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IN VITRO CYTOTOXIC EFFECTS AND QUALITY CONTROL STUDIES ON BLACK CUMIN SEED CAPSULES AND OILS IN TURKEY MARKET

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Abstract:

Objective

Black cumin seed is a species of Nigella sativa from the Ranunculacea family. thymoquinone, which is most important components of black cumin seed oil, is preferred as a traditional source of healing considering its potential medicinal properties.

Methods

Black cumin seed oil and soft capsules with different formulations produced by different companies in our country were purchased, quality control tests, in vitro cytotoxicity tests and antioxidant tests were performed on these oils and capsules.

Results

The highest amount of thymoquinone was seen in black cumin seed fat 2 (O2) (2.9728 mg / ml), while the least amount of thymoquinone was in soft capsule 1 (SC1) group (0.8917 mg / ml). According to the cell viability results obtained by MTT test, O2 experimental group had the highest cytotoxic effect with 90.21%, while SC1 experimental group had the lowest cytotoxicity with 94.98%. When the antioxidant values were examined, the highest value (913,363 μg GAE / mg) was found in fat 1 (O1) and the lowest value (180,181 μg GAE / mg) was obtained in SC1.

Conclusion

Our study suggests that the cytotoxic effect of black cumin seed can be a promising aspect, especially in the development of new treatment methods and anti-cancer drugs.

Keywords: black cumin seed oil, soft capsule, cytotoxicity, quality control studies

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Introduction:

Today, the rise in consumption of natural origin products, food additives, and the use of various cosmetic and pharmaceutical preparations are frequently used, many medicinally effective plant species have an important share in the world market, which is constantly increasing. Few plant species cultivated in our country, some of them are in the nature of herbal drugs and some of them are medicinal and aromatic plants. However, the use of medicinal and aromatic plants in our country is limited (1). However, in many European countries, these plants are used either as wellness products or in food supplements. Black cumin seeds have been used in Middle East Asia and

Africa for a long time, in recent years, in Europe, rather than treating diseases, it is used for prevention, supporting healthy living and improving the quality of life, active and healthy aging. Black cumin seeds vary according to the climate conditions and include essential oils, fixed oils, proteins, amino acids, alkaloids, tannins, saponins, fibers, carbohydrates, minerals, ascorbic acid, thiamine, niacin, pyridoxine and folic acid. Fixed oil of black cumin seed, oleic acid, linoleic acid, eicosadienoic, arachidonic acid and linolenic acid from unsaturated fatty acids; saturated fatty acids include myristic acid, palmitic acid and stearic acid. Essential oil contains nigellone, carvacrol, p-cymene, d-limonene, α and β-pinene and thymoquinone, dithioquinone, thymohydroquinone and thymol which are the most important pharmacologically active components (2,3). The antioxidant properties of essential oils in the structure of black cumin seed were investigated and it was found that these were the free radical scavengers of carvacrol, anethole and 4-terpineol, especially thymoquinone. In addition, the free radical scavenging effects of thymol, thymoquinone, and dithioquinone have been demonstrated in a study of superoxide radicals, singlet oxygen and hydroxyl radicals (4). Thymoquinone, the most important active ingredient of black cumin seed, has been shown to be the scavenger of many reactive oxygen species including superoxide radical anion and hydroxyl radicals by various mechanisms and this is due to its antioxidant properties. It is described that thymoquinone shows antioxidant properties and inhibits nephropathy by inhibiting lipid peroxidation in doxorubicin-induced nephropathy (5). According to the *in vivo* and *in vitro* studies, it was determined that the active components of black cumin seed have anti-tumor effects. Essential oils of black cumin seed have been shown to have cytotoxic effects on different human cancer cell lines. Seedlings of black cumin seed extracted with water or alcohol as solvent alone or in combination with hydrogen peroxide have been shown to have strong anti-carcinogenic effect on MCF-7 breast cancer cells in cell culture medium (6,7).

In addition to the anti-tumoral effect of black cumin seed extract, its active ingredients such as thymoquinone and dithymoquinone have been shown to have cytotoxic effects. For example, the active ingredient extracted with ethyl acetate extracted with column chromatography fraction 5 (CC-5) or with ahedrine, the rapid depletion of intracellular GSH and subsequent degradation of mitochondrial membrane potential due to increased production of reactive oxygen species, leukemia, anti-tumor effect against Lewis lung carcinoma has been reported (8).

As a result of many studies, thymoquinone, an active component of black cumin seed; colorectal cancer, breast and ovarian adenocarcinoma, neoplastic keratinocytes, fibrosarcoma, human osteosarcoma, lung carcinoma, prostate cancer and many other cancer types such as proliferation of cancer cells have been shown to inhibit the effect. Inhibition of cell growth was achieved by increasing gene expression and protein expression of the p53 gene and inhibiting the anti-apoptotic Bcl2 protein (9).

In a study by Roepke et al., the antiproliferative and pro-apoptotic effects of thymoquinone in two human osteosarcoma cell lines with different p53 mutation status were examined and described as a promising compound due to the relatively low toxicity of thymoquinone to normal osteoblasts (10).

Various *in vivo* and *in vitro* animal studies have been conducted to report the therapeutic effect of black cumin seed on diabetes, metabolic syndromes, lipid profile impairment,

atherogenesis, endothelial dysfunction, cardiac mass and contractility abnormality, platelet aggregation, heart rate, blood pressure disorder and cardiotoxicity. Therefore, black cumin seed can be used as a protective and therapeutic agent in cardiovascular disorders as a potential plant due to its strong antioxidant and anti-inflammatory properties (11).

In this study, black cumin seed oils and soft capsules of different formulations sold by different companies in our country were obtained, quality control tests were carried out and thus their efficacy and safety were compared. In this context; GC analysis was carried out to investigate the components and ratios of fatty acids contained in black seed oil and HPLC analysis was performed to determine the amount of thymoquinone which is one of the main components of black seed oil. In this way, the richness of black cumin seed soft capsules and oils in our country in terms of content was compared. In addition, MTT test was performed by applying commercially available black cumin seed capsule and fat forms to MCF-7 breast cancer cell line in order to investigate the cytotoxic effect of black cumin seed *in vitro*, and the results were compared with each other and this was a contribution to the literature on the anticancer effect of black cumin seed. In addition to these studies, antioxidant values of black cumin seed oils and soft capsules were compared by using total phenolic content and DPPH radical scavenging activity methods.

Materials and Methods:

Obtaining materials

In this study, black cumin seed oil which is marketed by 3 different companies and black cumin seed soft capsules supplied by the same companies were used. Black cumin seed oils O1, O2 and O3; soft capsules were coded as SC1, SC2 and SC3. The human breast cancer cell line MCF-7 used for cytotoxicity analysis was obtained from Mersin University Faculty of Medicine Department of Pharmacology.

Characterization of Black Seed Oil

Fatty acid composition analysis by GC analysis

GC analysis was performed using the DB-23 capillary column (60m x 0.25mm x 0.25μm) in combination with the Shimadzu GC 2010 Plus AF (Shimadzu, Japan). The column oven temperature was started at 165 °C and waited at this temperature for 10 minutes. It was then programmed to 180 °C, increasing by 2 °C per minute. It was then programmed to 205 °C, increasing by 2 °C per minute, after 10 minutes at 180 °C. Thereafter, it was allowed to stand for 30 minutes at 205 °C. The compounds were separated in a 1:100 ratio. The relative percentage amounts were calculated by the software as the total area under the peaks. The components of the fixed oil were determined by comparing GC retention times (Ret.Ti) with that of the reference methyl esters of fatty acid.

Determination of thymoquinone content by HPLC analysis

HPLC analysis was performed on an Agilent 1100 series HPLC system (Shimadzu, Japan) with UV detector at a wavelength of 254 nm. Mobile phase; water: methanol:2-propanol (50:45:5) and flow rate 0.6 ml / min, ACE 5C18 (25 cm x 4.6 mm) was used as the column. A UV detector operating at 254 and 294 nm wavelengths and diphenyl sulfone (DPS) as the calibration standard were used for the quantification. Thymoquinone (Sigma Aldrich, USA) was used as standard.

Cell Culture and in vitro Cytotoxicity Test

The MCF-7 breast cancer cell line was cultured in DMEM medium (Dulbecco's Minimum Essential Medium-Sigma Aldrich, USA) containing 10% fetal bovine serum (Sigma Aldrich, USA), 0.2 mM

glutamine (Biological Industries, Israel), 100 µg/mL streptomycin (Biological Industries, Israel) and 100 IU/mL penicillin (Biological Industries, Israel) at 37 °C, 5% CO_2 and 1 atmosphere pressure. Cells were monitored microscopically every other day and passaged with trypsin-EDTA (Biological Industries, Israel) when covering 70% of the culture vessel. The 96-well culture dishes were then seeded to $1x10^4$ cells/ml. The cells were allowed to incubate for 24 hours to proliferate by holding them in wells. After removal of the medium under sterile conditions, the cells were incubated for 48 hours in medium containing 10 µM dissolved black cumin seed oil and capsules. Medium containing 0.5% DMSO was used as negative control and medium containing 10 µM thymoquinone standard was used as positive control. All experiments were performed with 3 replicates. At the end of incubation, the cells were incubated with 0.5 mg/ml MTT stock solution (3-(4,5-dimethyl-thiazoyl) -2,5-diphenyl-SH-tetrazolium bromide) (Sigma M2128, USA) for 4 hours. After incubation, the MTT solution was discarded and 100µl of DMSO was added to each well to dissolve formazan crystals formed in the wells. The plate was shaken on the horizontal shaker for 1-2 minutes. The absorbance values of the samples were read at 570 nm wavelength with Elisa reader (Thermo Fisher Scientific, USA). As the dye deteriorated in light, each step of the experiment was performed as dark as possible. The absorbance value for each thymoquinone concentration was calculated by multiplying the ratio of the absorbance value to the control value by 100% and the inhibitor concentration (IC 50) at which 50% of the cells died.

% Cell viability (IC 50) = 100 x 1- (A/A0)

A = Mean absorbance of samples

A0 = Control absorbance

Determination of Antioxidant Effects

Determination of total phenolic matter

Soluble phenolic substances in ethanol extracts from different black cumin seed oil and fat capsules were determined by Folin-Ciocalteu reagent (FCR). In this method, the phenolic compounds and the Folin-Ciocalteu reagent form a compound in blue green color. This colored compound is directly proportional to the amount of polyphenolic material and shows maximum absorbance at 760 nm. 0.1 mL of each of the extracts obtained from different commercial black cumin seed oil and oil capsules were taken. 4.5 mL of distilled water was added followed by 0.1 mL of FCR. After 3 minutes, 0.3 mL of 2% sodium carbonate solution was added and incubated at room temperature for 2 hours in a turbulent water bath at 250 rpm. At the end of the period, the absorbance of the samples was read on spectrophotometer (Thermo Fisher Scientific, USA) at 760 nm and the results obtained were determined by using the equation obtained from the gallic acid standard graph.

Determination of DPPH radical removal activity

This method is based on the determination of the purple discoloration of the free radical DPPH (1,1-diphenyl-2-picrilhydrazil) by the antioxidant by measuring the spectrophotometer. The opening of the purple color of the radical indicates the presence of antioxidant activity. The reduced absorbance of the reaction mixture is indicative of high free radical removal activity. 4 mL of 0.1 mM DPPH (in ethanol) solution was added to each mL of ethanol extracts obtained from different commercial black cumin seed oils. After vortexing the mixture, it was allowed to stand in the dark and at room conditions for 30 minutes. At the end of the time, the spectrophotometer (Thermo Fisher Scientific, USA) was read at 517 nm absorbance. The percentage of DPPH radical scavenging activity was determined by the following formula:

% DPPH Radical Removal Activity = 100 x (A0-A)/A0

A = Sample absorbance

A0 = Control absorbance

Results:

Findings of GC Analysis

Linoleic acid, oleic acid and palmitic acid were the most common fatty acid components found in black cumin seed preparations on the market. When these preparations were compared with each other, SC2 and SC3 preparations were found to be rich in fatty acids compared to SC1, and unsaturated fatty acids were higher in all three capsules compared to saturated fatty acids. As in capsules, linoleic acid, oleic acid, and palmitic acid were the most common fatty acids found in black cumin seed oil preparations (Table 1). Likewise, unsaturated fatty acids in black cumin seed oils are much more than saturated fatty acids. When the findings obtained in our study were compared with the literature data, we found that the main components, linoleic and oleic acids, were quantitatively in the range reported for black cumin seed and our results are consistent with the literature data (Table 2).

Table 1. Composition of fatty acids methyl esters of black cumin seed soft capsules and oils

Fatty Acid Components	Black Cumin Seed Soft Capsules			Black Cumin Seed Oils		
	Soft Capsule 1 (SC 1)	Soft Capsule 2 (SC 2)	Soft Capsule 3 (SC 3)	Oil 1 (O 1)	Oil 2 (O 2)	Oil 3 (O 3)
Butyric Acid C4:0	0,011	0,065	0,061	0,084	0,249	0,161
Caproic Acid C6:0	0,002	0,0071	0,007	0,010	0,026	0,019
Caprilic Acid C8:0	0,003	0,015	0,013	0,021	0,055	0,013
Capric Acid C10:0	0,004	0,001	0,005	0,001	0,005	0,010
Lauric Acid C12:0	0,012	0,006	0,054	0,010	0,005	0,094
Myristic acid C14:0	0,079	0,154	0,176	0,160	0,130	0,194
Pentadecanoic Acid C15:0	0,011	0,024	0,020	0,021	0,016	0,037
cis-10-Pentadecanoic C15:1	-	0,236	-	0,014	-	-
Palmitic Acid C16:0	**6,607**	**11,903**	**11,764**	**12,262**	**10,301**	**12,172**
trans-Palmitoleic Acid C16:1	0,020	0,014	0,013	0,015	0,014	0,023
Palmitoleic Acid C16:1	0,119	0,177	0,177	0,183	0,181	0,218
Margaric Acid /Heptadecanoic Acid C17:0	0,047	0,056	0,050	0,058	0,044	0,062
Margoleic Acid/Heptadesenoic Acid C17:1	0,052	0,038	0,030	0,040	0,030	0,045
Stearic Acid C18:0	3,654	3,176	3,082	3,340	2,922	3,053
Oleic Acid C18:1 (cis-9)	**29,312**	**24,103**	**24,119**	**25,013**	**25,910**	**23,392**
tr-Linoelaidic C18:2 (trans- 9,12)	0,113	0,053	0,052	0,046	0,116	-
Linoleic Acid C18:2 (cis-9,12)	**33,610**	**56,487**	**56,803**	**55,237**	**57,749**	**57,021**
tr-Linolenic Acid	0,227	0,149	-	0,019	-	-
Linolenic Acid C18:3 (cis-9,12,15)	**25,156**	**0,353**	**0,351**	**0,453**	**0,175**	**0,226**
Arashidic Acid C20:0	0,262	0,182	0,177	0,212	0,164	0,201
Eikosenoic /Gadoleic Acid C20:1	0,371	0,330	0,314	0,427	0,234	0,281

cis 11,14-Eicosadienoic Acid C20:2	0,320	2,460	2,465	2,367	1,664	2,501
Docasahekzanoic Acid C22:6	-	-	0,256	-	-	0,266

Table 2. Saturated and unsaturated fatty acids ratios of black cumin seed oils and soft capsules

Fatty Acids	Soft Capsule 1 (SC 1)	Soft Capsule 2 (SC 2)	Soft Capsule 3 (SC 3)	Oil 1 (O 1)	Oil 2 (O 2)	Oil 3 (O 3)
Saturated fatty acids (g/100g)	10,70	15,60	15,41	16,18	13,92	16,02
Monounsaturated Fatty Acids (g/100g)	29,88	24,90	24,66	25,70	26,37	23,96
Polyunsaturated Fatty Acids (g/100g)	59,43	59,50	59,93	58,12	59,71	60,02
Total Trans Fatty Acids (g/100g)	0,36	0,22	0,07	0,08	0,13	0,024
Total Fatty Acids (g/100g)	100,00	100,00	100,00	100,00	100,00	100,00

Results of HPLC Analysis

The amounts of thymoquinone (TQ), the main active ingredient of black cumin seed, in the soft capsule forms and oils on the market, were shown by HPLC analysis (Figure 1).

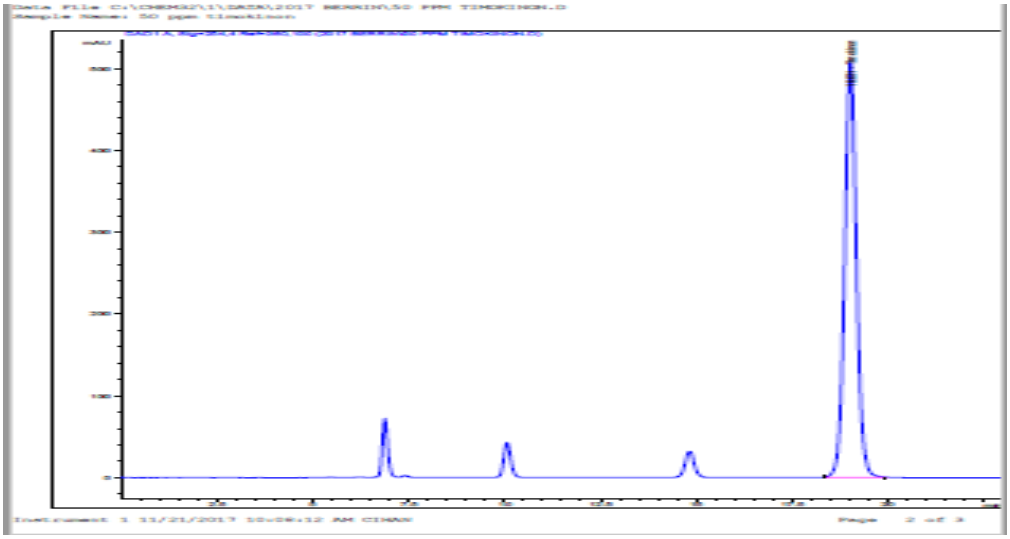

Figure 1. HPLC chromatogram of thymoquinone standard.

According to the results obtained, the comparison of the amounts of thymoquinone in the contents of the preparations is shown in Table 3.

Table 3. The amount of thymoquinone obtained by HPLC analysis of black cumin seed soft capsules and oils.

	Ret Time (minute)	Area (MAU)	Amount (mg/L)
Thymoquinone (TQ)	19,031	1084	49,74
Soft Capsule 1 (SC 1)	18,986	6,161	0,8917
Soft Capsule 2 (SC 2)	18,958	84,25	1,244
Soft Capsule 3 (SC 3)	18,985	41,67	1,0518
Oil 1 (O 1)	18,988	47,94	1,0801
Oil 2 (O 2)	19,002	467,7	2,9728
Oil 3 (O 3)	18,982	174,3	1,6501

According to the results obtained by HPLC analysis, thymoquinone content was found to be highest in O2 (2.9728 mg/ml). The minimum amount of thymoquinone was found in SC1 (0.8917 mg/ml). In the light of the information available in the literature, 18-24% of the essential oils in the structure of black seed are composed of thymoquinone. There was no statistically significant difference between the two groups (capsule-oil) in terms of HPLC (p=0.215).

Findings of Cytotoxicity Analysis

Table 4 shows the absorbance values and mean absorbance levels of the data obtained from the experimental and control groups at the end of the 48th hour by MTT method. IC50 values (concentration of inhibitor at which 50% of the cells die) were calculated from the mean of these obtained absorbances. The data obtained from the calculations are shown in Figure 2.

Table 4. Absorbance values obtained from MTT analysis.

	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance Average
Positive control	0,0255	0,0394	,029	0,0313
Soft Capsule 1 (SC 1)	0,0009	0,0013	,008	0,001
Soft Capsule 2 (SC 2)	0,0061	0,0048	,005	0,0053
Soft Capsule 3 (SC 3)	0,0039	0,0047	,004	0,0042
Oil 1 (O 1)	0,0053	0,0042	,0052	0,0049
Oil 2 (O 2)	0,0097	0,0072	,0077	0,0082
Oil 3 (O 3)	0,0072	0,0067	,0065	0,0068
Negative control	0,1061	0,0711	,0741	0,0838

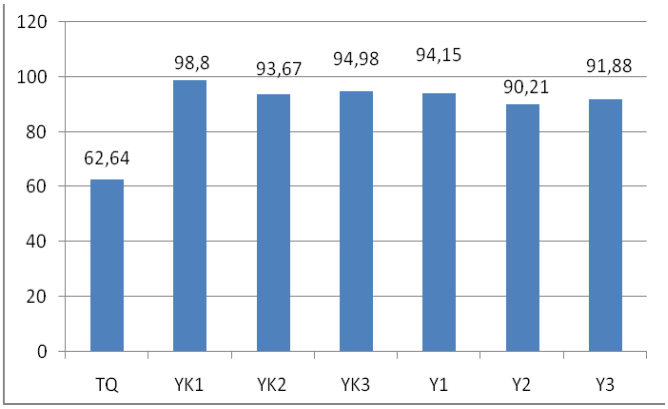

Figure 2. % Expression of IC50 values obtained from MTT analysis. TQ: thymoquinone (positive control group); IC50: 50% cell proliferation inhibition percentage

Results from three independent experiments were expressed as cell viability. Cell viability was evaluated according to the negative control (cell viability was 100% for the group without thymoquinone). There was no statistically significant difference in IC50 between the two groups (capsule-oil) (p=0.134). 0.5 % DMSO was used as the negative control and 10 µM thymoquinone as the positive control. As a result of MTT analysis, it was observed that the O 2 experimental group had the highest cytotoxic effect compared to the negative control according to IC50 values calculated from the absorbance values obtained from each experimental group at 48 hour. The lowest cytotoxicity group was found to be SC 1 experimental group with 98.8 % ratio.

Antioxidant Effects Findings

Total soluble phenolic substances in ethanolic extracts obtained from different black seed oil and oil capsules were determined by Folin-Ciocalteu reagent (FCR). If the phenolic substance is present in the medium, the maximum absorbance value is observed at 760 nm in FCR added extracts. The high value in absorbance is directly proportional to the amount of phenolic substance. For this purpose, gallic acid graph was prepared using standard gallic acid (Figure 3).

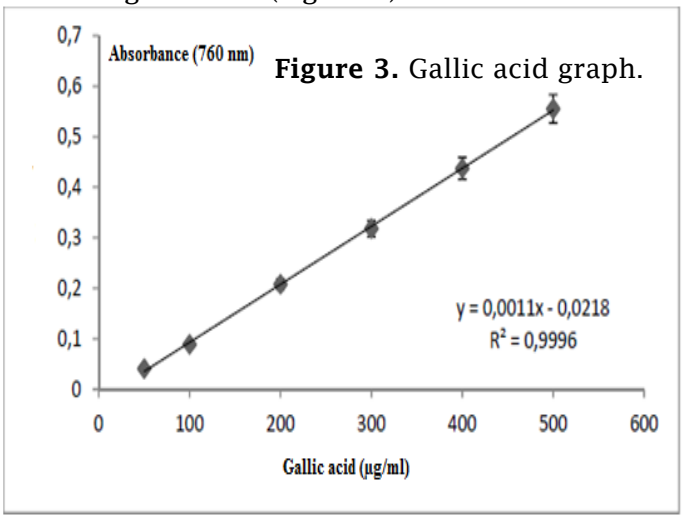

Figure 3. Gallic acid graph.

With the correct equation obtained from this standard graph, total phenolic contents of the samples were calculated as mg gallic acid (mg GAE / g extract) equivalent. In the analysis of the extracts obtained from different black seed oil and capsules according to Folin Ciocalteu method, the findings of total phenolic substances are given in Table 5. The highest value was found in O1, and the lowest value was found in SC1 with 180,181 µg GAE/mg. According to these results, it was observed that O1 extract was richer in terms of phenolic matter than other extracts.

Table 5. Total phenolic content

Extracts	Total phenolic content (µg GAE/mg)
Oil 1 (O1)	913,363
Oil 2 (O2)	241,090
Soft Capsule 1 (SC1)	180,181
Standard (thymoquinone)	684,000

Thymoquinone was used as standard for DPPH free radical removal activity. The DPPH solution in ethanol is purple. If the substances added to the solution have antioxidant properties, they remove the DPPH radical and the color of the purple solution turns into a yellow color. The lighter the color of the DPPH solution, the less absorbance is observed. This means higher absorbance with low activity. O1 of the extracts prepared in this method, DPPH radical removal activity compared to the standard was observed to be the highest (41%) (Figure 4).

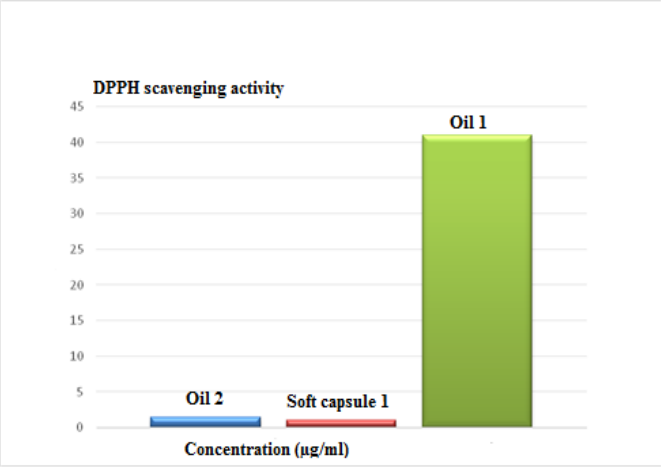

Figure 4. DPPH free radical scavenging activity values.

Discussion:

The use of plants in treatment is as old as human history. Thousands of years ago, people recognized the therapeutic power of plants and took advantage of it to live healthy. Black cumin seeds have been used for healing since ancient times. The chemical contents of black cumin seeds vary depending on the geographical region and climate. In general, the seeds include fixed oil, volatile oil, proteins, amino acids, reduced sugar, mucilage, alkaloids, organic acids, tannins, resin, toxic glucoside, metarbin, pungent substances, glycosidal saponins, crude fibers, vitamins, minerals and essential oil (12,13). Many in vivo and in vitro studies have shown that both essential oil and active components of black seed have antitumor effects (14,15). Research on different types of cancers in humans has been shown to have a cytotoxic effect against some of the essential oil of black cumin seed (8,16,17). It has been reported that thymoquinone, the most important bioactive component of black cumin seed, is a potential chemotherapeutic and chemopreventive component and stimulates apoptosis, which is of great importance in the cellular cycle due to its antiproliferative effect (19,20). Studies have shown that thymoquinone inhibits cancerous cell proliferation in many types of cancer such as prostate cancer, breast cancer, liver cancer, colorectal cancer, and human osteosarcoma (21,22, 23).

Epidemiological studies show that the anticancer agent, thymoquinone, reduces the risk of developing cancer, but it is an effective antiproliferative compound when used alone or in combination with various chemotherapeutic drugs

in cancer patients. Overall, thymoquinone demonstrates its antiproliferative efficacy in three ways. These; induction of apoptosis, inhibition of angiogenesis and cell cycle arrest (2, 24, 25). There are some studies in the literature to investigate the presence of thymoquinone in the methanol extract of black cumin seed by HPLC and to determine the amount of substances found (26). However, the difference of our study from the literature is that we have worked on the soft capsules and oils of black cumin seed which are used in the market as a herbal product for healing purposes and we have made an idea in terms of the effectiveness and quality of these products by comparing them in terms of the amount of thymoquinone which is the active ingredient. It is stated in the literature that thymoquinone is responsible for many pharmacological and biological effects of black cumin plant, which indicates that the marketed Nigella sativa preparations of natural origin will make an important contribution to the literature in this respect.

Previous studies have shown that black cumin seed has a cytotoxic effect on cancer cells. In our study, MTT test was performed in MCF-7 cancer cell line to investigate the cytotoxicity of black cumin seed. Our cytotoxicity results supported us with HPLC analysis. In other words, the highest thymoquinone product (Y2) produced by HPLC test showed the highest cytotoxicity in MTT analysis and caused the highest cell death. As a result of the HPLC experiment, the market product with the lowest amount of thymoquinone showed low cytotoxicity and showed low cell death.

When the antioxidant values of different black cumin seed oil and capsules were examined, the highest value was found in O1 and the lowest value was found in SC1. The antioxidant values of black cumin seed oil and oil capsules were found to be similar. When the findings of DPPH free radical scavenging activity were compared with the literature, the data obtained from the analysis were close to each other (27). This situation shows that the formulation of the capsule has no negative effect on the antioxidant activity of the oil. Therefore, the preparation of black cumin seed oil in soft capsule form may be recommended for efficacy. On the other hand; when the total amount of phenolic substances were examined, different results were obtained from the studies in the literature (28,29). As it is seen in the results of different studies, it is thought that the difference between oil and capsules may be due to the effect of factors such as differences in the techniques applied in agriculture of the plant (frequency of application of irrigation, fertilization, harvesting etc.), soil structure difference, different climate and environmental conditions (28).

As a result, our opinion about the cytotoxic effect of black cumin seed in MCF-7 cell culture medium has been strengthened in the light of previous studies. This study is a research in cancer disease called as the disease of the age. As suggested by many studies, the cytotoxic effect of black cumin seed can be a promising aspect, especially in the development of new treatment methods and anti-cancer drugs.

With this study, we believe that the use of black cumin seed in daily life should be expanded and the black cumin seed should be consumed consciously. However, our study has been an effective single dose study, so we believe that this study can be improved by studying at different concentrations and even by studying different cell lines. Because a few studies have been reported that when black cumin seed is applied to healthy liver it causes serious fattening. Black cumin seed is known to be useful, but when used more than a certain dose has been observed to cause fatty liver. This finding is very important in this process

starting with fatty liver and leading to cancer formation. We believe that this effect can be avoided if the use of black cumin seed is limited to certain daily doses. Therefore, this point should be considered in the treatment methods and drug development.

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Conflict of Interest: The authors declared that this article has not had any conflict of interest during the preparation and publication of this manuscript.

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