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Fatty acid, tocopherol, mineral composition, total phenolic, flavonoid and thymoquinone content, and antioxidant potential of *Nigella stellaris*

Sibel Silahtaroğlu [a], Serife Selma Uras Güngör [a], Ahmet İlçim [b], and Gamze Kökdil [a,*]

[a] *Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Yenisehir Campus, 33169 Mersin, Turkey*
[b] *Department of Biology, Faculty of Arts and Science, Mustafa Kemal University, 31000, Antakya, Hatay, Turkey*

**Corresponding author at: Department of Pharmacognosy, Faculty of Pharmacy, Mersin University, Yenisehir Campus, 33169 Mersin, Turkey.*
Tel.: +90.324.3413021. Fax: +90.324.3413022. E-mail address: gkokdil@gmail.com (G. Kökdil).

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ABSTRACT

In the present study, the fatty acid composition of the fixed oil from seeds of *Nigella stellaris* has been investigated by GC-MS. Linoleic and oleic acids were the major fatty acids. The tocopherols determined by HPLC and α- and δ-tocopherols were detected in the oil. The seeds also were analyzed by ICP-MS to determine mineral content. Ca, P, and K were main elements among seventeen minerals in the seeds. Total phenolic and flavonoid contents of the aqueous methanolic extracts of different plant parts were assessed by Folin-Ciocalteu method and the $AlCl_3$ assay. The seed extract was also analyzed by RP-HPLC analysis for its thymoquinone content. The present study showed that the seed extract contained low amount of thymoquinone. The seed extract exhibited the significantly higher total phenolic and flavonoid content than the extract of aerial part. DPPH radical scavenging activity was used to evaluate the antioxidant capacity of the extracts. Both of the extracts showed high antioxidant activity.

1. Introduction

The genus *Nigella* L. (Ranunculaceae) includes about 22 species distributed from the Mediterranean regions to West Asia [1-3]. Some *Nigella* species especially *N. sativa* have been used traditionally in Arabian countries, Indian subcontinent and Europe for culinary and medicinal purposes. The seeds of *N. sativa* (black seed/ black cumin) are used as natural remedy for many ailments including asthma, cough, bronchitis, headache, rheumatism, hypertension, fever and influenza [4,5]. Black cumin has been extensively studied phytochemically and pharmacologically [5,6] and it was known that the plant contain fixed and volatile oil, quinonic compounds, vitamins, phenolic compounds, alkaloids, saponins, sterols, minerals, aminoacids, proteins and carbonhydrates [6-9]. Recent studies showed that *N.sativa* have antimicrobial, anticestode, antitumor, antidiabetic, antioxidant, antiallergic, anti-inflammatory, immunomodulatory, antiaflatoxine, spasmolytic, bronchodilator, hypocholesterolemic, hypotensive, hepatoprotective, antinociceptive, neuroprotective and anticonvulsant effects [5-12].

N. sativa seeds have been intensively investigated to determine its nutritional and therapeutic value whereas little information is known concerning the chemical composition and biological activity about other *Nigella* species [13-17]. The genus *Nigella* comprises about 15 taxa in Turkey [2,3,18]. *N. stellaris* is one of these taxa, which grows in East Mediterranean region. It is an annual plant grows to 5-20 cm tall with laciniae linear-setaceous leaf, lavender-blue sepals, follicles acutely keeled, styles longer than the fused follicles, spreading horizontally to form a rigid star. This species has not been studied phytochemically and pharmacologically. The aim of the present study was to investigate *N. stellaris* seeds in detail chemically for possible nutritional value. In this study, the total phenolic and flavonoid content and its antioxidant activity of the methanolic extracts of *N. stellaris* seed and herbs were also tested.

2. Experimental

2.1. Plant material

Nigella stellaris Boiss. was collected from Southern Turkey (C6 Maraş: Avşar village, 580-600 m, fields) in June 2008 by one of the authors (A. İlçim). Voucher specimen was deposited in the Herbarium of the Department of Biology, Faculty of Arts and Sciences, Kahramanmaraş Sütçü İmam University (Ahmet İlçim 1762 KSUH) and identified by Dr. Ahmet İLÇİM (Department of Biology, Faculty of Arts and Sciences, Mustafa Kemal University, Antakya-Hatay, Turkey)

2.2. Reagents and chemicals

All chemicals were analytical-reagent grade and obtained from the following sources: Methanol, *n*-hexane and Folin-Ciocalteu's phenol reagent were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), gallic acid and thymoquinone were obtained from Sigma Chemical Company (Sigma, MO, USA). Rutin was obtained from Fluka Chemie (Buchs, Switzerland).

2.3. Extraction procedure

The seeds were powdered mechanically and extracted with *n*-hexane for 6 h in a Soxhlet apparatus. Removal of the solvent under reduced pressure gave the fixed oil. The fatty acid content of the fixed oil was investigated by GC and GC-MS analysis of their methyl esters. Oil (0.5 g) was dissolved in 10 mL *n*-hexane in a centrifuged tube and 2 mL 2 M methanolic KOH was added. The mixture was shaken for 2 min and centrifuged at 4000 rpm for 15 min. The upper layer was removed, washed with water, and 1 mL used for analysis [19].

For thymoquinone analysis, a powdered *N. stellaris* seed sample of 0.1 g was extracted with 10 mL methanol, vortexed for 1 min and sonicated for 20 min. After that, it was left overnight in constant rotamix, sonicated for 1 hour and vortexed for 1 min and centrifuged for 25 min at 1600 rpm. The supernatant phase was transferred to a volumetric flask. The volume was completed to 10 mL with methanol. An aliquot of 20 μL was injected into the HPLC [8].

2.4. Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analyses were carried out on an Agilent Technologies 6890 N Network GC System equipped with a DB-Wax capillary column (60 m × 0.25 mm, 0.25 μm) and DB-23 capillary column (60 m × 0.25 mm, 0.25 μm) and interfaced with an Agilent 5973 Network Mass Selective Detector. The oven temperature was kept at 140 °C for 5 min, programmed to 165 °C at a rate of 5 °C/min and kept at 165 °C for 10 min, then programmed to 190 °C at a rate of 5 °C/min and kept at 190 °C for 55 min. The split ratio was 30:1. Transfer line temperature 280 °C; ion source temperature 210 °C; carrier gas helium at a linear velocity of 1.5 mL/min.; ionization energy 70 eV; scan range 15-550 amu. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

The constituents of the fixed oil were identified by comparison of their GC retention times with those of reference methyl esters of the fatty acid and also by comparison of their mass spectra with published spectra (Famedb23.L: Mass spectral library for FAMEs DB-23 column, NIST02.L mass spectral library).

2.5. High performance liquid chromatographic analysis

Tocopherols were analyzed by high-performance liquid chromatography (HPLC) equipped with a FLD (E_x = 295 nm E_m = 330 nm) and an Atlantis HILIC silica column (25 cm × 4.6 mm, 5 μm). The fixed oil was used for the analysis. Separation of all tocopherols was based on isocratic elution with the mobile phase hexane containing 4% 1,4-dioxane and 0.04% acetic acid. The system was operated at a flow-rate of 1 mL/min. Standard solutions of α-tocopherol acetate, α, β, γ and δ-tocopherols. The tocopherols content of the sample was quantified by the external standard method

Thymoquinone analyses were carried out by RP-HPLC. A system of Agilent 1100 series with UV detector at a wavelength of 275 nm was used. The mobile phase was water:methanol:2-propanol (50:45:5, *v*:*v*:*v*) at 0.6 mL/min flow rate. The column used was an ACE 5 C18 (25 cm x 4.6 mm i.d.) [8].

2.6. Mineral analysis

A microwave oven Anton PAAR Multiwave 3000 model were used for microwave-assisted digestion of plant materials. The mineral constituents in examined plant materials were analyzed by using Agilent 7500a ICP-MS.

2.7. Determination of total phenolic and flavonoid content

Powdered aerial parts (6 g) and seeds (0.75 g) were dispersed with 80% aqueous methanol sonicated for 60 min at 30 °C. The supernatants were filtered through a Whatman Grade 1 filter paper. The volumes of the extract of seeds and aerial parts were adjusted to 100 mL and 25 mL by adding the appropriate volume of 80% aqueous methanol, respectively. The extracts were stored at 4 °C for the analysis [20]. The concentration of total phenolic contents was performed by the Folin-Ciocalteu method [21]. The total phenols were expressed in milligram equivalents of gallic acid (GAE) (400-1000 mg/L) per grams of sample. The amount of total flavonoids in the extracts was measured as the method described by Kim *et al*. (2003) with some modification based on that of the method described by Subhasree *et al*. [22]. The measurement was based on reaction with $AlCl_3$ and spectrophotometric technique. All determinations were performed in triplicate. Results were expressed as mg/g rutin equivalents (RE). The values are presented as means of triplicate analyses.

2.8. DPPH radical-scavenging activity

Radical scavenging activity of the extracts was determined as the method described by Yen and Duht (1994) [23]. Spectrophotometric analysis was used to measure the free radical scavenging capacity. The DPPH solution was prepared 6×10^{-5} M concentration. 0.1 mL of the each extract and standard solutions of butylated hydroxyanisole (BHA) (200-500 mg/L) were added 2.9 mL the methanolic solution of DPPH. The mixtures were shaken vigorously and incubated in the dark for 45 min at room temperature and the decreases in the absorbance values were measured at 517 nm with a spectrophotometer [24]. All analysis was carried out in triplicate. The percentage of DPPH scavenging activity was calculated using the equation (1).

$$\% \text{ DPPH scavenging activity: } 100(A_{control} - A_{sample} / A_{control}) \quad (1)$$

where $A_{control}$ is the absorbance of the control reaction mixture without the test compounds, and A_{sample} is the absorbance of the test compounds.

3. Results and discussion

The yield of the fixed oil from *N. stellaris* was 34.3%. The fatty acid composition of the oil was presented in Table 1. The oil was characterized by high amounts of unsaturated fatty acids. The most important fatty acids were linoleic and oleic acids which accounted for 71% of the total FAME. The oil contains $C_{20:2}$ eicosadienoic acid (4.9%) that its presence was characterized to be a specific chemotaxonomic marker for *Nigella* species [13,25]. In this study, the total saturated fatty acids of the oil were 14.2%. Palmitic acid was the main saturated fatty acid. In addition, total unsaturated fatty acids accounted for 79.1% of total fatty acids.

In literature, mainly *N. sativa* seed oil from different geographic origins was investigated for their fatty acid composition. *N. sativa* seeds contain a fixed oil (15.6-41.6%) which is composed mainly unsaturated fatty acids including $C_{20:2}$ eicosadienoic acids [6,9,19,25-28]. The fatty acid composition of the other ten *Nigella* species has been investigated and the authors found that all the oils characterized by high amounts of unsaturated fatty acids [13].

Table 1. Fatty acid composition of *N. stellaris* seed oil.

Fatty acid [a]	% [b]
Saturated	
$C_{16:0}$ (Palmitic acid)	10.3±0.2
$C_{18:0}$ (Stearic acid)	3.7±0.2
$C_{20:0}$ (Arachidic acid)	0.2±0.1
TSFA	14.2±4.43
Monounsaturated	
$C_{18:1n9}$ (Oleic acid)	16.6±0.5
$C_{20:1}$ (11-Eicosenoic acid)	0.5±0.1
TMUFA	17.1±8.84
Polyunsaturated	
$C_{18:2n6}$ (Linoleic acid)	54.4±1.2
$C_{18:3n3}$ (α-Linolenic acid)	0.3±0.1
$C_{18:3n6}$ (γ-Linolenic acid)	0.4±0.0
$C_{20:2n6}$ (11,14-Eicosadienoic acid)	4.9±1.3
$C_{20:3n3}$ (11,14,17-Eicosatrienoic acid)	0.8±0.5
$C_{20:4n6}$ (Arachidonic acid)	0.6±0.1
$C_{22:2}$ (Docosadienoic acid)	0.6±0.4
TPUFA	62.0±19.13

[a] TSFA, total saturated fatty acids; TMUFA, total monounsaturated fatty acids; TPUFA, total polyunsaturated fatty acids.
[b] All values given are means of three determinations.

The present study shows that the qualitative composition of the oil from *N. stellaris* is in agreement with previously published data for fixed oils of *Nigella* seeds. This is the first report of composition of the oil from *N. stellaris*. The oil was rich in polyunsaturated fatty acids which play an important role in human health.

In this study, *N. stellaris* seeds were analyzed for their thymoquinone content by RP-HPLC in the methanolic extract. The seeds had a thymoquinone (TQ) concentration of 0.02±0.0003 μg/mL. In the literature, the presence of TQ in essential/fixed oil and the methanolic extract of *N. sativa* seed were determined by different methods [8,29]. It was found that TQ content ranged between 3098.5-1274.6 mg/kg in the seeds from different origin [8]. It has been shown to be the principal active ingredient in the seeds of *N. sativa* [9]. To our knowledge this is the first report for TQ content in the seed of *N. stellaris.* The amount of pharmacologically active thymoquinone was lower than reported that of *N. sativa* seed in the literature.

The analyses of the oil showed that the oil contains two kinds of tocopherols which are α-tocopherol and δ-tocopherol. The oil contained α-tocopherol (2.04±0.07 mg/100 g) and δ-tocopherol (0.17±0.01 mg/100 g). Our results were quite different from the tocopherol composition of *N. sativa* seed oil that the oil had mainly α- and γ-tocopherols [8,16].

The concentrations of twelve elements determined in *N. stellaris* seeds. Table 2 summarizes the element composition of examined seeds. The seeds contained significant amounts of important mineral elements. Phosphorus was relatively high in the seeds. Calcium and potassium were other predominant macrominerals among twelve elements analyzed in the seeds. The other important elements, in descending order by quantity, were Mg, Na, Zn, Mn and Fe.

Table 2. Mineral content of *N. stellaris* seeds.

Minerals	Content (μg/g) *
Macro minerals	
Calcium (Ca)	4214±98
Magnesium (Mg)	1612±49
Phosphorus (P)	6913±117
Potassium (K)	3637±99
Sodium (Na)	427.90±13
Essential trace minerals	
Chromium (Cr)	5.77±0.11
Copper (Cu)	12.93±0.32
Iron (Fe)	20.99±2.02
Manganese (Mn)	21.16±1.3
Nickel (Ni)	2.48±0.07
Selenium (Se)	1.37±0.01
Zinc (Zn)	58.93±3.41

* Values are means±standard deviations (n = 3).

In the literature, mineral composition of *N. sativa* was studied extensively because of its using as a spice but there is no information about mineral content of *N. stellaris* as well as the other *Nigella* species [30,31]. *N. stellaris* seeds provide relatively high amounts of the minerals such as P, K and Ca whereas *N. sativa* seeds are a source of Ca, Fe and K [7].

The content of the total phenolic and flavonoids of the extracts from *N. stellaris* determined using Folin-Ciocalteu and $AlCl_3$ spectrophotometrically method.

The seed extract of the plant had highest total phenolic content than the phenol content of the aerial parts (Table 3). The extracts of seeds and aerial parts of the flavonoid content was similar each other. Both extracts from the seeds showed high radical scavenging activity with DPPH radical. The flavonoid content exhibited a positive correlation with the antioxidant activity.

Table 3. Total phenolic and flavonoid content, and free radical scavenging activity of test samples from *N.stellaris*.

Sample	Total phenolic content (mg of GAE/g)	Total flavonoid content (mg of RE/g)	Inhibition (%)
Seed extract	394.06±3.33	130.24±0.04	93.59±0.02
Aerial parts extract	187.70±037	92.58±0.02	92.79±0.01
BHA (0.5 mg/mL)	-	-	96.32±0.01

4. Conclusions

To our knowledge, we report fatty acid and mineral composition, tocopherol and thymoquinone, total phenol and flavonoid content for the first time from *N. stellaris*. In conclusion, *N. stellaris* seeds contain fixed oil which is rich sources of unsaturated fatty acids, minerals and phenols. The extracts have also strong antioxidant activity. Some further studies are necessary to the possibility of using *N. stellaris* seeds and the extracts as a potential source of nutraceuticals and to isolate the active constituents in the extract. Not only antioxidant activity but also other biological activity studies of the extracts from *N. stellaris* should be done to evaluate potential therapeutic uses of the plant.

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