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The Fatty Acid Composition of *Nigella sativa* from Turkey

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Abstract

Nigella sativa, "black seed" or "black cumin" belongs to the Ranunculaceae family. It has been applied in folk medicine since ancient times. The phytochemical contents of black cumin are of interest to researchers in agriculture, pharmacology, and energy. Nowadays, it is so popular to be considered losing weight diets. In this study, the oil content and fatty acid composition of a commercial genotype of Nigella sativa L. from Turkey besides the fixed oil antioxidant activity were evaluated. Omega-6 fatty acid was the major fatty acid in the seed oil of studied black cumin, followed by Omage-9, free acid. However, the major composition of the oil was unsaturated fatty acids and could be considered a healthy fat for daily consumption. Moreover, the seed oil showed relatively high antioxidant activity by DPPH scavenging capacity.

Key words

Black cumin, Fatty acids, Antioxidants, Fat.

Introduction

The presence of medicinal plants in nature is one of the great blessings of God. Since man is a part of nature, nature has certainly provided medicinal plants for every disease. For this reason, the more human beings turn to nature and benefit from its blessings, the faster, better and more assuredly they will be treated for their illness. One of the magic medicinal plants which have been used throughout history is black cumin. Black cumin (Nigella sativa L.) is an important perennial medicinal and aromatic plant from the Ranunculaceae family and is one of the three commonly known species of the Nigella genus.

The plant, also known as "karamuk" and "siyah susam" in Turkish, is an annual and herbaceous plant that grows to about 20-30 cm and has black seeds. Among 12 different species of black cumin grown in the rich flora of Turkey, only Nigella sativa L. is cultivated (Baytop, 1984; Aslan, 2019).

The seeds of black caraway are widely used as spices besides their application in the food, medicine, and cosmetics industries (Baytöre, 2011).

Black cumin is a miraculous medicinal plant with pharmacological, biological, and therapeutic properties due to containing compounds such as fixed oils, essential oils, alkaloids, saponins, and proteins (Moghimi et al., 2018; Mukhtar et al., 2019). Based on previous studies, the seeds of black cumin have been accepted as an important medicine in Asian and Middle Eastern countries for centuries in traditional medicine and are useful for many diseases such as chronic headache, backache, hypertension, diabetes, infection, paralysis, and inflammation (Randhawa and Alghamdi, 2011; Yimer ve ark., 2019). Besides its seed oil which contains high levels of oleic, linoleic, and linolenic fatty acids has antioxidant, anti-inflammatory, anticancer, antidiabetic, antitumoral, antimicrobial, and immune-stimulating activities and gastroprotective properties. (Khan et al., 2011; Ahmad et al., 2013; Adam et al., 2016; Mazaheri et al., 2019). In Turkey, the plant is grown extensively in Mersin, Burdur, Istanbul, Gaziantep, Amasya, and Kahramanmaraş provinces in our country (Baytöre, 2011; Kılıç and Arabacı, 2016).

The antimicrobial property of black cumin is associated with the essential oil containing significant amounts of phenolic compounds (Ahmad et al., 2013). Black cumin contains essential oils such as thymoquinone, thymohydroquinone, carvacrol, dithymoquinone, 4-terpineol, p-cymene, t-anethole, sesquiterpene longifoline, α-pinene and various pharmacological active ingredients such as thymol (Ahmad et al., 2013; Gholamnezhad et al., 2015). Thymoquinone, the most abundant essential oil, is included in pharmacological toxicity studies due to its strong antioxidant, anti-inflammatory, and immune-strengthening capacity. Thymoquinone is also beneficial for liver health. In addition, nigelone present in black cumin prevents the release of histamine leading to reducing the allergic symptoms to a great extent. (Salem, 2005; Darakhshan et al., 2015).

Maideen (2020) reported that the antiviral, anti-inflammatory, antioxidant, immunomodulatory, bronchodilatory, antihistaminic, and antitussive activities of black cumin may make the plant a candidate for herbal treatment of COVID-19, the pandemic disease which caused the death of millions of people all over the World and affected the economy of World in the 21st century. The author also reported that nigellidine and α-hederin as the active compounds of N. Sativa showed an inhibitory effect on SARS CoV-2. N and anti-hypertensive, anti-diabetic, anti-obesity, anti-ulcer, anti-hyperlipidemic, and antineoplastic activities of N.sativa also would help the COVID-19 patients to relieve side effects of the disease in patients. Moreover, Sativa could be used as a side therapy along with offered conventional drugs to relieve the symptoms of COVID-19 in patients besides reducing the adverse effects of conventional medicines.

The fatty acid composition of the seed oil (32-40%) has been reported to consist of approximately 50% linoleic acid, 20% oleic acid, and 10% palmitic acid. Along with these, arachidonic, eicosadienoic, stearic, myristic, linolenic, and palmitoleic acids are some other fatty acids (Gharby et al., 2015; Aziz et al., 2017; Hosseini et al., 2019). The most popular monounsaturated fatty acid is oleic acid with unique characteristics in lowering blood cholesterol. Omega 6 and omega 3 (Linoleic acid and linolenic acid, respectively) are polyunsaturated fatty acids with proven effects on reducing the risks of blood pressure and vascular diseases (Hosseini et al., 2019). Therefore, black seed oil as a rich source of essential fatty acids is considered an extremely valuable and nutritious oil source to be an alternative to traditional oils.

Similarly, in different studies, health benefits have been reported because the fatty acids composition of pomegranate and fig seeds contain oleic acid, α-linolenic acid, linoleic acid, and punicic acid, and it has been emphasized that they should be included more in human diets (Ergun and Bozkurt, 2020; Bozkurt and Ergun, 2021).

In a study by Piras et al. (2013), linoleic acid was higher than other fatty acids in three Turkish and one Egyptian local breeds of N. Sativa. Based on previous studies, the essential oil composition and fatty acid profiles differ by region (Hosseini et al., 2019). Moreover, studies reported that N. sativa L. seed oil can be a good source of biodiesel replacing fossil fuel which is a big challenge for fighting global warming besides meeting increasing population needs. N. sativa L. seed oil had a conversion of more than 93% for transesterification with methanol (Aghabarari et al 2014; Khan et al 2015).

As black caraway is a precious plant with pharmaceutic properties and is consumed in daily health nutrition in Turkey, this study aimed to investigate the fatty acid composition of commercial genotypes of black caraway from Turkey.

Materials and methods

Plant Material

The seeds of *Nigella sativa* L. used in this study were provided by a commercial company. The analyses were done at 5 replicates and the approximately 450-gram seed was used.

Oil Extraction

An automatic soxhlet device was used to extract the total lipids. One hundred fifty grams of dried seeds were used for oil extraction. Hexane (Merck KGaA, Darmstadt, Germany) was used as a solvent. The oil percent in the samples was determined by weighting the extracted oil and expressed as g100g-1 of dry samples. The esterification of fatty acids was done by solving the 100 mg of oil in 10 mL hexane followed by adding 2 N KOH (David et al., 2003).

Determination of Fatty Acids

The fatty acids were analyzed by a GC ("Perkin Elmer, Shelton, USA"). Chromatographic separation was performed using a ("30 m × 0.25 mm ID, 0.25 μm film thickness DB-Wax") column equipped with a "flame ionization detector" (FID). The initial oven temperature was set at 50°C and after 1 min it was raised to 25°C every minute until 200°C, followed by raising 3°C per min to 230°C and was held for 18 min. The" injector" and the "detector" temperatures were set at 280°C and 250°C, respectively. The results were expressed in "GC area %" as a "mean value and ± standard deviation" (David et al., 2003).

Determination of DPPH radical scavenging capacity

"The free radical scavenging activity" of the seed extracts was analyzed using "2,2-diphenyl-1- picrylhydrazyl" (DPPH) assay (Kostiæ et al., 2013). The color loss of DPPH solution was measured at 517 nm scavenging the reaction of DPPH radical with the sample. One hundred microliters of methanolic extract of seed samples were reacted with fresh methanolic DPPH solution followed by incubating for 30 min at room temperature. Then, the absorbance was read at 517 nm against the blank. The ability of the extracts to inhibit DPPH (% RSC) was computed from the decrease in absorbance. The data were expressed as milligrams of Trolox equivalent (TE) "per 100 g of sample"x.

Results and discussion

The physicochemical and nutritional characteristics of each oilseed are influenced by its fatty acid composition. The total fat, fatty acid composition, and antioxidant activity of the studied black caraway are shown in Table 1. The total fat obtained from the seeds of black caraway was 39.5 g/100g. Hosseini et al. (2019) determined the total fat of 16 black caraway from different countries between 27.57-33.04 g/100g. Gharby et al., (2015) obtained the oil yield of black cumin 37% and 27% from solvent or cold press, respectively. Similarly, Atta (2003) compared "solvent" or "cold press" extraction methods and yielded 24.76% and 34.78% of oil content in black cumin, respectively. However, many studies on the total fat content of black cumin from different regions generally showed that the fat content differs in the range of %20-%30 (Burits and Bucar, 2000; Hamrounı-Sellamı et al., 2008; Cheikh-Rouhou et al., 2007; Sultan et al., 2009; Ali et al., 2012; Hosseini et al., 2019, Kiani et al., 2020). The results obtained in this study are the highest among results from previous studies. As reported solvent extraction method was more successful in the determination of total fat in black cumin as it was the preferred method in this study. The high amount of obtained total fat may be due to the extraction method with equipped soxhlet besides the genotype and location of the material.

Totaly 10 saturated and five unsaturated fatty acids were detected in the studied genotype of *Nigella sativa* L. The major fatty acids in *Nigella sativa* were found as linoleic, oleic, and palmitoleic acids (52.89%, 25.12%, and 13.62%), respectively. Approximately half percent of seed oils were polyunsaturated fatty acids mainly linoleic acid. The results are in agreement with previous studies (Burits and Bucar, 2000; Hamrounı-Sellamı et al., 2008; Cheikh-Rouhou et al., 2007; Sultan et al 2009; Ali et al., 2012; Hosseini et al. 2019; Kiani et al., 2020). A high concentration of linoleic acid is a determining indicator of the nutritional value of plant oils and has healthy effects by reducing serum cholesterol leading to a remedy for cardiovascular disorders (Al-Jassir, 1992; Keles, 2020; Guney, 2020; Ergun 2021). Most percentages of seed oil (80.86%) in studied *Nigella sat*iva L. were comprised of unsaturated fatty acids. Palmitoleic acid was found as the main SFA in the seed oil of black cumin. Likewise, measurable amounts of caproic acid (C6:0) an SFA, eicosenoic acid (C20:1n9c), cis-11,14-eicosadienoic acids (C21:2) MUFAs were detected. Pereira et al., (2014) reported that unsaturated fatty acids eicosenoic and cis-11,14-eicosadienoic acids had inflammatory effects. However, genetic factors (cultivar, variety grown), environmental conditions, seed quality (maturity, harvesting, or storage conditions), oil extraction methods, and esterification methods during the determination of fatty acids may be the cause of possible differentiation among results from different studies on oil content and fatty acid composition of *Nigella sat*iva L. (Ramadan and Mörsel 2002; Kiani et al., (2020).

Free radicals generated through routine metabolism pathways may be the basis of many human diseases (Gundesli 2020; Okatan et al., 2021). Antioxidants have the ability to scavenge free radicals by exchanging electrons (Gundesli et al., 2020; 2021). 4-terpineol,t-anethole, and thymoquinone carvacrol isolated from the essential oil of *N. sativa* have considerable free radical scavenging properties (Ali and Blunden, 2003). There are several studies on the antioxidant capacity of black cumin oil. Among various antioxidant capacity measuring assays, DPPH has been widely used in the scavenging assessment of free radicals due to being an easy method (Erkan et al., 2008; Scherer and Godoy, 2009). Erkan et al., (2008) reported that black cumin seeds inhibited DPPH radical formation as 515 of IC50 (μM) mean. Sultan et al (2009) also studied the antioxidant capacity of fixed and essential oils of black cumin via DPPH assay and reported their inhabitation strength of DPPH as 32.32% and 80.25%, respectively. In, this study, the DPPH antioxidant activity of black cumin was determined as 284.70 mg TE/100g. Al Turkmani et al (2015) reported that scavenging DPPH radicals of *N. sativa* essential oil showed a significant difference in samples from two different regions in Syria as 0.607 $mg^{-1}ml$ and 0.240 $mg^{-1}ml$. Although there are differences in the DPPH antioxidant activity among previous studies, the results are in agreement with each other based on affecting factors such as region, genotype, and extraction solvents.

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Table 1. Fatty acid composition, total fat, and antioxidant activity of *Nigella sativa* L.

Fatty Acids	**%**	
Caproic acid (C6:0)	0.10±0.01	
Caprylic acid (C8:0)	0.04±0.01	
Myristic Acid (C14:0)	0.36±0.01	
Pentadecanoic acid (C15:0)	0.03±0.00	
Palmitic acid (C16:0)	13.62±0.36	
Palmitoleic acid (C16.1)ω−7	0.17±0.00	
Margaric Acid (C17:0)	0.07±0.00	
Stearic acid (C18:0)	3.55±0.01	
Arachidic acid (C20:0)	0.21±0.00	
Behenic acid (C22:0)	0.05±0.01	
ΣSFA	**18.2**	
Oleic acid (C18:1n9c)ω−9	25.12±0.06	
Eicosenoic acid (C20:1n9c)ω−9	0.32±0.01	
cis-11,14-Eicosadienoic acid (C21:2)	2.33±0.04	
ΣMUFA	**27.77**	
Linoleic acid (Cl8:2n6c) ω−6	52.89±0.01	
a-Linolenic acid (C18:3n3) ω−3	0.20±0.01	
ΣPUFA	**53.09**	
Total Fat	**39.50**	g/100g
DPPH Antioxidant Activity	**284.70±8.90**	mg TE/100g

Conclusion

The present study revealed that the commercial genotype from Turkey has a high content of fat besides a good source of linoleic–oleic type fatty acids which are so important for a healthy diet in the cosmetic industry. The study supported the findings of previous studies on the rich phytochemical content of *N.sativa* showing that commercial genotypes also can be used in breeding programs. However, studies on trueness to the name of these genotypes besides the breeding of genotypes with higher content of bioactive compounds should be carried out

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

Z.E. and M.Z. contributed equally in fatty acid analyses and the writing of the manuscript. M.T.E helped in writing the manuscript. The authors contributed to the discussion of the results and all of them read and approved the final manuscript.

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