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## Health Benefits of the Volatile Components of Black Seed (*Nigella sativa*) Analyzed by GC-MS

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**Abstract:** Black seed (*Nigella sativa*) is an annual herbaceous plant belonging to the Ranunculaceae family. The most commonly used part of the plant is its seeds, which are highly valued due to their chemical composition. In this study, the volatile components obtained from black seed (*Nigella sativa*) samples collected from the Amasya and Yozgat regions were analyzed. The obtained essential oils were analyzed using the GC-MS method. According to the GC-MS analysis results, some volatile chemical compounds identified in black seed (*Nigella sativa*) and their potential applications in the health field are summarized below; Thymoquinone (p-cymene-2,5-dione) (% composition 11-15) : It has been shown to improve therapeutic indices when used in combination therapies with anticancer drugs and has contributed to preventing chemotherapy-induced damage in non-tumor tissues. Alpha-phellandrene (2-methyl-5-propan-2-ylcyclohexa-1,3-diene) ((%composition 3,4): Its essential oil exhibits antimicrobial activity and has been found effective against human breast cancer (Hs 578T) and prostate tumor (PC-3) cell lines. Beta-pinene (6,6-dimethyl-2-methylene-bicyclo[ 3.1.1]heptane) (%composition 0,03-0,07): Used as a natural insecticide and associated with certain biological activities; however, it has a high toxicity potential. Thymol (5-methyl-2-propan-2-ylphenol) (% composition 0,03-0,06): Utilized as an effective agent in reducing gingivitis and dental plaque formation. Limonene (1-methyl-4-prop-1-en-2-ylcyclohexene) (% composition 0,03): Animal studies have shown that it can slow the growth of various cancer cells such as those in the skin, lungs, stomach, and pancreas. However, there is still insufficient data to confirm these effects in humans.

## 1. Introduction

The essential oils of many different plants, known for their scientific and commercial benefits, are widely used. The primary sectors where essential oils are utilized include pharmaceuticals, food, cosmetics, and aromatherapy. Essential oils are generally in liquid form at room temperature; however, some plants such as rose and anise have essential oils that are not liquid under normal conditions. In terms of physical properties, essential oils are generally similar to each other. They are optically active due to their high refractive indices, and their specific rotations help in their identification. Typically, essential oils are colorless or pale yellow, but when exposed to air for extended periods, their color tends to darken. Their chemical and biological properties have attracted significant interest. Research has shown that essential oils play crucial roles in plant chemistry. They are known to participate in intercellular communication and have protective effects as part of plants' defense mechanisms against external factors. They are also found in some important hormones. Essential oils can be obtained from nearly every part of a plant, with seeds being particularly efficient sources.

Biological Effects of *Nigella sativa* and the Antimicrobial Properties of Its Essential Oil  
It has been demonstrated that essential oils exhibit antimicrobial activity, particularly against antibiotic-resistant pathogenic microorganisms, which are unable to survive in their presence and fail to develop resistance even through mutation. The genetic systems of bacteria are not capable of developing defensive mutations against the wide range of natural antibacterial terpenes found in essential oils. Biological Activities of *Nigella sativa* Protective Effects Against Free Radicals: The oil of *Nigella sativa* has shown protective effects against damage caused by free radicals, as confirmed by in vivo (laboratory-based) experiments. Consequently, *N. sativa* oil may be applicable in the treatment of conditions related to oxidative stress such as brain and cardiac anoxia, rheumatism, and cancer (Houghton, 1995). Antioxidant Activity: Oxidative stress, driven by free radicals, is known to play a role in the onset and progression of various diseases, including hypertension, cancer, and AIDS. Since *Nigella* seeds began to be used in the treatment of inflammatory conditions, their potential free radical scavenging capacity has become a subject of research. It has been shown that two primary components of the essential oil—fixed oil and thymoquinone—exert inhibitory effects on lipid peroxidation in liposomes (oxidative degradation of fats) (Woo, 2012). Analgesic Effect: In one study, the anti-inflammatory effect of *Nigella sativa* was evaluated in rats in which paw edema was induced with carrageenan and ear edema with castor oil. The tests demonstrated that *Nigella sativa* possesses significant analgesic properties. Notably, naloxone, an opioid antagonist, was unable to reverse the analgesic effect of *N. sativa* observed in the formalin test, suggesting a non-opioid mechanism of action and indicating the presence of a strong analgesic effect. Effects on DNA Damage: In a study investigating the protective and DNA repair-inducing effects of *Nigella sativa* aqueous extract and its active component thymoquinone against hydrogen peroxide-induced oxidative DNA damage, the alkaline comet assay (single-cell gel electrophoresis) was employed. The study revealed that treatments with *N. sativa* aqueous extract at dilutions of 1:10, 1:20, 1:40, and 1:80 significantly reduced DNA damage in *S. cerevisiae* cells. Effects on the Digestive System: *Nigella sativa* has demonstrated antioxidant and anti-ulcer activities under different experimental conditions. In a study investigating its effects on gastric acid secretion in isolated rat stomachs, it was found that aqueous extract of *N. sativa* at doses of 2 mg/100 mL DMSO and 5 mg/100 mL DMSO significantly reduced acid secretion stimulated by histamine, and the 5 mg/100 mL DMSO dose also reduced secretion induced by bethanechol. However, none of the tested doses affected acid secretion induced by pentagastrin. These findings suggest that *Nigella sativa* possesses gastroprotective properties by reducing gastric acid secretion. Effects on the Immune System: Both *Nigella sativa* oil and thymoquinone have been reported to enhance the proliferation of T

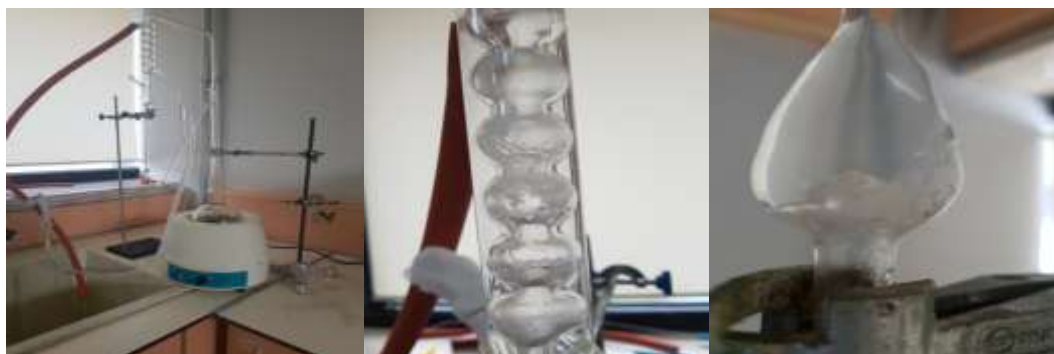
cells and cytotoxic cells involved in mediating the immune response, indicating a significant immunomodulatory effect.

## 2. Materials and Methods

The *Nigella sativa* plants used in this study, collected from the provinces of Amasya and Yozgat, were analyzed using GC-MS, as shown in Figure 1, to compare their volatile components. The chemical composition of *Nigella sativa* varies depending on factors such as the season of harvest, plant variety, and the geographical region in which it is cultivated. Previous studies have confirmed that the essential oil of *Nigella sativa* typically contains compounds such as  $\beta$ -pinene, p-cymene,  $\gamma$ -terpinene,  $\alpha$ -pinene, carvacrol, d-limonene, as well as thymoquinone, dithymoquinone, thymohydroquinone, and thymol. These compositions have been shown to differ significantly between regions and localities. Such variation is known to result from factors including the harvest season and cultivation methods. The most widely used method for obtaining volatile components is hydrodistillation. The best results are generally obtained from powdered samples. The principle of the method involves placing a specific amount of the sample—taken from the seed, fruit, or peel of the plant—into a round-bottom flask connected to a condenser. The plant material is then boiled for 2 to 8 hours. The volatile oil is obtained by condensing the oil molecules carried by the steam and separating them from the water. This is a traditional method commonly employed for small-scale production of volatile compounds.

For the Clevenger extraction, *Nigella sativa* samples collected from Amasya and Yozgat were first ground into a fine powder using a ball mill. Subsequently, 100 grams of each sample were accurately weighed using an analytical balance. Each weighed sample was placed into a round-bottom flask along with boiling chips, followed by the addition of 300 mL of water. The samples were then subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus. Once boiling commenced, the process was monitored, and the essential oils were collected into vials. The collected oils were then prepared for analysis by GC-MS.

Solvent extraction is a traditional method in which plant material is either directly immersed in a solvent at room temperature or subjected to reflux with an organic solvent in a Soxhlet apparatus (Zhang, 2014). For this study, 50 grams of *Nigella sativa* samples obtained from Amasya and Yozgat were accurately weighed using an analytical balance. Each sample was placed into a round-bottom flask, followed by the addition of 200 mL of hexane, and extracted for 8 hours using a solvent extraction apparatus. Hexane was used as the solvent in this study. At the end of the extraction, the organic solvent was removed and recovered from the mixture via distillation. The remaining oil phase contained the volatile compounds of interest. A known disadvantage of this method is the potential loss of low-molecular-weight volatile compounds during the concentration step.



**Figure 1.** Clevenger and Soxhlet extraction setup

**Table 1.** GC-MS Instrument Specifications

<b>Detector</b>	Agilent 5973 mass selective detector
<b>Column</b>	HP-5 capillary column, 0,25 mm x 60 m x 0,25 µm
<b>Injection temperature</b>	260°C
<b>Temperature program</b>	With a heating rate of 5°C/minute, it rises from 60°C to 150°C, and after waiting at this temperature for 10 minutes, it increases again by 3°C/minute to 260°C.
<b>Ion source temperature</b>	200 °C
<b>Carrier gas</b>	Helium
<b>Gas flow rate</b>	1,7 mL/min
<b>Injection volume</b>	2 µL

### 3. Results and Discussion

In the black cumin (*Nigella sativa*) sample from Yozgat province,  $\alpha$ -phellandrene, valencene,  $\beta$ -caryophyllene, eugenol, palmitic acid, stearic acid, myristic acid, decanoic acid, oleic acid, and linoleic acid were detected at levels above 1% using the Clevenger extraction method, as presented in Table 1. Thymol and carvacrol, which are among the main components of black cumin, were also observed. Among the fatty acids, the monounsaturated fatty acid oleic acid was found at a concentration of 11.30%. These findings indicate that the sample is rich in fatty acids. The total fatty acid content was 26.96%, while the eugenol content was 17.60%. Thymoquinone could not be obtained using this method.

**Table 2.** GC-MS results of Clavenger extraction of black cumin from Yozgat province

<b><u>Rt(Retention time)</u></b>	<b><u>Component Name</u></b>	<b><u>% Composition</u></b>
6.019	Alpha pelledren	3,29
8.222	Beta pinene	0,65
8.222	Alpha pinene	0,65
8.703	Sabinene	0,49
11.398	Alpha Terpinene	0,07
12.336	Limonene	0,73
14.350	Gamma Terpinene	0,14
14.350	Delta-3-Karene	0,14
22.967	Longipinen	0,43
25.783	Valensene	2,97
26.418	Beta Caryophyllene	1,60
28.220	Alpha Caryophyllene	0,31
29.542	Carvone	0,18
31.945	Tridecanone	0,04
37.747	Tetradecanal	0,15
50.479	Eugenol	17,60
55.079	Isoeugenol	0,97
53.111	Carvacrol	0,31
53.111	Thymol	0,31
55.606	Butyric Acid	0,10
56.304	Cyclododecene	0,31
68.863	Furoxane	0,77
69.733	Palmitic Acid	7,13
74.385	Stearic Acid	2,84
74.385	Myristic Acid	2,84
74.385	Decanoic Acid	2,84
75.186	Oleic Acid	11,30
76.605	Linoleic Acid	2,75

In the black cumin (*Nigella sativa*) sample from Amasya province, hexacosane, furoxan, linolenic acid, palmitic acid, stearic acid, and oleic acid were detected at concentrations above 1% based on the Clevenger extraction method, as shown in Table 2. The amount of the saturated fatty acid palmitic acid was found to be 8.95%. These results indicate that the sample is rich in saturated fatty acids. Thymoquinone was not obtained using this method.

**Table 3.** GC-MS results of Clavenger extraction of black cumin from Amasya province

Rt(Retention time)	Component Name	% Composition
15.249	Meta Simen	0,06
24.284	Linalool	0,02
26.441	Alpha - Caryophyllene	0,01
28.856	Hexacosene	0,02
32.226	Cuminal Aldehyde	0,09
57.683	Hexacocene	3,59
58.696	Decosene	0,39
60.372	Eicosen	0,49
62.507	Linoleic Asid	7,40
64.246	Octacosene	0,67
64.709	Trtriacontane	0,59
66.872	Linolenic Asid	6,79
69.733	Palmitic Asid	8,95
71.072	Furoxan	4,97
74.317	Stearic Asid	2,36
75.089	Oleic Asid	6,61

**Table 4.** GC-MS results of Soxhlet extraction of Yozgat black cumin

Rt(Retention time)	Component Name	% Composition
6.002	Alpha Tujen	0,28
8.171	Beta Pinene	0,07
12.285	Limonene	0,03
15.117	p-simene	0,88
23.019	Alpha Caryophyllene	0,01
25.823	Longifolene	0,12
41.129	Thymoquinone	15,02
52.659	Thymol	0,03
52.659	Carvacrol	0,03
56.126	Tetradecadiene	0,02
59.725	Butyric Asid	0,03
62.466	Methyl Linoleate	0,21
69.544	Palmitic Acid	11,14
74.980	Oleic Acid	19,48
76.600	Linoleic Acid	31,66
78.373	Linolenic Acid	1,96
86.007	Furoxan	0,88
92.764	Erucic Asid	2,31

According to Soxhlet extraction results presented in Table 3, the black cumin (*Nigella sativa*) sample from Yozgat province contained the following compounds in concentrations above 1%: palmitic acid (11.14%), oleic acid (19.48%), linoleic acid (31.66%), linolenic acid (1.96%), and erucic acid (2.31%). In addition, thymol and carvacrol—known as major components of black cumin—were detected in low amounts. The total fatty acid content was found to be higher compared to the components obtained through Clevenger extraction. Specifically, the total fatty acid content reached 66.58%, whereas it was 26.96% with the Clevenger method. Thymoquinone was successfully obtained using the Soxhlet method at a concentration of 15.02%, whereas it was not detected using the Clevenger extraction method. As shown in Table 4, the black cumin sample from Amasya province analyzed via Soxhlet

extraction contained the following compounds in amounts above 1%: thymoquinone (11.08%), myristic acid (1.48%), methyl linoleate (36.62%), linolenic acid (1.82%), eicosenoic acid (1.89%), linoleic acid (3.39%), palmitic acid (12.67%), and oleic acid (27.81%). Thymol and carvacrol were also observed in low quantities. Similar to the Yozgat sample, the total fatty acid content was higher than that obtained through Clevenger extraction, reaching 50.02% compared. Characteristics of Some Compounds Found in *Nigella sativa* (Black Seed) Identified by GC-MS Analysis

Thymoquinone (p-cymene-2,5-dione, TQ): Thymoquinone is the main active component of the volatile oil derived from *Nigella sativa* seeds. It selectively targets abnormal cells while sparing normal ones, and exhibits anti-neoplastic and anti-inflammatory effects. TQ enhances the body's defense mechanisms under adverse conditions and influences immune function. Although its therapeutic potential in humans has not yet been clinically tested, numerous studies have demonstrated promising anti-cancer effects in animal models. Combined use of TQ with clinically approved chemotherapeutic agents has shown improvements in treatment outcomes and protection of non-cancerous tissues from chemotherapy-induced damage (Gali-Muhtasib, 2006). Early laboratory studies conducted on mice have explored the effects of TQ in diseases such as diabetes, asthma, and cancer. TQ has been shown to act as a free radical and superoxide radical scavenger, and helps preserve the activity of antioxidant enzymes including catalase, glutathione peroxidase, and glutathione-S-transferase. TQ's anti-cancer properties are mediated through various mechanisms including cell regeneration, apoptosis (elimination of abnormal cells), reactive oxygen species (ROS) modulation, and inhibition of cancer cell metastasis. It has demonstrated anti-tumor effects in colon, prostate, pancreatic, and lung cancer models. TQ's antioxidant, anti-inflammatory, and anticancer activities suggest a potential therapeutic role in cancer treatment (Woo, 2012). In rat studies, TQ and calcium ionophore were administered, revealing that the ionophore prevents lipid peroxidation, while TQ inhibited non-enzymatic peroxidation in phospholipid liposomes approximately ten times more effectively. The pharmacological properties of the oil support its traditional use in the treatment of rheumatism and inflammatory disorders (Houghton, 1995). Laboratory analyses comparing TQ and the synthetic antioxidant tert-butylhydroquinone (TBHQ) showed both compounds effectively prevent lipid oxidation. TQ, however, was more active than TBHQ in scavenging superoxide anions. These findings highlight the strong antioxidant potential of both agents due to their radical-scavenging capacity, with TQ acting primarily as a potent superoxide scavenger (Badary et al., 2003).

The protective effects of TQ against doxorubicin (DOX)-induced nephrotoxicity in rats were also investigated. TQ treatment reduced triglycerides (TG), total cholesterol (TC), and lipid degradation in renal tissues. These results underscore TQ's high antioxidant potential and its protective role against DOX-induced nephropathy. Additionally, *Nigella sativa* has demonstrated analgesic activity comparable to aspirin, though it lacks antipyretic effects (Badary et al.2000).

Numerous studies have confirmed the anticancer properties of TQ across various cancer cell lines, mediated through complex molecular mechanisms. During cancer progression, free radicals attack and damage healthy cells (oxidative stress), which TQ counteracts via antioxidant and anti-inflammatory pathways. By enhancing antioxidant enzyme activity, TQ mitigates ROS-induced oxidative DNA damage, thereby reducing cancer risk. TQ has also been reported to exert resistance-modulating activity when combined with anticancer drugs such as DOX and etoposide. In rats, TQ suppressed the initiation and progression of gastric cancer. Its antitumor activity has been found comparable to 5-fluorouracil in colon cancer treatment, particularly in the SW-626 colon cancer cell line. Proteins from *N. sativa* have also been shown to significantly increase total lymphocyte counts, indicating immunomodulatory effects in both

humans and animals. A study in mice showed that *N. sativa* administration prevented decreases in hemoglobin and leukocyte levels, with a 3.2% increase in total leukocyte count observed.

**Table 5.** GC-MS results of Soxhlet extraction Amasya black cumin

Rt(Retention time)	Component Name	% Composition
8.651	Beta Terpinene	0,03
11.369	Alpha Terpinene	0,01
12.325	Limonene	0,03
14.293	Gama Terpinene	0,05
15.146	p-simene	0,76
25.840	Longifolene	0,10
32.140	Anethole	0,02
42.130	Thymoquinone	11,08
52.625	Thymol	0,06
52.625	Carvacrol	0,06
56.126	Tetradecadiene	0,02
61.476	Methyl Elaidate	0,06
65.808	Myristic Acid	1,48
69.596	Palmitic Acid	12,67
75.152	Oleic Acid	27,81
76.834	Methyl Linolelaidate	36,62
78.419	Linolenic Acid	1,82
80.662	Arachidic Acid	0,96
81.709	Eicocenic Acid	1,89
83.970	Linoleic Acid	3,39

**Alpha-Phellandrene:** Alpha-phellandrene is a monoterpene found in allspice and is known for its pleasant aroma. Approximately 50% of childhood cancers are lymphomas and leukemias, with leukemia being the second most common type. The majority of anticancer drugs used in clinical settings are derived from natural plant products. Alpha-phellandrene ( $\alpha$ -PA), an essential oil component reported in cancer research, has demonstrated antimicrobial activity. Recent studies have shown that  $\alpha$ -PA exhibits cytotoxic effects against human breast cancer (Hs 578T) and prostate tumor (PC-3) cell lines. Several reports support the classification of  $\alpha$ -PA as a biologically active compound( Lin et al., 2014).

**Beta-Pinene:** Beta-pinene is a flavor-aroma compound and a monoterpene found in allspice, ginger, nutmeg, bitter fennel, rosemary, and sage. The antimicrobial activities of the  $\alpha$ - and  $\beta$ -isomers of pinene were evaluated using the agar diffusion test against bacterial and fungal cells. Both  $\alpha$ -pinene and  $\beta$ -pinene were found to exhibit significant toxicity. In plants, pinenes have demonstrated fungicidal activity. Pinenes have been used for centuries in the production of flavors and fragrances and are also associated with various biological activities, including their use as natural insecticides (Rivas da Silva et al., 2012).

**Alpha-Pinene:** Alpha-pinene is known to cause irritation to the nose and throat and has been found to be an irritant when ingested, inhaled, or absorbed through the skin. Symptoms of exposure may include a burning sensation, coughing, wheezing, shortness of breath, headache, nausea, and vomiting. Pinenes are found in essential oils of coniferous trees (such as pine) and herbs like rosemary. These compounds exist as non-superimposable optical isomers or enantiomers, which differ only in their interaction with polarized light. The enantiomers may exhibit different toxicological and biological activities. Pinenes are monoterpenes with two active structural isomers,  $\alpha$ - and  $\beta$ -pinene, with  $\alpha$ -pinene being more widely studied and more prevalent in European pines. It is also commonly found in North America (Rivas da Silva et al., 2012).

**Sabinene:** Sabinene is a monoterpene that accumulates in small amounts in natural organisms. It is considered a potential component for next-generation jet fuels. Biosynthetic pathways are being developed to produce sabinene from sugars. Sabinene has also been tested for its antimicrobial properties and has shown antibacterial and antifungal activities. In rats, sabinene exhibited anti-inflammatory effects against paw edema (Zhang et al., 2014) .

**Gamma-Terpinene:** Gamma-terpinene is a monoterpene naturally present in the essential oils of various plants, including species of the *Eucalyptus* genus, and has been reported to possess anti-inflammatory activity. It is one of three isomeric hydrocarbons, is naturally occurring, and has been isolated from various plant sources. Gamma-terpinene possesses strong antioxidant activity and a lemon-like aroma, and is widely used in the food, flavor, soap, cosmetic, pharmaceutical, tobacco, confectionery, and perfume industries. In a mouse model, gamma-terpinene pretreatment was administered before inducing paw edema with histamine and thickening agents. Results showed a reduction in paw swelling within 1–24 hours after treatment. In another mouse model, acute liver injury was induced, and gamma-terpinene treatment led to increased leukocyte production compared to untreated controls. These findings suggest that gamma-terpinene enhances cell migration to the site of inflammation and exhibits potent anti-inflammatory properties across different models of inflammation(Ramalho et al., 2015).

**Alpha-Terpinene:** Alpha-terpinene is a flavoring agent extracted from the essential oils of cardamom (a member of the Zingiberaceae family) and marjoram. As a monoterpene,  $\alpha$ -terpinene is used as a fragrance compound and is found in various essential oils. Upon exposure to open air, it is known to form allergenic compounds more readily than other monoterpenes. It is considered a true antioxidant and contributes to the antioxidant activity of tea tree oil. However, due to its potential allergenic effects, its suitability for use in cosmetics and skincare products may be questioned. Structurally, it is similar to limonene (Rudbäck et al., 2012).

**Delta-3-Carene:** Delta-3-carene is a bicyclic monoterpene composed of cyclohexane and cyclopropane rings. Cyclopropane is inherently toxic and environmentally hazardous. Some volatile organic compounds (VOCs) that contain such structures exhibit high levels of toxicity. VOCs are commonly present in construction materials such as wall paints, varnishes, and floorings used in homes and workplaces. Even at low concentrations, VOCs and formaldehyde can cause neurological symptoms such as drowsiness, headaches, and fatigue, and chronic exposure may lead to carcinogenic effects. Prolonged exposure to low concentrations of VOCs can also contribute to respiratory diseases and asthma.

**Limonene:** Limonene, a monoterpene, is most abundantly found in lemon peel and is also present in the rinds of other citrus fruits. Epidemiological observations suggest that in regions with extensive citrus cultivation, the incidence of cancer and cardiovascular diseases is lower, although limonene has not been proven to cure such diseases. Studies have shown that limonene promotes wound healing and reduces tumor size. Laboratory experiments also indicate that limonene induces programmed cell death (apoptosis) in abnormally proliferating cancer cells. Animal studies have demonstrated that limonene can slow the growth of cancer cells in the skin, lungs, stomach, and pancreas. However, these results are limited to animal models. Limonene is listed in the Code of Federal Regulations for use in perfumes, soaps, foods, chewing gum, and beverages, and is generally recognized as safe (GRAS) for flavoring purposes (Sun, 2007). According to the GC-MS analysis results, some volatile chemical compounds identified in black seed (*Nigella sativa*) and their potential applications in the health field are summarized below; Thymoquinone (p-cymene-2,5-dione): It has been shown to improve therapeutic indices when used in combination therapies with anticancer drugs and has contributed to preventing chemotherapy-induced damage in non-tumor tissues. Alpha-

phellandrene (2-methyl-5-propan-2-ylcyclohexa-1,3-diene): Its essential oil exhibits antimicrobial activity and has been found effective against human breast cancer (Hs 578T) and prostate tumor (PC-3) cell lines. Beta-pinene (6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane): Used as a natural insecticide and associated with certain biological activities; however, it has a high toxicity potential. Thymol (5-methyl-2-propan-2-ylphenol): Utilized as an effective agent in reducing gingivitis and dental plaque formation. Limonene (1-methyl-4-prop-1-en-2-ylcyclohexene): Animal studies have shown that it can slow the growth of various cancer cells such as those in the skin, lungs, stomach, and pancreas. However, there is still insufficient data to confirm these effects in humans.

**Beta-Caryophyllene:** Beta-caryophyllene is a natural bicyclic sesquiterpene found in the essential oils of clove, rosemary, and hops. It is notable for containing both a cyclobutane ring and a rare trans-double bond in a nine-membered ring, both of which are uncommon in nature.  $\beta$ -Caryophyllene is a sweet and dry flavor compound present in food items such as allspice, fig, potted marjoram, and Roman chamomile, making it a potential biomarker for the consumption of these foods. It can also be detected in human feces and saliva.

**Hexanoic Acid:** Also known as caproic acid, hexanoic acid has a strong odor. In a study, hexanoic acid was found to protect tomato plants against *Botrytis cinerea* (grey mold). A glycosidic derivative of adipic acid monoethyl ester and 1,2,3,4-tetra-O-acetyl- $\beta$ -D-glucopyranose was shown to protect tomato plants against *Alternaria solani* and *Phytophthora citrophthora*. In pepper plants, acid-derived resistance was observed against *A. solani*.

**Carvacrol:** Carvacrol is a derivative of p-cymene hydride. Cyclophosphamide (CP) is a widely used chemotherapeutic agent for the treatment of cancer and malignant tumors in clinical settings. However, the use of CP is associated with toxicity in one or more tissues. According to the results of various studies, carvacrol has been shown to mitigate the toxic effects of CP and may help prevent tissue damage occurring during and after chemotherapy. Carvacrol is present in the structure of certain pharmaceutical formulations and is used therapeutically for a wide range of conditions. It has been employed in the treatment of influenza, throat and ear pain, lung infections, infectious diseases, pain from injuries, headaches, meningitis, allergies, bronchitis, diarrhea, dental and gingival diseases, sinusitis, asthma, aphthous ulcers, eczema, nerve-related pain, fatigue, prostatitis, parasitic infections, back pain, psoriasis, skin rashes, colitis, insect bites, gastritis, fungal diseases, and as an antibacterial agent in meat products.

**Thymol:** Thymol is a terpene compound found in thyme oil. The herb *Origanum vulgare* and related species are known for their characteristic thyme aroma. Thymol is frequently used as a flavoring agent in products characterized by a "Mediterranean taste." It has limited solubility in water but dissolves readily in alcohols and oils. Beyond its flavoring use, thymol is known for its antifungal properties. It is also used to reduce bacterial plaque in gingivitis treatment. Thymol is effective against bacteria and fungi, and is included at concentrations of about 1% in deodorizing formulations for use in the mouth, throat, and vaginal area. In general, during the autoxidation of lipids at room temperature, thymol is a more effective and active antioxidant than carvacrol (Yanishlieva et al., 1999). Thymol is a phenolic compound commonly used to combat oral bacteria. Although limited data are available on its effects on microorganisms, ongoing studies suggest that thymol reduces glucose uptake by bacterial cells (Evans, 2000). The use of microencapsulated thymol and carvacrol in polymer films for food packaging has been shown to be suitable (Guarda et al., 2011). Thymol is also used as a preservative in pharmaceuticals and as a flavor enhancer in food. Thymol exhibits antifungal activity and has been evaluated for genotoxic and cytotoxic effects in both mammalian cells and microorganisms. Depending on the dose, thymol has been shown to completely inhibit the

viability and proliferation of certain cell types. In DNA repair assays, thymol displayed clear dose-dependent toxicity. At lower concentrations, thymol reduced DNA damage, whereas at approximately 0.2 mM it caused significant DNA strand breaks, while concentrations around 0.1 mM did not induce noticeable damage, as demonstrated by gel electrophoresis.

**Alpha-Caryophyllene(Humulene):** Alpha-caryophyllene, also known as humulene, is a naturally occurring sesquiterpene. Studies have investigated the anti-inflammatory properties of two sesquiterpenes isolated from essential oils rich in  $\alpha$ -humulene (caryophyllene). Oral treatment with both compounds demonstrated significant inhibitory effects in mice and rats, with different responses observed between species. In certain studies conducted on mice, caryophyllene induced mortality and symptoms such as reduced activity, lethargy, and impaired coordination, particularly in females compared to males. Despite these observations, caryophyllene exhibits low toxicity, poses no mutagenic risk, and does not cause skin irritation in humans (Fernandes et al., 2007).

**Alpha-Thujone:** Alpha-thujone is a monoterpene that was a popular yet toxic compound with known adverse health effects during the 19th and early 20th centuries. It also serves as an active ingredient in certain herbal medicines. It is recognized for its insecticidal activity (Höld et al., 2000).

**p-Cymene:** p-Cymene is an aromatic organic compound used as a flavor enhancer and preservative in pharmaceuticals and food products. Studies evaluating its genotoxic potential—specifically its ability to cause damage to DNA, chromosomes, or nuclear structures—have shown that p-cymene does not exert insecticidal or genotoxic effects.

**Linalool:** Linalool is a naturally occurring terpene alcohol found in numerous aromatic plants and used in many commercial applications for its pleasant fragrance. It is produced by over 200 plant species, including those from the Lauraceae (e.g., bay laurel, cinnamon, rosewood), Rutaceae (e.g., citrus), and Lamiaceae (e.g., mint, aromatic herbs) families, across tropical and temperate regions. Linalool and linalyl acetate are known to be the principal volatile constituents of these species. Essential oils rich in these compounds are used in traditional medicine systems for their relaxing effects. Studies conducted on mice have demonstrated that linalool exhibits pharmacological and psychopharmacological activity, with dose-dependent sedative effects on the nervous system (Peana et al., 2002).

**Furoxan:** Furoxan is a nitric oxide donor. Due to this property, furoxan and its derivatives are actively investigated as potential new pharmaceuticals and insensitive high-density explosives (Gasco et al., 2004).

**Oleic Acid:** Oleic acid is an octadec-9-enoic acid with a Z (cis) double bond at the C-9 position. Chemically, it is classified as a monounsaturated omega-9 fatty acid. It is the most abundant fatty acid in the human body, accounting for approximately half of the fatty acid content. Oils rich in oleic acid are considered highly beneficial for health. It is primarily used in the soap industry, followed by medical, leather, and textile applications. Research has shown that as a monounsaturated fatty acid, it helps reduce the risk of hypertension, balances cholesterol levels, and provides protection against cardiovascular diseases. Furthermore, oleic acid reduces insulin demand in diabetic patients and may have protective effects against certain types of cancer. When treated with small amounts of nitrous acid ( $\text{HNO}_2$ ), oleic acid is used in the production of solid elaidic acid. Clinical studies involving the combination of oleic acid and erucic acid (as in Lorenzo's oil, containing 20% erucic acid and 80% oleic acid) have shown significant reductions in platelet counts in some patients. Oleic acid is synthesized in the endoplasmic reticulum (Aparna et al., 2012). Its triglycerides constitute the majority of olive oil. However, free oleic acid imparts a rancid taste to olive oil. It is also abundant in various animal fats. Interestingly, oleic acid is secreted by decomposing corpses of bees and

*Pogonomyrmex* ants, triggering a behavioral response in live workers to remove the dead bodies from the hive. When live insects are coated with oleic acid, they are mistakenly identified as dead and are dragged away accordingly.

**Erucic Acid :** Erucic acid is a very long-chain monounsaturated fatty acid consisting of 22 carbon atoms and a single double bond. Patients treated with erucic acid should be closely monitored by platelet count determination. It has been suggested as a potential therapeutic agent in the treatment of Alzheimer's disease. Findings indicate that erucic acid may inhibit the mitochondrial oxidation of harmful byproducts resulting from the metabolism of other fatty acids (Christophersen, 1972).

*Nigella sativa* seeds are traditionally used for a variety of purposes, including as an appetite stimulant, galactagogue, menstrual regulator, jaundice remedy, carminative, diuretic, bronchodilator, and immune system enhancer. When finely ground and mixed with honey, the seeds are used in folk medicine; the oil is reported to be applied in the treatment of alopecia areata and hemorrhoids. Research has demonstrated that black seed exhibits cytotoxic effects against various cancer cell lines and stimulates the production of tumor-specific antibodies. Furthermore, it has been found to increase both the number and activity of macrophage cells. Due to its rich content of bioactive constituents commonly found in medicinal plants, *Nigella sativa* is considered an important plant in traditional and modern medicine.

Interpretation of GC-MS results of Clavenger extraction of Amasya black cumin: It is observed that it has a rich structure in terms of fatty acids, long-chain hydrocarbons, and storage components. Volatile and Aroma Compounds (Low Percentage) Meta Cymen (0.06%) Linalool (0.02%)  $\alpha$ -Caryophyllene (0.01%) Cuminal Aldehyde (0.09%) These are found in plant-derived oils. They have aroma, odor, and biological activity (antimicrobial, antioxidant) potential. Since their percentages are low, they do not determine the main character of the sample, but they support its plant origin. Unsaturated and Saturated Fatty Acids (Main Fraction) Linoleic Acid (7.40%) – Omega-6 Linolenic Acid (6.79%) – Omega-3 Oleic Acid (6.61%) – Omega-9 Palmitic Acid (8.95%) Stearic Acid (2.36%) The sample contains a high percentage of fatty acids. It is quite high in unsaturated fatty acids (linoleic, linolenic, oleic). It has nutritional value, antioxidant and biological activity, and potential for cosmetic and pharmaceutical use. The presence of palmitic and stearic acids indicates structural and energy storage properties. Long-chain hydrocarbons Hexacosene (3.59%), Octacosene (0.67%), Decosene (0.39%), Eicosene (0.49%), Tritriacontane (0.59%) show that black cumin has a hydrophobic, stable, and oxidation-resistant structure. It may be important in cosmetic and coating applications. Notable compound Furoxan (4.97%) Furoxan derivatives are known for their biological activity (NO release, antimicrobial/anticancer research). It is rarely found in natural products; it is rich in fatty acids and valuable for its unsaturated fatty acids, with potential for nutritional, cosmetic, and pharmaceutical use.

Interpretation of Soxhlet extraction and GC-MS results of Amasya black cumin: The results show that black cumin is quite rich in volatile components and has a distinct chemical character in terms of fatty acid profile. Volatile Components (Low Retention Time – Aroma and Biological Activity): Components observed at low retention times (8–15 min) are generally volatile terpenes:  $\beta$ -Terpinen,  $\alpha$ -Terpinen, Limonene,  $\gamma$ -Terpinen. Found in low concentrations but contribute to the aromatic profile and antioxidant activity. p-Cymene (0.76%): Terpene derivative; known for its antimicrobial and anti-inflammatory effects. Longifolene (0.10%): Woody aroma, plant-derived component. This group indicates that black cumin has volatile oil characteristics, but they are not dominant. Phenolic and Biologically Active Components Thymoquinone (11.08%) The most notable component: Powerful antioxidant, anti-inflammatory, antimicrobial and potential anticancer effects. This percentage indicates that

*Nigella sativa* (black cumin) has a high pharmacological value. Thymol (0.06%) & Carvacrol (0.06%) Phenolic monoterpenes possess antiseptic and antimicrobial properties. Fatty Acid Profile (High Rt – Lipid Content) The sample is clearly rich in lipids: Unsaturated Fatty Acids (Dominant) Methyl Linoleate (36.62%) Oleic Acid (27.81%) Linoleic Acid (3.39%) Linolenic Acid (1.82%) It contains over 70% unsaturated fatty acids in total. This is a very positive profile in terms of nutritional value, cardiovascular benefit, and antioxidant capacity. Saturated Fatty Acids Palmitic Acid (12.67%) Myristic Acid (1.48%) Arachidic Acid (0.96%) The ratios are balanced, not excessively high. According to this GC-MS profile, black cumin seeds: possess high biological activity, are classified as a functional oil/herbal extract, and are suitable for pharmaceutical, nutraceutical, or food supplement use. They are particularly valuable due to their thymoquinone and unsaturated fatty acid content. The results for black cumin seeds from Yozgat show similar characteristics.

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