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NIGELLA SATIVA L.'NIN YAĞ ASİDİ, TOKOFEROL, MİNERAL BİLEŞİMİ, TOTAL FENOLİK, FLAVONOİT, TİMOKİNON MİKTARI VE ANTİOXİDAN AKTİVİTESİ

FATTY ACID, TOCOPHEROL, MINERAL COMPOSITION, TOTAL PHENOLIC, FLAVONOID, THYMOQUINONE CONTENT, AND ANTIOXIDANT ACTIVITY OF *NIGELLA SATIVA* L.

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ÖZET

Nigella sativa L. *tohumlarından elde edilen sabit yağın yağ asidi ve tokoferol bileşimi sırasıyla GC-MS ve HPLC analizleri ile saptandı. Linoleik ve oleik asitler başlıca yağ asitleridir. Major tokoferoller γ- and δ-tokoferollerdir. Bitkinin farklı kısmlarının sulu metanollü ekstrelerinin total fenol ve flavonoit bileşimleri ve primer antioksidan aktivitesi saptandı. Total fenol bileşimi Folin-Ciocalteu metodu ve total flavonoit bileşimi $AlCl_3$ deneyi ile spektrofotometrik yöntemle tayin edildi. Primer antioksidan aktivite açısından ekstreler DPPH yöntemi ile değerlendirildi. Yapılan çalışma tohum ekstresinin total fenol bileşiminin toprаküstü ekstresinden daha yüksek miktarda olduğunu gösterdi. Total flavonoit miktarı 106.10±0.10-118.06±0.02 mg (rutin equivan/g) arasında saptandı. Tohum timokinon miktarı RP-HPLC ile belirlendi ve konsantrasyonu 268.3 µg/mg olarak saptandı. DPPH radikal süpürücü testte ekstrelerin inhibisyonunun % 95.09-90.71 arasında olduğu belirlendi. Tohumların mineral bileşimi ise ICP-MS ile saptandı. Tohumlarda saptanan onyedi mineral arasında P, K, Ca ve Mg başlıca elementler olarak belirlendi.*

Anahtar kelimeler: *Nigella sativa, Yağ asidi, Tokoferol, Mineral, Fenolik bileşikler, Antioksidan etki*

ABSTRACT

The fatty acid and tocopherol composition of fixed oil from seeds of Nigella sativa L. *has been investigated by GC-MS and HPLC, respectively. Linoleic and oleic acids were the main fatty acids. The major tocopherols were γ- and δ-tocopherols. The total phenolic and flavonoid content and primary antioxidant activity of aqueous methanolic extracts of different parts of N.sativa were measured. Total phenolic content was assessed by Folin-Ciocalteu method and the content of flavonoids was measured spectrophotometrically by using the $AlCl_3$ assay. Primary antioxidant activity in terms of free radical scavenging activity of the extracts was evaluated by DPPH assay. The present study showed that the seed extract contained significantly higher amount of total phenolic compounds than aerial part extract. The total flavonoid content ranged between 106.10±0.10 and 118.06±0.02 mg rutin equivalent per g extracts. The thymoquinone content in seeds were quantified by RP-HPLC analysis and the concentration of thymoquinone was 268.3 µg/mL. In DPPH radical scavenging assay, inhibition % of the extracts was found to be 95.09-90.71. The seeds also were analyzed by ICP-MS to determine mineral content. P, K, Ca and Mg were predominant elements among seventeen minerals in the seeds.*

***Key words**: Nigella sativa* L.*, Fatty acid, Tocopherols, Mineral, Phenolics, Antioxidant activity*

INTRODUCTION

Nigella sativa L. (Ranunculaceae) is commonly known as black seed or black cumin has been used for medicinal purposes as natural remedy for treating of many ailments including asthma, cough, bronchitis, headache, rheumatism, hypertension, fever and influenza in many Middle Eastern countries and other parts of the world (1,2). *N.sativa* seeds are used as seasoning for foodstuffs like bread and pickles among Turkish people. Some *Nigella* species (*N. sativa* L., *N.damascena* L. and *N.arvensis* L., e.g.) are used as tonic, stimulating, carminative, diuretic and for delayed menses and lactation in Turkish folk medicine (3).

Many of the claimed folk medicinal uses of *N.sativa* have been extensively studied, which justifies its therapeutic value including antioxidant, antiallergic, anti-inflammatory, immunomodulatory, antimicrobial, anticestode, antitumor, antidiabetic, antiaflatoxine, estrogenic, spasmolytic, bronchodilator, hypocholesterolemic, hypotensive, hepatoprotective, antinociceptive, neuroprotective and anticonvulsant effects (2,4-14). In the literature, the occurrence of fats and fatty acids, essential oils, vitamins, phenolic compounds, alkaloids, saponins, sterols, minerals, amino acids, proteins and carbohydrates (1,2,6,9). Many of the pharmacological activities mentioned above have been attributed to fixed or essential oil or quinone constituents in the seed.

The genus *Nigella* comprises about 15 species in Turkey (15-17). The chemical composition pharmacological activities of cultivated *N. sativa* and some *Nigella* species growing wild in Turkey have been investigated (18-22). Many investigations on chemical composition, nutritional value, and pharmacological activities of *N. sativa* seeds can be found in the literature, there is no study has been carried out to determine thymoquinone content and detailed mineral composition, total phenolic and flavonoid contents and radical scavenging activity of *N. sativa* growing in Turkey. The aim of the present study was to evaluate nutritional value by means of fatty acid, tocopherols, mineral composition and total phenols, flavonoids in detail. In addition, the aqueous methanolic extracts obtained from different parts of the plant were investigated for their antioxidant activity by DPPH radical scavenging activity.

MATERIALS AND METHODS

Materials

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). *Nigella sativa L*. was collected from southern Turkey (C6 Maraş: Avşar) in June 2008. Voucher specimen was deposited in the Herbarium of the Department of Biology, Faculty of Arts and Sciences, Kahramanmaraş Sütçü İmam University (Ahmet İlçim 1759 KSUH) and identified by Assoc. Prof. Dr. Ahmet İLÇİM (Department of Biology, Faculty of Sciences, Mustafa Kemal University, Antakya-Hatay, Turkey)

Extraction of fixed oil

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. And allowed to stand for 10 min, the upper layer was removed, washed with water, and 1 µL used for analysis (21).

Gas chromatography-Mass spectrometry (GC-MS) Analysis

GC-MS analyses were carried out on an Agilent Technologies 6890 N Network GC System equipped with a DB-Wax capillary column (60 m x 0.25 mm x 0.25 µm) and DB-23 capillary column (60 m x 0.25 mm x 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 70eV; scan range 15-550 amu. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

Constituent Identification

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, NIST02.L)

Extraction and HPLC analysis of thymoquinone

A powdered *N.sativa* seed sample of 0.1 g was extracted with 10 mL methanol as the method described by Al-Saleh et al. (2006). HPLC analysis was performed on an Agilent 1100 series HPLC system with UV detector at a wavelength of 254 nm. The mobile phase was water: methanol: 2-propanol (50:45:5) at 0.6 mL/min flow rate. The column used was an ACE 5 C18 (25 cm x 4.6 mm i.d.) (22).

Tocopherol 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 x 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 were used. The tocopherols content of the sample was quantified by the external standard method.

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 using Agilent 7500a ICP-MS.

Determination of Total Phenolics and Flavonoids

Seeds (3 g) and aerial parts (6 g) were powdered mechanically and dispersed with 80% aqueous methanol sonicated for 60 min at 30 ^{o}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 50 mL and 100 mL by adding the appropriate volume of 80 % aqueous methanol, respectively. The extracts were stored at 4 ^{o}C for the analysis (23). The concentration of total phenolics in the extracts was determined using Folin-Ciocalteu procedure as described by Kim *et al*. (2003). The total phenols were determined as gallic acid equivalents (GAE) of per grams of each extracts. Gallic acid was prepared 400-1000 mg/L, and the values are presented as means of triplicate analyses (24).

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*. (25). The measurement was based on reaction with $AlCl_3$ and spectrophotometrical technique. All determinations were performed in triplicate. Results were expressed as mg/g rutin equivalents.

DPPH radical-scavenging Assay

Radical scavenging activity of the extracts was determined as the method described by Yen and Duht (26). The DPPH solution was prepared 6 x 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. All the analysis was carried out in triplicate. The percentage of DPPH scavenging activity was calculated using the following equation: % DPPH scavenging activity: 100 ($A_{control}$ - A_{sample} / $A_{control}$). 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 (26).

RESULTS AND DISCUSSION

The yield of the fixed oil from *N. sativa* was 43.8 %. The fatty acid composition of the oil is presented in Table 1. The dominating fatty acid was linoleic acid ($C_{18:2n-6}$) which accounted for 51.8 % of the total FAME. The second major fatty acid was oleic acid ($C_{18:1n9}$) (20 %). The oil was characterized by high amounts of unsaturated fatty acids. The oil also contains $C_{20:2}$ eicosadienoic acid (3.3 %).

The presence of this fatty acid in significant amounts together with the other unsaturated fatty acids was characterized to be a specific chemotaxonomic criterium for *Nigella* species (19,27).In this study, saturated fatty acids accounted for 20.5 % of total fatty acids. Among them, the main saturated fatty acids were palmitic and stearic acids. These results are in agreement with previously published data (21,28-30).

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

Fatty acid	%
Saturated	
$C_{14:0}$ (myristic acid)	0.2±0.0
$C_{16:0}$ (palmitic acid)	14.1±0.2
$C_{18:0}$ (stearic acid)	2.6±0.2
$C_{20:0}$ (arachidic acid)	0.8±0.1
$C_{22:0}$ (behenic acid)	2.1±0.0
$C_{24:0}$ (lignoseric acid)	0.7±0.1
TSFA	20.5
Monounsaturated	
$C_{18:1n9}$ (oleic acid)	20±0.5
$C_{20:1}$ (11-eicosenoic acid)	0.4±0.1
TMUFA	20.4
Polyunsaturated	
$C_{18:2n6}$ (linoleic acid)	51.8±0.4
$C_{18:3n3}$ (α- linolenic acid)	0.1±0.0
$C_{18:3n6}$ (γ- linolenic acid)	0.05±0.01
$C_{20:2n6}$ (11, 14- eicosadienoic acid)	3.3±0.2
$C_{20:3n3}$ (11, 14, 17-eicosatrienoic acid)	0.05±0.01
$C_{22:2}$ (docosadienoic acid)	0.5±0.1
TPUFA	55.8

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

The present results showed that the fixed oil from *N.sativa* seeds was rich in the unsaturated and essential fatty acids. The oil had appreciable amounts of linoleic acid which has serum cholesterol and blood pressure lowering effect (6,9). The oil is a valuable source of essential fatty acids.

The tocopherols were determined by HPLC in the fixed oil of *N.sativa* seeds. The oil mainly contains three kinds of tocopherols which are α-, γ- and δ-tocopherols. Among them γ- and δ-tocopherols were similar amounts 14.86±0.64 and 14.60±0.10 mg/100g, respectively). The concentration of α-tocopherol acetate was 2.11±0.08 mg/100g while the amount of α-tocopherol was 1.70±0.07 mg/100 g. Al-Saleh et.al reported that *N.sativa* seeds from different origin contained α-(5.65-11.39 mg/kg) and γ-tocopherols (2.26-6.95 mg/kg) (22). Matthaus and Özcan determined the total tocopherol content and composition in some *Nigella* species. They found that the oils contained between 1.70-4.12 mg/100g α-tocopherol and 0.97-4.51 mg/100g γ-tocopherol (31). Our results indicated that *N.sativa* seed oil analyzed had higher content of tocopherols than the literature data. The high content of tocopherols enhances antioxidant capacity of the oil. These results indicate that N.sativa oil has the potential source for pharmaceutical and food industry.

In this study, *N. sativa* seeds were analyzed for their thymoquinone content by RP-HPLC in the methanolic extract. The seeds had a thymoquinone (TQ) concentration of 268.3±0.4 µg/mL. In the literature, the presence of TQ in essential or fixed oil and the methanolic extract of *N. sativa* seed were detected using different chromatographic methods. (22,32,33). It was found that TQ content ranged between 3098.5-1274.6 mg/kg in the seeds from different origin and the seeds from Ethiopia contained the highest concentration of TQ. It has been shown to be main active ingredient in the seeds (22). To our knowledge this is the first report for TQ content in the seed of *N.sativa* from Turkish origin.

The concentration of twelve elements was determined in the seeds and the results for mineral composition of the seeds were presented in Table 2.

Table 2. Mineral content of *N. sativa* seeds from Turkey.

Minerals	Content (μg/g)
Macro minerals	
Sodium (Na)	367.40±11
Magnesium (Mg)	1387±54
Phosphorus (P)	5284±109
Potassium (K)	4218±97
Calcium (Ca)	4214±98
Essential trace minerals	
Chromium (Cr)	5.75±0.07
Manganese (Mn)	25.83±1.77
Iron (Fe)	77.37±14.23
Nickel (Ni)	3.97±0.09
Zinc (Zn)	78.79±3.29
Copper (Cu)	8.50±0.48
Selenium (Se)	1.72±0.10

Three macro minerals, namely, phosphorous, potassium and calcium were relatively high in the seeds. The other main minerals, in descending order by quantity were magnesium, sodium, zinc and iron. These findings are in agreement with previous data for *N.sativa* seeds (6,34-35). In this study, it was shown that *N.sativa* seeds contain relatively high levels of P, K, Ca and Fe which these elements plays an important role in maintenance of human health. The seeds are good alternative source of these mineral elements as a dietary supplement.

The results for total phenolic and flavonoid content, and the DPPH radical scavenging activity were presented in Table 3. The obtained results showed that the seed extract contains the higher total phenolic compounds. The flavonoid content of the extracts of seeds and aerial parts was similar to each extracts.

Table 3. Total phenol and flavonoid content, and DPPH scavenging activity (%inhibition) of the extract from *N.sativa*

Aqueous methanolic extract (20:80)	Total phenol (mg/g) GAE	Total flavonoid (mg/g) RE	inhibition % DPPH
seed extract	410.78±1.97	106.10±0.10	95.09±0.05
aerial parts extract	244.82±2.51	118.06±0.02	90.71±0.06

In the literature, different extracts from different parts of *N.sativa* had been investigated for their phenols and antioxidant activities. Total phenols and antioxidant activity of methanolic extract of different parts of *N.sativa* from Czech Republic had been investigated by Javorkova et al.(2011). They found that the seed extract had higher total phenolics than other parts and linear correlation determined between antioxidant activity and the total content of polyphenols (36). Meziti et al. (2012) reported that 70% aqueous methanolic extracts of *N.sativa* from Algeria had 33.63 µg GAE/mg of extract and moderate DPPH-scavenging activity. They also confirmed antioxidant activity of the extract *in vivo* assay (37).

In this study, thymoquinone content, selenium concentration, total phenol and flavonoid contents of *N.sativa* from Turkish origin are reported for the first time. Present results suggest that the plant can be considered as good sources of minerals and natural antioxidants. Our results were confirmed the antioxidant potential of the plant extract in terms of radical scavenging assay. Generally, both extracts showed significantly antioxidant activity and the phenolic content exhibited a positive correlation with the radical scavenging activity. Therefore, it is possible that the antioxidant activity of the extracts is due to high presence of phenolic and flavonoid compounds. Further studies on identification of all phenolics in seeds and aerial parts are needed to elucidate their antioxidant mechanisms and its extracts may be used in future antioxidative therapy. These results also show *N.sativa* from Turkish origin has a valuable source of nutracetuical supplements.

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