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Chemical Composition of *Nigella sativa* L. Seeds Used as a Medical Aromatic Plant from East Anatolia Region, Turkey

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In this study, chemical composition such as vitamins, fatty acids and trace elements of *Nigella sativa* L. seeds collected from East Anatolia Region of Turkey were investigated. Fatty acids in the lipid extracts were converted into methyl esters. The methyl esters were extracted with *n*-hexane, they were separated and quantified by gas chromatography. Fat soluble vitamins were determined by HPLC and the mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase. For determination of trace elements levels, solutions from microwave digestion of a certain amount of seeds were analyzed with inductively coupled plasma optical emission spectrometry. Main fatty acids of *Nigella sativa* L. seeds were found as 66.5 and 23.5 (as relative % peak area) for linoleic acid (18:2) and oleic acid (18:1), respectively. The contents of Co, Ni, Fe, Zn, Cu, Mn and Cr were determined as 0.12, 1.48, 117.32, 41.42, 30.26, 28.56 and 2.55 µg/g (dry matter) respectively. The levels of vitamins were found as 10.19 µg/g for α-tocopherol, 2.28 µg/g for δ-tocopherol, 0.18 µg/g for retinol, 1.38 µg/g for vitamin D_2 1.85 µg/g for vitamin K_1 and 2.15 µg/g for vitamin K_2. The *Nigella sativa* L. seeds were found to be rich in unsaturated fatty acids, vitamins and trace elements, suggesting that they may be valuable for apoptosis and would be appropriate to further studies in this direction.

Key Words: *Nigella sativa*, Fatty acids, Trace elements, Vitamins.

INTRODUCTION

Throughout history, human beings used a variety of multi-plant and plant mixtures against various diseases. Seeds, leaves, fruits, stems and roots of these plants using grinding, dissolving, distillation and extraction such as pre-process after obtained mixtures was applied to patients. Extracts of the medicinal plants inhibit the disease increased organism resistance were determined with experiments and observations.

Various studies on the herbal plant have been interested because of adverse effect of drugs used for therapy of many diseases. Any adverse effects and toxic effects are not expected if recommended amounts of used plants or plant parts for trephies are applied to patients. *Nigella sativa* L. (Nigella) is an annual herbaceous plant belonging to the Ranunculaceae family growing in countries bordering the Mediterranean Sea. Nigella seeds are used for edible and medicinal purposes in many countries. This seed oil has been reported to possess activities of antioxidant, antitumor, antibacterial, antiinflammatory and a stimulatory effect on the immune system[1-3].

In order to obtain therapy potentials of medical plants, their chemical compositions should be determined by various instrumental techniques. Therefore, we have aimed to determine levels of trace element and fat soluble vitamins and fatty acids compositions of Nigella seeds commonly grown in East Anatolia Region of Turkey.

EXPERIMENTAL

Nigella seeds grown East Anatolia were purchased as grinded fresh from closed bazaar in Elazig-Turkey. The material was transported in polypropylene bags and held at room temperature and analyzed into two days.

The standard metal solutions of Co, Ni, Fe, Zn, Cu, Mn and Cr (1000 mg/L, analytical grade, Merck) were diluted to the desired concentrations with 0.2 mol/L HNO_3. Concentrated HNO_3 and $HClO_4$ for digestion samples were of analytical grade (Merck). Ultra-pure water (deionized, 18 MΩ-cm) was obtained using a Milli-Q system (Millipore Corporation, Billerica, MA, USA). The vitamin standard stock solutions were prepared by dissolving 10 mg of each reagent obtained from Sigma-Aldrich (St. Louis, USA) in 100 mL of methanol using dark brown volumetric flasks. These solutions were stable for at least 1 month when stored in the dark at 4 ºC.

Working solutions were prepared from the stock solutions by appropriate dilution with ethanol and shielded from light.

Oil extraction and preparation of fatty acid methyl esters: The seed samples were ground using a ball mill into powder. Lipids were extracted with hexane/isopropanol (2/1 v/v). The lipid extracts were centrifuged at 10 g for 5 min and filtered; then the solvent was removed on a rotary evaporator at 40 ºC. Lipids were extracted with heptane in a straight through extractor. Fatty acids in the lipid extracts were converted into methyl esters by means of 2 % sulphuric acid (v/v) in methanol[4]. The fatty acid methyl esters were extracted with *n*-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionization detection (Schimadzu GC, 17 Ver. 3) coupled to a glass GC 10 software computing recorder. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Machery-Nagel, Germany) using nitrogen as carrier gas (flow rate, 0.8 mL/min). The temperatures of the column, detector and injector valve were 130-220 ºC and 240- 280 ºC, respectively. Identification was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Determination of vitamins: The chromatographic system was equipped with a Shimadzu HPLC and photodiode array detector. Supelcosil LC 18 DB column (250 mm × 4.6 mm, 5 µm; Sigma, USA) were used for separation of vitamins. Extractions of fat-soluble vitamins were done according to studies of Perales *et al*.[5]. One g seed samples, 0.5 g of ascorbic acid and 10 mL of KOH- ethanol solution (prepared by dissolving 50 mL of ethanol in 15 mL of 60 % [(w/v) KOH] were mixed and shaken continuously overnight at room temperature. Thereafter, the samples were transferred into a separating funnel where liquid extraction with 10 mL of hexane and shaking the funnel for 5 min was carried out. This procedure was repeated two more times. The organic phases were combined and washed two times with 10 mL of water. Then, the organic phase was collected and evaporated to dryness in a vacuum rotary evaporator at 40 ºC and the residue was re-dissolved in 1 mL of mobile phase[6]. Samples were transferred to autosampler vials of the HPLC instrument. The mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 mL/min. The temperature of column was kept at 40 ºC. Detection was performed at 202 nm for vitamins. Identification of the individual vitamins was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions[7].

Determination of trace elements: Perkin-Elmer 3100 inductively coupled plasma optical emission spectrometer (ICP-OES) (Norwalk, USA) with a Gem Cone nebulizer on a cyclonic spray chamber and an auto sampler AS 91 (Perkin-Elmer) was used in the current study.

Samples were digested microwave system: Samples were cleaned by pure water and then, 2 g portion of each sample dried at 80 ºC was accurately and 0.50 g directly weighted into PTFE (polytetrafluorethylen) bombs. For the samples decomposition concentrated 4 mL HNO_3 (65 %, w/w) and 1 mL of $HClO_4$ (60 %, w/w) were added[8]. In a tightly closed system, the following six-step microwave digestion program was applied. PTFE bomb was waited for an hour to cool and was carefully opened. Colourless solution was transferred into beaker and evaporating to dryness with hot plate. Afterwards final volume was diluted to 20 mL with 0.1 mol/L HNO_3. The blank digests were carried out in the same way. Sample solutions were analyzed by ICP-OES.

RESULTS AND DISCUSSION

The concentrations of the elements, vitamins and fatty acid compositions in the Nigella seeds are presented in Tables 1-3. All data are the results of average of three measurements on each sample.

In the fatty acids profile of Nigella the main fatty acids were found as 66.5 and 23.5 (as relative % peak area) for linoleic acid (18:2) and oleic acid (18:1), respectively. Other fatty acids in Nigella were found as 5.4 % for (16:0), 2.7 % for (20:2), 1.1 % for (18:0), 0.8 % for (18:3) (Table-1). In fully ripened seeds, saturated fatty acids represent 6.5 % of Fatty acids, while unsaturated ones form 93.5 %. Ibtissem *et al*.[9] were found as 61.3 % for linoleic acid and 12.7 % for oleic acid in Nigella seeds from Tunisian. In other a study, levels of linoleic acids were determined as 50.3 % for Nigelle of Tunisia and 49.1 % for Nigella of Iran[10]. Nigella seeds from East Anatolian in term of levels of linoleic acid and oleic acid have been seemed richer than given literatures. As different these fatty acids profile, Al-Jassir was obtained as 0.18 % (14:1) and 1.08 % (24:0)[11].

TABLE-1
FATTY ACID COMPOSITIONS OF *Nigella Sativa* L.

Fatty acid	Relative % peak area
16:0	5.4
18:0	1.1
18:1	23.5
18:2	66.5
18:3	0.8
20:2	2.7
ΣUSFA	93,5
ΣSFA	6,5

ΣUSFA: Total unsaturated fatty acids, ΣSFA: Total saturated fatty acids

Nigella seeds are rich in term of linoleic acid. Hence, when it has been taken by through diets, it prevents cardiovascular disorders such as coronary heart diseases, atherosclerosis and high blood pressure[12].

Metal ions and metal complexes materials having an important role in vital functions of organisms. Because of this, various inorganic, organic, analytical and physical techniques are stated to be used for these substances synthesis, structures, formations, stabilities and analysis Zinc, Mn and Fe are important co-enzymes; Cu is bound to amino acids. Iron and cobalt are cause substantial catalytic activity and have been shown to be essential elements for nitrogen fixation in addition to their usefulness for the formation of vitamin B_{12}. Iron is an essential activator for enzymes catalyzing reactions involved in chlorophyll synthesis and for ferrodoxin nitrate reductase. Generally, the main characteristics of essential elements

depend on the regulatory mechanisms, which are able to keep the elements at the nutrition level[13].

Trace metal levels in the analyzed samples are listed in Table-2. Trace element concentrations were determined on dry weight as μg g^{-1} and the relative standard deviations were less than 10 % for all elements. The contents of Co, Ni, Fe, Zn, Cu, Mn and Cr were determined as 0.12, 1.48, 117.32, 41.42, 30.26, 28.56 and 2.55 μg/g (dry matter), respectively.

TABLE-2
LEVELS OF TRACE ELEMENTS *Nigella Sativa* L.
(μg/g DRY MATTER)

Co	Ni	Fe	Zn	Cu	Mn	Cr
0.12 ± 0.02	1.489 ± 0.125	117.32 ± 18.7	41.42 ± 5.8	30.26 ± 4.5	28.56 ± 2.5	2.55 ± 0.18

TABLE-3
LEVELS OF FAT –SOLUBLE VITAMINS
Nigella Sativa L. (μg/g DRY MATTER)

α-Tocopherol	δ-Tocopherol	Retinol	Vitamin D_2	Vitamin K_1	Vitamin K_2
10.19	2.28	0.18	1.38	1.85	2.15

Analysis of Nigella seed samples from other region of Turkey were found as 130, 56 and 18 μg/g for Fe, Zn and Cu, respectively[14]. In other a study, levels of Fe, Zn and Cu were determined as 8.6, 8.04 and 1.65 μg/g for Nigella of Tunisia, respectively and 9.42, 7.03 and 1.48 μg/g for Nigella of Irani, respectively[10].

These data are showed that Nigella seeds from east Anatolia contained significant amounts of important trace elements and *N. sativa* L. seeds are also a source of iron, copper, zinc, manganese and chrome. In addition, levels of trace element in Nigella seeds are critical components of many antioxidant and metabolism processes.

Vitamins are biologically active controlling agents for an organism's health and growth. These agents are often accompanied by an excess of compounds with similar chemical properties. Human diet should contain appropriate amount of vitamin for its essential functions. Systematic absence of vitamins in human diet can results in deficient growing and development[15].

The levels of vitamins were found as 10.19 μg/g for α-tocopherol, 2.28 μg/g for δ-tocopherol, 0.18 μg/g for retinol, 1.38 μg/g for vitamin D_2 1.85 μg/g for vitamin K_1 and 2.15 μg/g for vitamin K_2.

Iman *et al.*[16] were determined α-tocopherol and retinol levels of Nigella seeds in different country. It was found that α-tocopherol and retinol levels were 10.4 and 0.21 μg/g for Ethiopia, 11.3 and 0.27 μg/g for India, 9.52 and 0.20 μg/g for Saudi Arabia, 5.65 and 0.42 μg/g for Syria and 7.04 and 0.15 μg/g for Sudan, respectively. Found α-tocopherol and retinol levels by us are compatible with other studies in the literature.

Vitamin E (α-tocopherol) and vitamin A are lipid-soluble vitamins essential for human health. Both groups have free-radical-scavenging properties that allow them to function as physiologic antioxidants in protecting a number of chronic diseases such as cancer and cardiovascular disease[17].

Due to a lot of useful material in the composition of Nigella seed, it has antioxidant and anti-toxic effects. *In vitro* studies have been showed that Nigella seeds protect the cells against osmotic pressure, throat cancer, cortisol and lipopolysaccharide and protect liver against *t*-butyl hydroperoxide. In additional, Nigella seeds prevent observed internal bleeding and hemolysis as a results snake and scorpion poisoning and decreases levels of ALT and AST[18].

It was observed that the oil of Nigella decreased the production of nitric oxide in mices[18], prevented liver fibrosis in rabbits[19], decreased the toxicity of kidney from gentamycine and its protective role may be against methyonine[20]. Nigella decreased the level of lipid peroxidation in renal toxicity induced by $KBrO_3$[21].

Conclusion

Positive effects of *Nigella sativa* as results of studies in literature may be explained by synergy effects from its chemical compositions such as, vitamins, trace elements, fatty acids, anti-oxidants and other substances. For this reason, the plants used in the field of medicine chemical contents should be determined with multi-discipline studies performed together of analytical chemists, biochemists and microbiologists using various instrumental techniques. The data of our study may also be useful for the evaluation of nutritional information.

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