

ANTIOXIDANT ACTIVITIES OF BLACK CUMIN (*NIGELLA SATIVA* L.) SEEDS CULTIVATING IN DIFFERENT REGIONS OF TURKEY

NEJDET ŞEN^{1,4}, YAKUP KAR² and YENER TEKELİ³

¹Department of Chemistry, Faculty of Science, Selcuk University, 42075, Selcuklu, Konya, Turkey

² Department of Petroleum and Natural Gas Engineering, Faculty of Engineering, Mustafa Kemal University, 31200, İskenderun, Hatay, Turkey

³ Department of Chemistry, Faculty of Science, Mustafa Kemal University, 31040, Hatay, Turkey

Accepted for Publication September 29, 2008

ABSTRACT

In this study, the antioxidant activity of the methanolic extracts of the black cumin (Nigella sativa L.) seeds collected from Konya, Isparta, Çorum, Burdur, Afyon and Samsun was evaluated using three different methods, specifically the β -carotene and linoleic acid system, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power assays. Among the six black cumin samples of different origins, the black cumin sample origins from Konya city showed the most potent radical scavenging activity in each assay, showing 94.59% (at 1 mg/mL) in the β -carotene bleaching method and 44.44% in the DPPH radical scavenging method (at 0.5 mg/mL). Positive correlations were found between the total phenolic content in the black cumin extracts and their antioxidant activities. The values of their antioxidant activity were either equivalent to or higher than those of positive BHA and BHT controls. These satisfying results lead to expect the use of black cumin seeds as health-promoting ingredients such as dietary supplements and nutraceuticals.

PRACTICAL APPLICATIONS

Nigella sativa L. seeds have been used for nutritional and pharmaceutical purposes, such as a condiments in bread and other dishes, and as anthelmintic

⁴ Corresponding author. TEL: 90-332-2231885; FAX: 90-332-2410106; EMAIL: nsen@selcuk.edu.tr

and antibacterial agent. In addition, its seeds have been also used traditionally in the preparation of a sweet dish, which is made up of black cumin paste sweetened with honey or syrup, and especially in the flavoring of foods such as bakery products and cheese. Because *N. sativa* L. seeds include phospholipids, aroma compounds have many pharmaceutical properties; they could also be used for a many new multi-purposes in industrial areas such as food, cosmetic and pharmaceutical.

INTRODUCTION

Lipid oxidation may bring about to the forming of a many negative circumstances (rancidity, lower quality and nutritional value). The majority of these can occur during the processing, distribution, storage and final preparation of foods. Moreover, this oxidation is connected with aging, heart disease, membrane damage, cancer and many pathogenic human diseases. To control and delay this oxidation, there is a growing demand for antioxidants. In this context, various synthetic antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary butyl hydroquinone (TBHQ) and nordihydroguaiaretic acid (NDGA) have been approved and are routinely used as food protecting agents (McMullen *et al.* 1991). However, it has recently been reported that these agents possess probable toxic and carcinogenic properties (Ito *et al.* 1985). Hence, the recent growing public demand for replacement of synthetic with natural antioxidants has initiated intense research activity (Hras *et al.* 1992). In addition, the reactive oxygen species (ROS) generated beyond the antioxidant capacity of a biological system lead to oxidative stress (Maxwell 1995). As a result of this stress, it may create a variety of pathogenic human diseases, such as arteriosclerosis, diabetes mellitus, hypertension, inflammation, cancer, AIDS and accelerating with the process of aging (Burits and Bucar 2000). The use of natural antioxidants would support biological resistance to free radicals and, as a consequence of this resistance, the process of aging, as well as the risk of age-associated degenerative diseases, will noticeably decrease (Assimopoulou *et al.* 2005).

In recent years, public demand and interest in natural products has gradually increased because of natural products' potential medicinal properties, as well as negative perception (about potential toxic and carcinogenic properties) of synthetic products. As natural product, plant species especially seeds are an important natural antioxidant sources for many pharmacological effects such as anti-tumor activity, anti-oxidative activity, anti-inflammatory activity, etc. (Michelitsch *et al.* 2004). A large number of plants with seeds have been investigated, and some of these have been cultivated. Among these seeds, it has

been immensely researched that black cumin seed (*Nigella sativa* L.) and its constituents have different areas of use, including pharmaceutical, nutritional (as a condiment and oil) and industrial (as cosmetic product, sweet dish, paste, etc.). In many recent studies, it has been attributed that black cumin seeds and/or its oil have many medical properties: antihelmenthic, antineoplastic, antifungal and antibacterial effects (Hasan *et al.* 1989; Hussein 1990; Hanafy and Hatem 1991; Salomi *et al.* 1992; Ramadan 2007). Further studies showed the presence of activities such as bronchodialator, immunopotentiating, hypotensive, antispasmodic, hypertensive, etc. (Aqel 1992a,b).

Many studies with black cumin and its constituents in literature are related to either their pharmacological properties or their physical and chemical properties. For example, Gad *et al.* (1963) researched the physical and chemical characteristics and fatty acid composition of oil extracted from Egyptian black cumin seeds. Babayan *et al.* (1978) researched the proximate analysis, fatty acid and amino acid composition of the black cumin seeds. Ramadan and Morsel (2002) particularly studied the contents of triacylglycerol (TAG) and sterol (ST) of *Nigella sativa* L. seed oil. Takruri and Dameh (1998) studied the nutritional values (element, protein, mineral, vitamin, etc.) of five samples of black cumin seeds from five different sources. Michelitsch *et al.* (2004) studied the determination of isopropylmethylphenols (carvacrol and/or thymol) in black seed oils. Atta (2003) researched the physicochemical properties of black cumin seeds cultivated in Egypt and their oil properties (lipid classes, fatty acid composition and sterol content). Cheikh-Rouhou *et al.* (2007) researched the physicochemical properties of two black cumin seeds cultivated in Tunisia and Iran. In recent years, the antioxidant and antimutagenic activities of methanolic extracts from shoots and roots of Tunisian *Nigella sativa* were investigated by Bourgou *et al.* (2008).

N. sativa L. has been grown in many regions in Turkey especially Afyon, Burdur, Isparta, Çorum and Konya (Baytop 1984). To our knowledge, there are no detailed studies about the antioxidant properties of black cumin seeds grown in different regions of Turkey. The studies about the black cumin seeds grown in Turkey are relating volatile oil and fatty acid compounds. For instance, Turker and Bayrak (1997) investigated the compositions of volatile and fixed oils of 20 black cumin seeds grown in different regions of Turkey. Nergiz and Otles (1993) researched the proximate chemical composition and some micro-constituents of *N. sativa* L. seed grown in the İzmir region of Turkey.

The aim of this study is to make a detailed research on antioxidant activity of black cumin seeds grown in six different regions of Turkey: Çorum, Afyon, Konya, Burdur, Samsun and Isparta, compare with each other and synthetic antioxidants: BHA and BHT in terms of the antioxidant activity.

MATERIALS AND METHODS

Plant Material, Standards and Reagents

The seeds of black cumin (*N. sativa* L.) were obtained from six regions: Çorum, Afyon, Konya, Burdur, Samsun and Isparta in Turkey. Before extraction, these seeds were finely ground with a small domestic electronic coffee grinder (Sinbo, Istanbul, Turkey) and the grounded seed (particle size: +0.60–0.85 mm) preserved in hermetic bags; these bags stored at +4C until extraction.

All solvents used were of analytical grade. Methanol, n-hexane, chloroform, BHA, BHT, trichloroacetic acid, ferric chloride, Folin-Ciocalteu reagent, gallic acid, sodium carbonate, potassium ferricyanide and potassium phosphate buffer (pH 6.6) were purchased from Merck Co. (Darmstadt, Germany). β -carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween 40) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radicals were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Preparation of Methanolic Extracts from Black Cumin Seeds

First, the ground seed samples (particle size: +600–850 μ m) were defatted by extraction with n-hexane in a Soxhlet apparatus at 80C for 6 h following the procedure described in Cheikh-Rouhou *et al.* (2007) and Kar (2008). Then, the defatted and dried cakes were extracted with 70% methanol following slight modification of the procedure described by Koşar *et al.* (2002) to obtain the methanolic extracts for the determining tests of total antioxidant activity and phenol content. Nearly 15 g of each cake was extracted with 60 mL of 70% methanol at 40 ± 5 C for 0.5 h in a glass conical flask in a shaking water bath. The mixture was then filtered through a filter paper using a Buchner funnel. Then, the residue was extracted twice with 60 mL of 70% methanol as described previously. The methanolic extracts obtained were combined and the solvent was evaporated under vacuum at 45C using a rotary evaporator (Laborota 4000, Heidolph Inst. GmbH & Co. KG, Schwabach, Germany), and then dried. Finally, the dry extract obtained from each cake was weighed and kept in a dark-colored and hermetic bottle at +4C until testing.

Determination of Total Phenolic Content in Methanolic Extracts

The total phenolic content of each extract was determined according to the slight modification of the method described by Singleton *et al.* (1999) involving Folin-Ciocalteu reagent and gallic acid as standard. Five hundred microliters of the test solution, which was prepared with 0.5 mg from each dried extract, was dispersed in 1 mL of 70% methanol and added to a

volumetric flask. Then, 46 mL of distilled water and 1 mL of Folin-Ciocalteu reagent was added and was vigorously shaken for 3 min. After this shaking time, 3 mL of 20% Na_2CO_3 was added and the mixture was allowed to stand at room temperature with intermittent shaking on a shaker (Nüve, 350 SL, Ankara, Turkey) for 2 h. Absorbance of each test solution was measured at 760 nm with a spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) against a blank consisting of all reagents and solvents without extracts. The concentration of phenolic compounds in all the extracts was calculated according to the following equation obtained from the standard gallic acid graph, which was determined in the range of 3.1 to 31 mg/mL, and the final results were expressed as mg of gallic acid equivalents per gram of dry weight. Samples were analyzed in triplicate.

$$\text{Absorbance} = 0.1024 \times \text{gallic acid (mg/mL)} + 0.1018, R^2 = 0.9809 \quad (1)$$

Determination of DPPH Free Radical Scavenging Activity

The free radical scavenging or hydrogen-donating ability of each methanolic extract was determined using the stable radical (DPPH•) according to the modified method described by Singh *et al.* (2005). Each methanolic extract or a standard (BHA and BHT) was dissolved with 70% methanol and prepared at various concentrations ranging from 0.50 to 0.03125 mg/mL sample solutions. Three milliliters of various concentrations of the extracts or the standards was mixed with a 1-mL solution of DPPH (final concentration of DPPH was 3.72×10^{-5} M) methanolic solution of DPPH. Then, the mixture was shaken vigorously and left to stand for 30 min at room temperature in the dark, and the absorbance of each test solution was read at 515 nm using the ultraviolet-visible spectrophotometer (Shimadzu UV-1700). The control (containing all reagents except the test compound) and standards were subject to the same procedure. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following the formula. Samples were analyzed in triplicate.

$$\% \text{ DPPH radical scavenging effect} = [(\text{Ac}_{515} - \text{As}_{515}) / (\text{Ac}_{515})] \times 100 \quad (2)$$

Where Ac_{515} is the absorbance of control at 515 nm and As_{515} is the absorbance of sample at 515 nm.

Determination of Ferric Reducing Power of Methanolic Extracts

The reducing power of each extract or the standards (BHA and BHT) was determined according to the method of Oyaizu (1986). One milliliter of

each extract or the standard solution prepared with 70% methanol at various concentrations ranging from 0.05 to 0.5 mg/mL was mixed with 2.5 mL of 0.2 M potassium phosphate buffer (0.2 M and pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. A 2.5 mL amount of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride, and the absorbance was read at 700 nm against the blank. Samples were analyzed in triplicate.

Antioxidant Activity Assay using a β -Carotene-Linoleate Model System

The antioxidant activity of black cumin extracts was evaluated according to the β -carotene-linoleic acid (linoleate) model system described in the literature (Miller 1971; Amarowicz *et al.* 2004; Topcu *et al.* 2007). A 1 mL amount of the stock β -carotene solution, prepared by dissolving 2 mg of β -carotene in 10 mL of chloroform, was transferred into a round-bottom flask containing an emulsifier mixture formed by 0.02 mL of 60% linoleic acid and 200 mg of Tween 40. The mixture was then evaporated at 40C under vacuum for 12 min via a rotary evaporator (Laborota 4000) to remove the chloroform. After the removal of chloroform, the mixture was immediately diluted with slow addition of 100 mL of distilled water saturated with oxygen and agitated vigorously to form an emulsion. A 5 mL amount of this emulsion was added to a test tube containing 0.2 mL of the antioxidant (black cumin seed extract, BHA or BHT) solution (1 mg/mL in 70% methanol) or 0.2 mL of 70% methanol solution as the control. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a UV-VIS spectrophotometer (Shimadzu UV-pharmaSpec 1700) against the blank and the tubes were incubated in a water bath at 50C for 2 h, for thermal oxidation, and the measurement of absorbance was continued at 15 min intervals until the end ($t = 120$ min).

Antioxidant activity was expressed as the rate of β -carotene bleaching (R) calculated using the following the equation according to first-order kinetics, as described by Al-Saikhan *et al.* (1995).

$$R = \ln(A_{t=0}/A_{t=t}) \times 1/t \quad (3)$$

where $\ln$ is the natural log, $A_{t=0}$ is the initial absorbance (470 nm) of the emulsion at time 0, $A_{t=t}$ is the absorbance (470 nm) at 15, 30 and 45 min and t is the time in minutes.

An average rate for each sample was calculated based on the determined for $t = 15, 30$ and 45 min time intervals. The antioxidant activity (ANT) was calculated as the percent inhibition of the R relative to the control using the equation as follows:

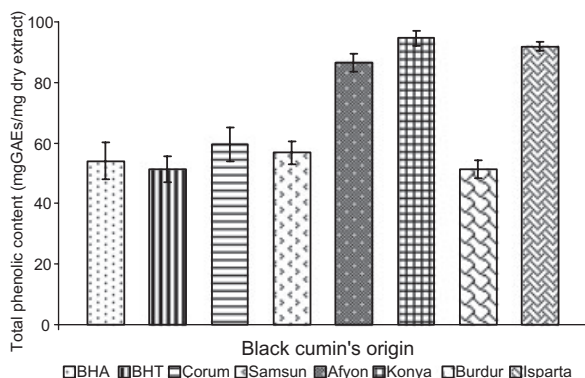


FIG. 1. THE TOTAL PHENOLIC CONTENT OF THE METHANOLIC EXTRACTS FROM BLACK CUMIN SEEDS NATIVE TO DIFFERENT CITIES IN TURKEY

$$\% \text{ ANT} = \left[\frac{(R_{\text{control}} - R_{\text{sample}})}{(R_{\text{control}})} \right] \times 100 \quad (4)$$

where R_{control} and R_{sample} are the average bleaching rates of β -carotene in the emulsion without antioxidant and with extract or standard (BHA or BHT), respectively. Samples were analyzed in triplicate.

Statistical Analysis

All experiments were performed in triplicate. Data are reported as mean $\pm$ standard deviation (SD) of triplicate determinations (data not shown). Experimental results were analyzed statistically using one-way analysis of variance (Sokal and Rohlf 1973). P values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Total Phenolic Content

The total phenolic contents (mg of GAEs/mg of dry extract) of the black cumin samples obtained from six different cities in Turkey – Konya, Isparta, Afyon, Samsun, Çorum and Burdur – are shown in Fig. 1. Among all the extracts, the sample of black cumin seeds from Konya demonstrated the highest phenolic content, with an average of 2.92 mg of GAEs/mg of dry extract, followed by Isparta, Afyon, Samsun, Çorum and Burdur. There is a significant difference value in the total phenolic content between black cumin seeds.

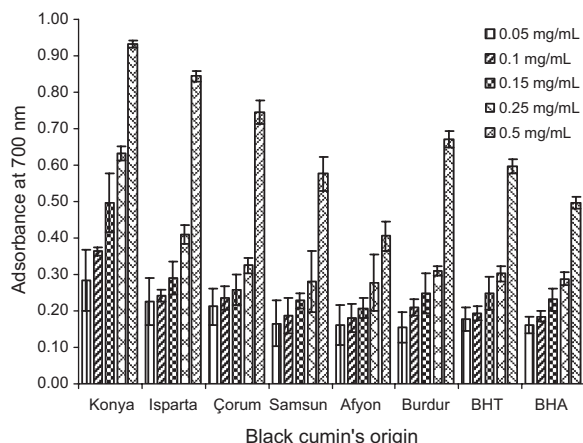


FIG. 2. REDUCING POWER EFFECT OF DIFFERENT CONCENTRATIONS OF BLACK CUMIN EXTRACTS, BHT AND BHA

The differences in the total phenolic content among the six studied samples might be due to many differences, such as the environmental conditions, genetic background or agricultural techniques applied (i.e., fertilizer application and frequency of irrigation).

Antioxidant Activities of Methanolic Extracts

In this study, the antioxidant activity of the methanolic extracts from black cumin seeds was evaluated using reducing power (FRAP), DPPH scavenging, and β -carotene bleaching assays.

Reducing Power Assay (FRAP)

In reducing power assay, the yellow color of test solution changes to blue when adding reducers (i.e., antioxidants) to the test solution medium. As a result of this, color change (formation of Perl's Prussian blue) based on the conversion of the Fe^{3+} /ferricyanide complex to Fe^{2+} /ferrous and the concentration of Fe^{2+} can be established by measuring at 700 nm. A higher absorbance at 700 nm indicates a higher reducing power (Barros *et al.* 2007).

The reducing powers of all the black cumin extracts were excellent and increased with increasing concentration, as shown in Fig. 2. In general, all extracts and synthetic antioxidants have been shown high reducing power. BHT and BHA, and the Konya sample's reducing power values are higher than the others (Fig. 2).

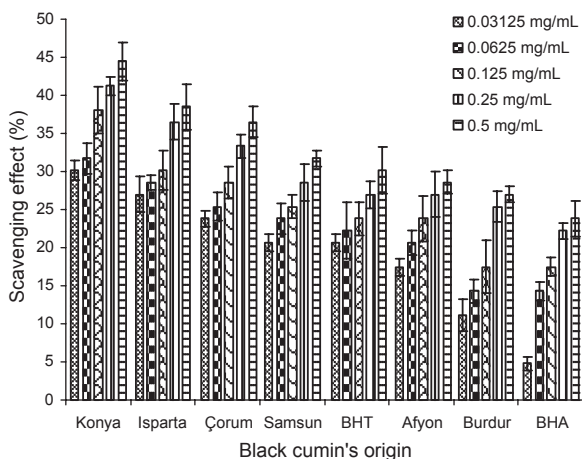


FIG. 3. SCAVENGING ACTIVITY (%) OF BLACK CUMIN METHANOLIC EXTRACTS ON DPPH RADICALS

In the literature, it is clarified that the reducing properties are generally associated with the presence of reductones possessing the hydrogen-donating ability (Shimada *et al.* 1992; Barros *et al.* 2007). They show antioxidant action by breaking the free radical chain via this property. Based on the reducing power results, we can say that the extract of the Konya sample contain higher amounts of reductone compounds (i.e., carvacrol, thymol, 4-*tert*-butylcatechol, etc.) than the others.

Radical Scavenging Activity Assay

The decrease in absorbance of the DPPH radical owing to the scavenging capability of black cumin extracts and synthetic antioxidants (BHA and BHT) is illustrated in Fig. 3.

It can be seen from Fig. 3 that the extracts prepared by methanol showed varying degrees of scavenging capacities. Also, the results showed that scavenging activity was increased with increasing concentration for all the extracts and positive controls BHT and BHA. At all concentrations, the sample of Konya showed higher scavenging activity than the others and synthetic antioxidants. This high activity power result from its having much higher total phenolic content than the others (see Fig. 1). Also, it reached the highest scavenging activity level (44.44%) at a concentration of 0.5 mg/mL.

Singh *et al.* (2005) reported that the scavenging capability of acetone extract from black cumin (native to India) on DPPH radicals increased linearly with increasing concentration and the extract exhibited good to excellent

scavenging activity in comparison with positive BHA and BHT controls. This result was similar to our study results obtained from the DPPH assay.

The correlation between the radical scavenging activity and phenols content of the six samples was studied using a linear regression analysis. The correlation coefficient between total phenolic content and DPPH scavenging activity ($R^2 = 0.785$, $y = 0.070x + 21.35$) was found to be very strong (higher than 0.5). As a result, we think that the high scavenging activity results from the phenolic compounds: carvacrol, 4-terpineol, thymol, thymohydroquinone, etc. situated large quantity in black cumin extracts.

β -Carotene Bleaching Assay

Figure 4 shows the antioxidant activities of black cumin methanolic extracts and positive controls with the coupled oxidation of β -carotene and linoleic-acid-generating free radicals. As seen from the figure, all extracts indicated good antioxidant activity comparable to positive controls. Among all extracts and positive controls, the extract of the sample from Konya showed the highest antioxidant activity (94.59%) comparable to those of other extracts and positive controls. There was a significant difference in the antioxidant activity ($P < 0.05$), especially in the activities of the black cumin seeds from Konya, Isparta and Afyon were higher than the others. The observed differences in the antioxidant activity of all extracts may be related to their phenolic content, as reported by Thippeswamy and Naidu (2005), Bourgou *et al.* (2008). The antioxidative effectiveness in natural sources was mostly due to the phenolic compounds found in their structures (Hayase and Kato 1984).

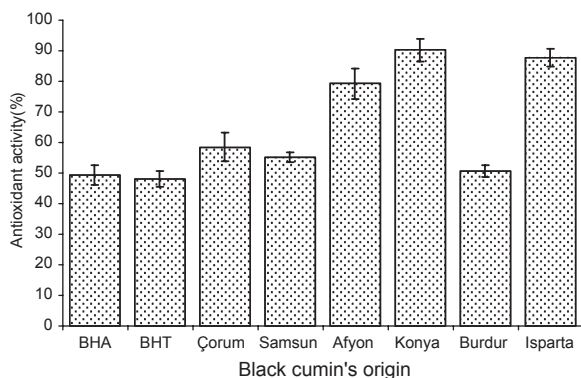


FIG. 4. ANTIOXIDANT ACTIVITY (%) OF THE BLACK CUMIN METHANOLIC EXTRACTS BY β -CAROTENE BLEACHING METHOD

As seen from the Figs. 1 and 2, it was determined that there was a positive correlation between antioxidant activity and total phenolic content for all methanolic black cumin extracts ($R^2 = 0.563$, $y = 0.167x + 43.09$). Also, it was observed that the results were in agreement with the others (Tsushida *et al.* 1994; Velioglu *et al.* 1998; Kaur and Kapoor 2002; Alali *et al.* 2007; Sarikurkcü *et al.* 2007).

All in all, from both this positive correlation resulting from the relationship between antioxidant activity and phenolic content, and the information of literature including phenolic group in natural products play an important role in antioxidant activity (Huang and Frankel 1997), we can say that this high activity results very likely from phenolic compounds such as carvacrol, thymol and 4-*tert*-butylcatechol in the black cumin extracts in agreement with the literature reported by Singh *et al.* (2005).

CONCLUSION

In conclusion, it was determined that positive correlation was between antioxidant activity and amount of total phenolic content in all black cumin extracts. Hence, phenolic compounds (i.e., carvacrol, thymol and 4-*tert*-butylcatechol etc.) could make a significant contribution to the antioxidant activity in these extracts. Also, antioxidant activities of all extracts in all test systems were found equal to or higher than those of synthetic antioxidants (BHA and BHT). As a result, it can be said that the black cumin extract can be regarded as a promising plant-derived natural antioxidant and it is also potential resource for its use in different fields such as foods, cosmetics, and pharmaceuticals.

ACKNOWLEDGMENT

We thank the Scientific Research Projects Foundation of Selcuk University (SUBAP-Grant Number: 06101004) for the financial support of this study.

REFERENCES

- AL-SAIKHAN, M.S., HOWARD, L.R. and MILLER, J.C.J.R. 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*). *J. Food Sci.* 60, 341–343.
- ALALI, F.Q., TAWAHA, K., EL-ELIMAT, T., SYOUF, M., EL-FAYAD, M., ABULAILA, K., NIELSEN, S.J., WHEATON, W.D., FALKINHAM,

- J.O. and OBERLIES, N.H. 2007. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: An ICBG project. *Nat. Prod. Res.* 21, 1121–1131.
- AMAROWICZ, R., PEGG, R.B., RAHIMI-MOGHADDAM, P., BARL, B. and WEIL, J.A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* 84, 551–562.
- AQEL, M.B. 1992a. The calcium antagonistic effect of the volatile oil of *Nigella sativa* seeds. *Dirasat* 19B(1), 119–133.
- AQEL, M.B. 1992b. The relaxing effect of the volatile oil of *Nigella sativa* seeds on vascular smooth muscle. *Dirasat* 19B(2), 91–100.
- ASSIMOPOULOU, A.N., SINAKOS, Z. and PAPAGEORGIOU, V.P. 2005. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytother. Res.* 19, 997–1000.
- ATTA, M.B. 2003. Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. *Food Chem.* 83, 63–68.
- BABAYAN, V.K., KOOTTUNGAL, D. and HALABY, G.A. 1978. Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* L. seeds. *J. Food Sci.* 43, 1314–1315.
- BARROS, L., FERREIRA, M.J., QUEIROS, B., FERREIRA, I.C.F.R. and BAPTISTA, P. 2007. Total phenols, ascorbic acid, β -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem.* 103, 413–419.
- BAYTOP, B. 1984. Türkiye’de kullanılan tıbbi bitkiler. In *Çörekotu*, (B. Baytop, ed.) pp. 211–212, İstanbul Üniversitesi Yayınları 3255, İstanbul, Turkey.
- BOURGOU, S., KSOURI, R., BELLILA, A., SKANDRANI, I., FALLEH, H. and MARZOUK, B. 2008. C.R. Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Biologies* 331, 48–55.
- BURITS, M. and BUCAR, F. 2000. Antioxidant activity of nigella sativa essential oil. *Phytother. Res.* 14, 323–328.
- CHEIKH-ROUHOU, S., BESBES, S., HENTATI, B., BLECKER, C., DEROANNE, C. and ATTIA, H. 2007. *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food Chem.* 101, 673–681.
- GAD, A.M., EL-DAHNY, M. and HASSAN, M. 1963. Studies on the chemical composition of Egyptian *Nigella sativa* L. oil. *Planta Medica* 11, 34–138.

- HANAFY, M.S.M. and HATEM, M.E. 1991. Studies on the antimicrobial activity of black cumin. *J. Ethnopharmacol.* **34**, 275–278.
- HASAN, C.M., AHSAN, M. and ISLAM, N. 1989. In vitro antibacterial screening of the oils of *Nigella sativa* seeds. *Bangladesh J. Bot.* **18**, 171–174.
- HAYASE, F. and KATO, S. 1984. Antioxidative components of sweet potatoes. *J. Nat. Sci. Vitaminol.* **30**, 37–46.
- HRAS, A.R., HADOLIN, M., KNEZ, Z. and BAUMAN, D. 1992. Comparison of antioxidative and synergistic effects of rosemary extract with α -tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chem.* **71**, 229–233.
- HUANG, S.W. and FRANKEL, E.N. 1997. Antioxidant activity of tea catechins in different lipid systems. *J. Sci. Food Agric.* **40**, 3033–3038.
- HUSSEIN, A.S.M. 1990. Antibacterial and antifungal activities of some Libyan aromatic plants. *Planta Medica* **56**, 644–645.
- ITO, N., FUKUSHIM, S. and TSUDA, S. 1985. Carcinogenicity and modification of carcinogenic response by BHA, BHT and other antioxidants. *Crit. Rev. Toxicol.* **15**, 109–150.
- KAR, Y. 2008. Çörekotu (*Nigella sativa* L.) tohumunun doğal antioksidan ve alternatif enerji kaynağı olarak incelenmesi. Doktora Tezi, Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, ss. 226, Konya, Turkey.
- KAUR, C. and KAPOOR, H.C. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol* **37**, 153–161.
- KOŞAR, M., BOZAN, B., TEMELLI, F. and BAŞER, K.H.C. 2002. Sumak (*Rhus Coriaria*)’ın fenolik bileşikleri ve antioksidan etkileri. 14. *Bitkisel İlaç Hammaddeleri Toplantısı*, (K.H.C. Baser and N. Kirimer, eds), pp. 113–124, Anadolu University: Eskisehir, Turkey.
- MAXWELL, S.R.J. 1995. Prospects for the use of antioxidant therapies. *Drugs* **49**, 345–361.
- MCMULLEN, L.M., HAWRYSH, C. and TOKARSKA, B. 1991. Ascorbyl palmitate efficiency in enhancing the accelerated storage stability of canola oil. *J. Food Sci.* **56**, 1651–1659.
- MICHELITSCH, A., RITTMANNBERGER, A., HÜFNER, A., RÜCKERT, U. and LIKUSSAR, W. 2004. Determination of isopropylmethylphenols in black seed oil by differential pulse voltammetry. *Phytochem. Anal.* **15**, 320–324.
- MILLER, H.E. 1971. A simplified method for the evaluation of antioxidant. *J. Am. Oil. Chem. Soc.* **48**, 91–94.
- NERGIZ, C. and OTLES, S. 1993. Chemical composition of *Nigella sativa* L. seeds. *Food Chem.* **48**, 259–261.

- OYAIZU, M. 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* 44, 307–315.
- RAMADAN, M.F. 2007. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): An overview. *Int. J. Food Sci. Technol.* 42, 1208–1218.
- RAMADAN, M.F. and MORSEL, J.T. 2002. Neutral lipid classes of black cumin (*Nigella sativa* L.) seed oils. *Eur. Food Res. Technol.* 214, 202–206.
- SALOMI, N.J., NAIR, S.C., JAYAWARDHANAN, K.K., VARGHESE, C.D. and PANIKKAR, K.R. 1992. Antitumour principles from nigella sativa seeds. *Cancer Lett.* 63, 41–46.
- SARIKURKCU, C., TEPE, B., DAFERERA, D., POLISSIOU, M. and HARMANDAR, M. 2007. Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. *globosum* (lamiaceae) by three different chemical assays. *Bioresour. Technol.* 99, 4239–4246.
- SHIMADA, K., FUJIKAWA, K., YHARA, K. and NAKAMURA, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* 40, 945–948.
- SINGH, G., MARIMUTHU, P., DE HELUANI, C.S. and CATALAN, C. 2005. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds. *J. Sci. Food Agric.* 85, 2297–2306.
- SINGLETON, V.L., ORTHOFER, R.M. and RAMUELA-RAVENTOS, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299, 152–178.
- SOKAL, R.R. and ROHLF, J.F. 1973. *Introduction to Biostatistics*, pp. 164–185, W. H. Freeman and Company, San Francisco, CA.
- TAKRURI, H.R.H. and DAMEH, M.A.F. 1998. Study of the nutritional value of black cumin seeds (*Nigella sativa* L.) *J. Sci. Food Agric.* 76, 404–410.
- THIPPESWAMY, N.B. and NAIDU, K.A. 2005. Antioxidant potency of cumin varieties-cumin, black cumin and bitter cumin-on antioxidant systems. *Eur. Food Res. Technol.* 220, 472–476.
- TOPCU, G., AY, M., BILICI, A., SARIKURCU, C., OZTÜRK, M. and ULUBELEN, A. 2007. A new flavones from antioxidant extracts of *Pistacia terebinthus*. *Food Chem.* 103, 816–822.
- TSUSHIDA, T., SUZUKI, M. and KUROI, M. 1994. Evaluation of antioxidant activity extracts and determination of some active compounds. *Nippon Sokuhin Kogyo Gakhaishi* 41, 611–618.

- TURKER, L. and BAYRAK, A. 1997. Çörekotu (*Nigella sativa* L.)'nun sabit and uçucu yağ kompozisyonunun araştırılması. Standart Ekim Sayısı 430, 128–134.
- VELIOGLU, Y.S., MAZZA, G., GAO, L. and OOMAH, B.D. 1998. Antioxidative activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 46, 4113–4117.