with petroleum ether for 4 h in a Soxhlet apparatus. The extract was concentrated under reduced pressure.

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1 ml concentrated extract was dissolved in 20 ml petroleum ether and 2 ml 2 M methanolic KOH added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed and washed with water. This oil (as the methyl esters of the fatty acids) was analyzed by GC using a Shimadzu GC-17A system equipped with a FID detector, a capillary SGE BX-70 column (30 m × 0.25 mm) and nitrogen as the carrier gas. The oven temperature was kept at 130 °C for 1 min, programmed to 185 °C at a rate of 5 °C/min and kept at 185 °C for 2 min, then programmed to 220 °C at a rate of 15 °C/min and kept at 220 °C for 3 min. The injection volume was 0.1 μl in the split mode. The constituents were identified by comparison of their retention times with those of reference samples. Reference solutions of 1 % w/v of the methyl esters of the following fatty acids were used: Lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and eicosadienoic acid.

Extraction and analysis of volatile oil

25 ml of concentrated extract (prepared by the above method) were hydrodistilled for 4 h. The distillate was extracted with *n*-hexane. The organic layer was separated, concentrated to 1 ml under reduced pressure, and dried over anhydrous sodium sulfate. This volatile oil was analyzed by GC/MS using a Hewlett-Packard 6890/5972 system with HP-5MS capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The carrier gas was helium with a flow of 0.8 ml/min. The split ratio was 1:10. The column temperature was programmed from 60–260 °C at 4 °C/min. Mass spectra were taken at 70 eV. Mass range was from *m/z* 35–350 amu. The constituents were identified by matching their mass spectra in the Wiley275.L library and by comparison of their retention indices with literature values (Adams, 1995). Retention indices were determined using retention times of *n*-alkanes that have been injected to the same instrument and under the same chromatographic conditions. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

Table I. Fatty acid composition of the fixed oil of *Nigella sativa* L.

Fatty acid	RT	Percentage
Lauric acid	4.68	0.6
Myristic acid	5.91	0.5
Palmitic acid	7.48	12.5
Stearic acid	9.37	3.4
Oleic acid	9.79	23.4
Linoleic acid	10.52	55.6
Linolenic acid	11.95	0.4
Eicosadienoic acid	12.71	3.1
Total fatty acids		*99.5*

Table II. Chemical composition of the volatile constituents of *Nigella sativa* L.

Compound	RI	Percentage
n-Nonane	901	1.7
3-Methyl nonane	931	0.3
1,3,5-Trimethyl benzene	969	0.5
n-Decane	1001	0.4
1-Methyl-3-propyl benzene	1052	0.5
1-Ethyl-2,3-dimethyl benzene	1087	0.2
n-Tetradecane	1400	0.2
n-Hexadecane	1600	0.2
Nonterpenoid hydrocarbones		*4.0*
α-Thujene	928	2.4
α-Pinene	935	1.2
Sabinene	975	1.4
β-Pinene	979	1.3
Myrcene	992	0.4
α-Phellandrene	1007	0.6
p-Cymene	1026	14.8
Limonene	1030	4.3
γ-Terpinene	1059	0.5
Monoterpenoid hydrocarbons		*26.9*
Fenchone	1097	1.1
Dihydrocarvone	1206	0.3
Carvone	1245	4.0
Thymoquinone	1251	0.6
Monoterpenoid ketones		*6.0*
Terpinen-4-ol	1179	0.7
p-Cymene-8-ol	1186	0.4
Carvacrol	1302	1.6
Monoterpenoid alcohols		*2.7*
α-Longipinene	1353	0.3
Longifolene	1408	0.7
Sesquiterpenoid hydrocarbones		*1.0*
Estragole	1200	1.9
Anisaldehyde	1255	1.7
trans-Anethole	1289	38.3
Myristicin	1523	1.4
Dill apiole	1627	1.8
Apiole	1684	1.0
Phenyl propanoid compounds		*46.1*
Total compounds		*86.7*

Results and Discussion

The solvent extraction of *N. sativa* gave a green oily extract with a strong aromatic odor. Eight fatty acids were identified in the extract, which represented about 99.5 % of the total fatty acid composition (Table I). The extract was consisted of four saturated fatty acids (17.0 %) and four unsaturated fatty acids (82.5 %). Linoleic acid (55.6 %), oleic acid (23.4 %), and palmitic acid (12.5 %) were the major components. The fatty acid composition determined by the present investigation is similar to literature values (Houghton *et al.*, 1995).

The hydrodistillation of the extract from the seeds of *N. sativa* gave a yellowish volatile oil. The chemical composition of the volatile oil is listed in Table II. Thirty-two compounds, constituting 86.7 % of the volatile oil, were identified. The oil consisted of six phenyl propanoid compounds (46.1 %), nine monoterpenoid hydrocarbons (26.9 %), four monoterpenoid ketones (6.0 %), eight nonterpenoid hydrocarbons (4.0 %), three monoterpenoid alcohols (2.7 %), two sesquiterpenoid hydrocarbons (1.0 %). So, the oil is characterized by a large amount of phenyl propanoids. The oil presented high levels of *trans*-anethole (38.3 %) and *p*-cymene (14.8 %). Other important constituents were limonene (4.3 %) and carvone (4.0 %). These results are nearly similar to the qualitative results obtained from other investigations (Mozaffari *et al.*, 2000).

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