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Antioxidant and Biocidal Activities of *Carum nigrum* (Seed) Essential Oil, Oleoresin, and Their Selected Components†

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In the present study, chemical constituents of the essential oil and oleoresin of the seed from *Carum nigrum* obtained by hydrodistillation and Soxhlet extraction using acetone, respectively, have been studied by GC and GC-MS techniques. The major component was dillapiole (29.9%) followed by germacrene B (21.4%), β-caryophyllene (7.8%), β-selinene (7.1%), and nothoapiole (5.8%) along with many other components in minor amounts. Seventeen components were identified in the oleoresin (**Table 2**) with dillapiole as a major component (30.7%). It also contains thymol (19.1%), nothoapiole (15.2.3%), and γ-elemene (8.0%). The antioxidant activity of both the essential oil and oleoresin was evaluated in mustard oil by monitoring peroxide, thiobarbituric acid, and total carbonyl and *p*-anisidine values of the oil substrate. The results showed that both the essential oil and oleoresin were able to reduce the oxidation rate of the mustard oil in the accelerated condition at 60 °C in comparison with synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene at 0.02%. In addition, individual antioxidant assays such as linoleic acid assay, DPPH scavenging activity, reducing power, hydroxyl radical scavenging, and chelating effects have been used. The *C. nigrum* seed essential oil exhibited complete inhibition against *Bacillus cereus* and *Pseudomonas aeruginosa* at 2000 and 3000 ppm, respectively, by agar well diffusion method. Antifungal activity was determined against a panel of foodborne fungi such as *Aspergillus niger*, *Penicillium purpurogenum*, *Penicillium madriti*, *Acrophialophora fusispora*, *Penicillium viridicatum*, and *Aspergillus flavus*. The fruit essential oil showed 100% mycelial zone inhibition against *P. purpurogenum* and *A. fusispora* at 3000 ppm in the poison food method. Hence, both oil and oleoresin could be used as an additive in food and pharmaceutical preparations after screening.

KEYWORDS: ***Carum nigrum*; essential oil; oleoresin; antioxidant activity; antimicrobial activity**

INTRODUCTION

Antioxidants are substances that, when added to food products, especially lipids and lipid-containing foods, can increase shelf life by retarding lipid peroxidation, which is one of the major processes producing deterioration of food products during processing and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been used as antioxidants since the beginning of the 20th century. However, restrictions on the use of these compounds are being imposed because of their carcinogenicity (*1*). Consequently, the need to identify alternative natural and safe sources of food antioxidant arose (*2*), and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (*3*). There are studies reported regarding the chemical constituents, antioxidant properties, and antimicrobial activity of *Carum* species (*4*−*6*), but there seems to be no report on *Carum nigrum* (*7*), which is categorized as a weed. In addition, the antioxidant potential of a particular substance varies according to the system in which the activity of the substance is studied. Hence, in the present investigation an attempt to study the antioxidant and antimicrobial properties of *C. nigrum* essential oil and oleoresin has been made. Different methods have been used to evaluate the antioxidant potential of caraway essential oil and oleoresin, and it would provide additional information to assess the antioxidant and antimicrobial activity of caraway.

MATERIALS AND METHODS

Chemicals. Diphenylpicrylhydrazyl (DPPH) and carbendazim were bought from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany);

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linoleic acid was from Acros (Fair Lawn, NJ); BHT, BHA, and 2,4-dinitrophenylhydrazine were purchased from s. d. Fine-chem Ltd., Mumbai, India; thiobarbituric acid, thymol, *p*-cymene, and carvacrol were received from Merck, Darmstadt, Germany. Ampicillin and cloxacillin were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India. Crude mustard oil was purchased from a local oil mill in Gorakhpur, India. All solvents used were of analytical grade.

Sample Extraction. There are 37 species of caraway reported in *Wealth of India* (*7*). Caraway seeds were purchased from a local spice market at Ooty (Hill Station), Tamil Nadu, India, during January 2004, and voucher specimens were kept at the Herbarium of the Faculty of Science, D.D.U. Gorakhpur University. Two hundred and fifty grams of caraway (100 mesh particle size) seeds was subjected to hydrodistillation using a Clevenger apparatus to yield the essential oil (2.1%).

Oleoresin was obtained by extracting 30 g of powdered spice with 300 mL of acetone for 3 h in a Soxhlet extractor. Evaporation of the solvent at reduced pressure furnished a viscous extract (3.9%). Both essential oil and oleoresin were stored in a freezer until further use.

Chemical Characterization. *Gas Chromatography (GC).* A Hewlett-Packard 5890 (Agilent Technologies, Buenos Aires, Argentina) gas chromatograph fitted with a capillary HP-5 column (5% phenyl methylsiloxane; length = 30 m, inner diameter = 0.32 mm, and film thickness = 0.25 μm) was used, with the injector and detector temperatures maintained at 240 and 250 °C, respectively. Injection volume was 1 μL with a split ratio 30:1; helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The oven temperature for both essential oil and extract was programmed linearly as follows: from 60 to 185 °C at 1.5 °C min^{-1}, held for 1 min, then again started at 9 °C min^{-1} to 275 °C. For RI measurements, the oven temperature program suggested by Adams (from 60 to 246 °C at 3 °C min^{-1}) was used (*8*).

Gas Chromatography−Mass Spectrometry (GC-MS). Analysis of caraway essential oil and oleoresin were run on a Hewlett-Packard 6890 GC-MS system (Agilent Technologies) coupled to a quadrupole mass spectrometer (model HP 5973) with an HP-5MS (5% phenyl methylsiloxane, length = 30 m, inner diameter = 0.25 mm, and film thickness = 0.25 μm) capillary column. The injector, GC-MS interface, ion source. and selective mass detector temperatures were maintained at 280, 280, 230, and 150 °C, respectively. Other conditions were as follows: ionization energy, 70 eV; injection size, 1.0 μL (in split mode); carrier gas, helium at a flow rate of 1.0 mL min^{-1}. The oven temperature for the essential oil was programmed linearly as follows: from 60 to 185 °C (1.5 °C min^{-1}), held at 185 °C (1 min), from 185 to 275 °C (9 °C min^{-1}), held at 275 °C (2 min). The oleoresin was held at 70 °C (5 min), programmed from 70 to 220 °C (3 °C min^{-1}) and from 220 to 280 °C (5 °C min^{-1}), and held at 280 °C for 5 min.

Components Identification. The components percentage was taken from capillary GC traces with FID. Identification of the individual components of essential oil and oleoresin was based on (a) comparison of their mass spectra and retention indices on an HP-5 column with published data (*8*, *9*), (b) computer matching with the Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC-MS system, and (c) co-injection with authentic samples (wherever possible). The retention index was calculated using a homologous series of *n*-alkanes.

Antioxidant Activity in Mustard Oil. *Sample Preparation.* The essential oil and oleoresin from seeds of caraway were added individually to crude (unrefined) mustard oil at a level of 0.02% (v/v). The initial peroxide value (PV) of oil is 1.1 mequiv of O_2/kg. Synthetic antioxidants (BHA and BHT) and selected components thymol and carvacrol were also added to the mustard oil at the level of 0.02% (v/v). Absolute ethanol (1 mL) was used for dilution of selected components and synthetic antioxidants. Oxidation was periodically assessed by the measurement of peroxide (PV), thiobarbituric acid (TBA), total carbonyl (TC), and *p*-anisidine values of the oil substrate.

PV and TBA Values. The rate of oil oxidation was monitored by the increase of peroxide values. Each oil sample was weighed (3 ± 0.1 g) and subjected to iodimetric determination (*10*). The TBA value of the samples was measured according to the method developed by Kikuzaki and Nakatani (*11*) with minor modifications. Oil substrate was weighed (10 ± 0.1 g), and 0.67% of aqueous TBA (20 mL) and benzene (25 mL) were added. The mixture was shaken in a wrist action shaker for 2 h and boiled in a water bath for 1 h. After cooling, the organic layer absorbance was measured at 540 nm on a Hitachi-U-2000 UV−visible spectrometer.

Total Carbonyl and p-Anisidine Values. Carbonyl and *p*-anisidine values were assessed according to methods described earlier (*12*, *13*). A calibration curve was drawn using capraldehyde (50−250 μg) in 50 mL of carbonyl-free benzene instead of the test sample. The TC values of the samples were calculated using the calibration curve and expressed as milligrams of capraldehyde per 100 g of sample. For the preparation of solutions, carbonyl-free benzene and alcohol were used. To measure the *p*-ansidine value of oil substrate, 0.5 ± 0.1 g of mustard oil was weighed in a 50 mL volumetric flask and the volume was made up with iso-octane; 5 mL of this solution was transferred into a test tube, and 1 mL of 0.25% *p*-anisidine reagent was added. Then it was kept in the dark for 10 min. The absorbance was measured at 350 nm (A_2). In the same manner, without the addition of reagent, absorbance was measured at 350 nm (A_1).

Individual Antioxidant Assays. *Antioxidant Activity in Linoleic Acid System.* The test samples of essential oil or oleoresin (20 μL) were dissolved in 1 mL of ethanol (v/v) mixed with linoleic acid (2.5%, v/v), 99.5% ethanol (4 mL, v/v), and 0.05 M phosphate buffer (pH 7, 4 mL). The solution was incubated at 40 °C for 175 h, and the degree of oxidation was measured every day according to the ferric thiocyanate method (*14*) with 75% ethanol (10 mL), 0.2 mL sample solution, and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) being added sequentially. A lesser value of absorbance indicates a higher the value of antioxidant capacity.

DPPH Assay. The DPPH assay was carried out as described by Cuendet and co-workers (*15*). Sample (5, 10, 15, 20, or 25 μL) was added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. The assay was carried out in triplicate, analyses of all samples were run in duplicate, and results are averaged.

Reducing Power. The reducing power was determined as described before (*16*). Various amount (5, 10, 15, and 20 μL) of essential oil or oleoresin (dissolved in 2.5 mL of methanol) were mixed with 2.5 mL of 200 mM phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After the addition of 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 200*g* for 10 min in a Sigma 3K30 model centrifuger. The organic layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride, and the absorbance was read at 700 nm in a UV−visible spectrophotometer.

Chelating Effect of Ferrous Ions. The chelating effect was determined according to the method of Shimada et al. (*17*). To 2 mL of the mixture consisting of 30 mM hexamine, 30 mM potassium chloride, and 9 mM ferrous sulfate were added 5, 10, 15, 20, or 25 μL of essential oil or oleoresin in methanol (5 mL) and 200 μL of 1 mM tetramethyl murexide. After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm. A lower absorbance indicates a higher chelating power. EDTA was used as a positive control.

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the sample for hydroxyl radicals generated from the Fe^{3+}/EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBA reactive substances formation (*18*). Sample (5, 10, 15, 20, or 25 μL; essential oil or oleoresin) was added to the reaction mixture containing 3 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2, and 20 mM phosphate buffer (pH 7.4), making up the final volume to 3 mL. The reaction mixture was incubated at 37 °C for 1 h. The formed TBARS were measured by using a method (*19*) reported earlier. One milliliter of 1% TBA and 1 mL of 2.8% trichloroacetic acid were added to test tubes and incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were conducted in triplicate. The percent inhibition (*I*) of deoxyribose degradation was measured.

Antimicrobial Activity. *Antibacterial Assay.* The following food-borne bacteria, *Staphylococcus aureus* (MTCC 3103), *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 1790), *Escherichia coli* (MTCC 1672), *Salmonella typhimurium* (MTCC 733), and *Pseudomonas aeruginosa* (MTCC 1942), obtained from Microbial Type Culture

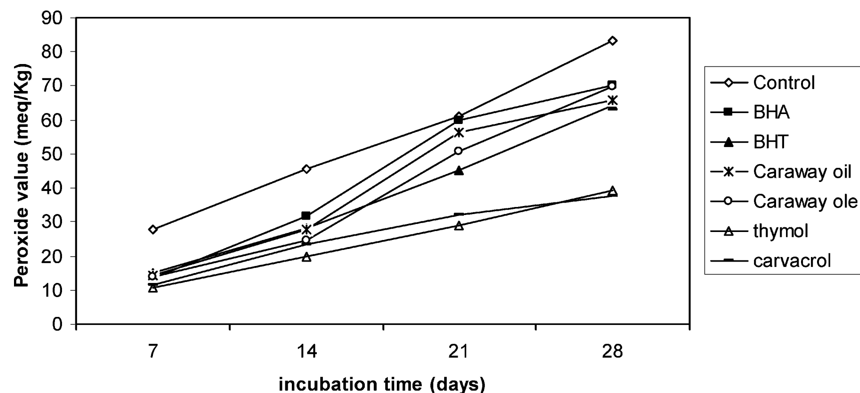

Figure 1. Antioxidant activities of essential oil and oleoresin in mustard oil in terms of peroxide value.

Table 1. Chemical Composition of *C. nigrum* Essential Oil Analyzed by GC-MS

compound	% FID	RI[a]	identification[b]
α-thujene	trace[c]	931	MS, RI, co-GC
α-pinene	trace	940	MS, RI, co-GC
camphene	trace	954	MS, RI, co-GC
sabinene	0.1	975	MS, RI, co-GC
β-pinene	0.1	980	MS, RI, co-GC
myrcene	trace	992	MS, RI, co-GC
α-phellandrene	trace	1004	MS, RI, co-GC
p-cymene	trace	1026	MS, RI, co-GC
limonene	0.1	1030	MS, RI, co-GC
β-phellandrene	trace	1031	MS, RI, co-GC
1,8-cineole	trace	1032	MS, RI, co-GC
γ-terpinene	0.1	1062	MS, RI, co-GC
p-cresol	0.2	1076	MS, RI, co-GC
terpinolene	trace	1089	MS, RI, co-GC
camphor	0.2	1147	MS, RI, co-GC
borneol	0.2	1170	MS, RI, co-GC
terpinen-4-ol	trace	1179	MS, RI, co-GC
cuminal	1.3	1242	MS, RI
bornyl acetate	1.8	1289	MS, RI, co-GC
thymol	0.2	1290	MS, RI, co-GC
cuminol (*p*-cymen-7-ol)	0.2	1293	MS, RI, co-GC
citronellyl acetate	0.2	1353	MS, RI, co-GC
α-copaene	trace	1377	MS, RI
geranyl acetate	trace	1381	MS, RI, co-GC
β-elemene	2.7	1392	MS, RI
β-caryophyllene	7.8	1420	MS, RI, co-GC
cuminyl acetate	1.6	1432	MS, RI
γ-elemene	4.0	1438	MS, RI
α-humulene	0.3	1456	MS, RI, co-GC
trans-β-farnesene	0.4	1457	MS, RI
germacrene D	0.6	1484	MS, RI
β-selinene	7.1	1491	MS, RI
α-selinene	1.8	1499	MS, RI
germacrene-A	0.5	1507	MS, RI
β-bisabolene	0.1	1512	MS, RI
cis-γ-bisabolene	0.4	1514	MS, RI
myristicin	0.5	1520	MS, RI, co-GC
trans-α-bisabolene	0.1	1544	MS, RI
germacrene B	21.4	1561	MS, RI, co-GC
spathulenol	0.2	1579	MS, RI, co-GC
caryophyllene oxide	0.3	1583	MS, RI, co-GC
dillapiole	29.9	1622	MS, RI, co-GC
apiole	1.1	1671	MS, RI, co-GC
α-bisabolol	0.5	1684	MS, RI, co-GC
isospathulenol	0.3	1706	MS, RI
nothoapiole[d]	5.8	1759	MS, RI
total	91.5		

[a] The retention index was calculated using a homologous series of *n*-alkanes C_8–C_{18}. [b] Co-GC, co-injection with an authentic sample. [c] Trace, <0.05. [d] Systematic name: 1,2-methylenedioxy-3,5,6-trimethoxy-4-allylbenzene, CAS Registry No. 22934-74-3.

Table 2. Chemical Composition of *C. nigrum* Oleoresin Analyzed by GC-MS

compound	% FID	RI[a]	identification[b]
p-cymene	3.2	1026	MS, RI, co-GC
p-cresol	1.4	1076	MS, RI, co-GC
bornyl acetate	0.5	1289	MS, RI, co-GC
thymol	19.1	1290	MS, RI, co-GC
carvacrol	1.3	1299	MS, RI, co-GC
β-elemene	1.1	1392	MS, RI
β-caryophyllene	2.4	1420	MS, RI, co-GC
γ-elemene	8.0	1438	MS, RI
β-selinene	4.5	1491	MS, RI
myristicin	1.5	1520	MS, RI, co-GC
dillapiole	30.7	1622	MS, RI, co-GC
apiole	1.3	1671	MS, RI, co-GC
nothoapiole[c]	15.2	1759	MS, RI
neophytadiene	0.3		MS
palmitic acid	0.5		MS, co-GC
phytol	0.6		MS
nonacosane	0.2		MS
total	91.8		

[a] The retention index was calculated using a homologous series of *n*-alkanes C_8–C_{18}. [b] Co-GC, co-injection with authentic sample. [c] Systematic name: 1,2-methylenedioxy-3,5,6-trimethoxy-4-allylbenzene; CAS Registry No. 22934-74-3.

Collection (MTCC), Institute of Microbial Technology, Chandigarh, India, were used for the present study. The agar well diffusion method was used to investigate antimicrobial properties of both essential oil and oleoresin. The strains were activated on nutrient agar media and stored at 4 °C. The bacterial strain was further diluted using Ringer's solution. Solution concentrations of 1000, 2000, and 3000 ppm of essential oil and oleoresin (or selected pure components) were prepared in absolute ethanol; 50 μL aliquots of diluted sample were placed into agar wells (9 mm diameter), and plates were incubated at 37 °C. All of the plates were replicated twice, and the results were averaged.

Antifungal Assay. The essential oil and oleoresin (or selected pure components) were individually tested against a panel of foodborne fungi such as *Aspergillus niger* (MTCC 2479), *Penicillium purpurogenum* (MTCC 1786), *Penicillium madriti* (MTCC 3003), *Acrophialophora fusispora* (MTCC 341), *Penicillium viridicatum* (MTCC 2007), and *Aspergillus flavus* (MTCC 1884). The antifungal activity was assessed by two different methods.

Poison Food Technique. This method plays an important role in bioassay methods (*20*) to evaluate antimicrobial activity. The calculated amount of sample was added to molten Czapek dox agar (CDA) medium (≈45 °C) to obtain the desired concentration. The pathogen of interest from the growing tips (punched in a fungal mat grown on CDA medium in sterile Petri dishes) was placed at the center, and all plates were incubated at 37 °C. Radial growth in terms of diameter (millimeters) was examined after 5 days.

Inverted Petri Dish Method. This method (*21*) allows the determination of antifungal activity of compounds in the vapor phase. In this method, a calculated quantity of sample was soaked on filter paper (Whatman no. 1, 10 mm diameter) and kept at the center of the lid of the inverted Petri dish. Control agar plates were prepared under the same conditions except for the addition of test compound. All of the plates were incubated at 37 °C, and results were measured in terms of diameter (millimeters) after 5 days.

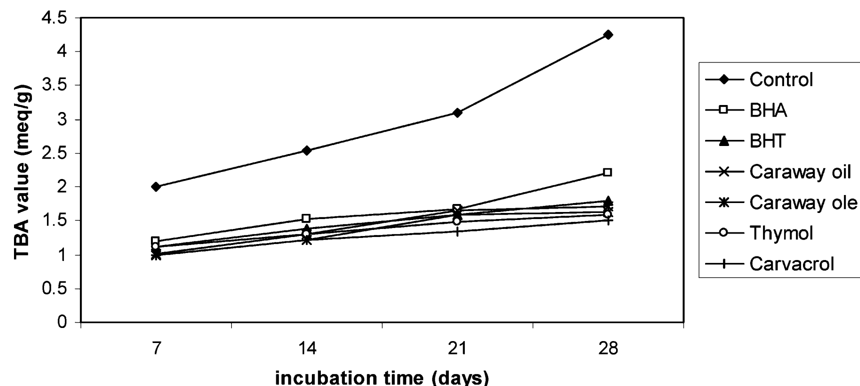

Figure 2. Antioxidant activities of essential oil and oleoresin in mustard oil in terms of thiobarbituric acid value.

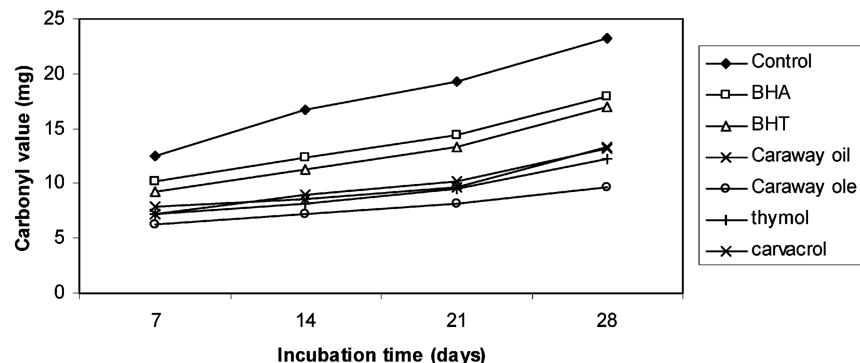

Figure 3. Antioxidant effect of caraway essential oil and oleoresin at 0.02% level in terms of carbonyl value.

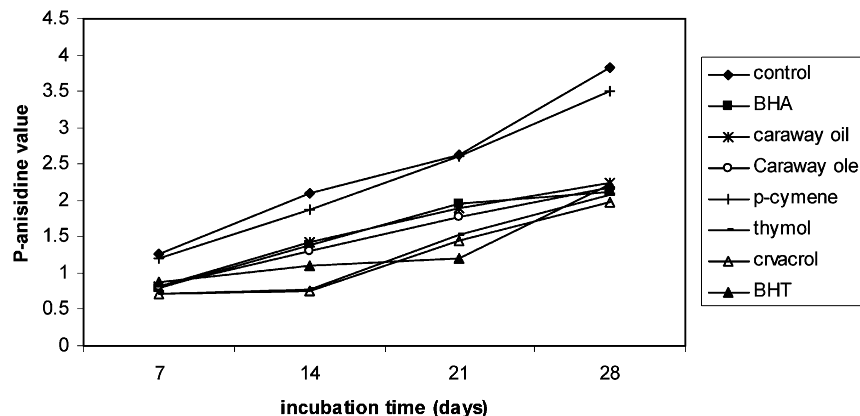

Figure 4. Antioxidant effect of caraway essential oil and oleoresin at 0.0 2% level in terms of *p*-anisidine value.

Statistical Analysis. For the essential oil or oleoresin, three samples were prepared for assays of every antioxidant and antimicrobial attribute. The data are presented as mean ± standard deviation of three determinations (data are not shown). Statistical analyses were performed using a one-way analysis of variance (*22*). A probability value of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Chemical Component Analysis. Chemical characterization of the essential oil (**Table 1**) resulted in the identification of 46 components. The major component was dillapiole (29.9%) followed by germacrene B (21.4%), β-caryophyllene (7.8%), β-selinene (7.1%), and nothoapiole (5.8%) along with many other components in minor amounts. Seventeen components were identified in the oleoresin (**Table 2**) with dillapiole as a major component (30.7%). It also contains thymol (19.1%), nothoapiole (15.2.3%), and γ-elemene (8.0%).

Antioxidant Activity in Mustard Oil. The addition of natural and synthetic antioxidant to mustard oil affected, to different degrees, the peroxide and TBA values during accelerated oxidation at 60 °C for 28 days (**Figures 1** and **2**) of storage. PV measures primary products of lipid oxidation, and TBA measures the formation of secondary oxidation products, mainly malonaldehyde, which may contribute off-flavors to oxidized oil (*23*). All samples with essential oil and oleoresin were more stable on heating at 60 °C than the control, when assessed by the change in peroxide (**Figure 1**) and TBA (**Figure 2**) values. In addition, secondary oxidation products such as carbonyls and 2-alkenals have been measured at the same time interval in terms of total carbonyl (**Figure 3**) and *p*-anisidine (**Figure 4**) values. The results agreed well with PV and TBA values, and they proved a stabilization effect of essential oil and oleoresin in the mustard oil system.

Antioxidant Activity by Individual Assays. When linoleic acid was readily oxidized by incubation at 40 °C for up to 175 h, concentrations of peroxides that were measured at 500 nm were abruptly increased in the control sample after 50 h (**Figure 5**). However, addition of essential oil and oleoresin effectively

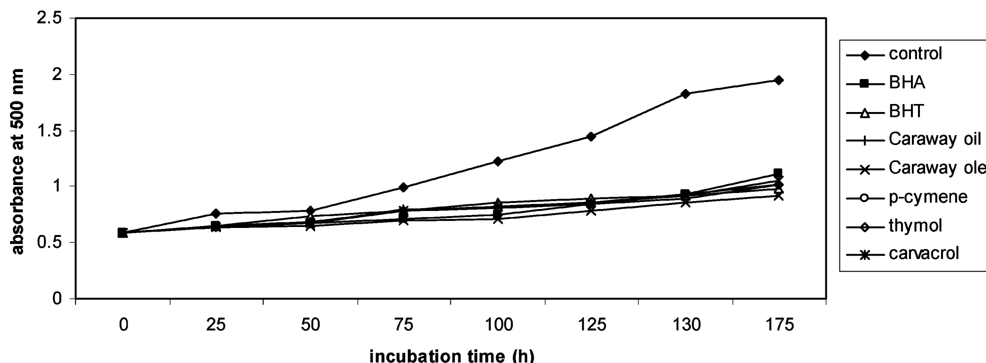

Figure 5. Antioxidant activity of caraway essential oil and oleoresin compared to standard antioxidants (BHA and BHT) in linoleic system.

Table 3. Changes in DPPH Radical Scavenging Activities According to Different Concentrations of Essential Oil and Oleoresin

	DPPH[a] radical scavenging activity (%)				
sample (μL)	BHA	BHT	caraway oil	caraway oleoresin	thymol
5	83.45	85.2	41.21	50.32	70.31
10	90.31	89.01	57.81	58.39	75.11
15	92.42	91.32	63.65	73.14	78.25
20	95.71	94.71	68.15	76.91	82.05
25	98.41	96.91	72.21	79.79	86.41

[a] Values represent means (DPPH) ($n = 2$). In all cases relative error was <9%.

Table 4. Reducing Power of Essential Oil and Oleoresin Expressed in Percentage along with Selected Component

	reducing power[a] (%)				
sample (μL)	BHA	BHT	caraway oil	caraway oleoresin	thymol
5	63.25	65.2	21.61	24.32	60.33
10	71.31	69.01	27.56	29.39	65.22
15	75.22	74.32	33.15	34.04	73.08
20	79.01	81.71	38.55	42.44	79.05
25	85.51	87.91	41.01	51.78	84.81

[a] Values represent means ($n = 2$). In all cases relative error was <9%.

suppressed the peroxide concentration in emulsion during incubation ($p < 0.05$).

The scavenging effect of the essential oil (41.21−71.21%) and oleoresin (50.32−79.79%) on DPPH radical linearly increased with increasing concentration (**Table 3**). Both essential oil and oleoresin showed strong percent scavenging activity in comparison with BHA (83.45−98.21%) and BHT (85.2−96.91%) at 5, 10, 15, and 20 mg levels. Reducing powers of both essential oil and oleoresin were moderate and increased with increasing concentration (**Table 4**). However, essential oil and oleoresin possess low scavenging and reducing power effects at lower concentration in comparison with synthetic antioxidants. The reducing power of essential oil and oleoresin might be due to their hydrogen-donating ability (*24*) and is generally associated with the presence of reductones (*25*). The component presents in the essential oil and oleoresin could act as good reductants, which could react with free radicals to stabilize and terminate radical chain reactions.

The chelating effect of the essential oil on ferrous ions was 17.75% at 5 μL and increased to 33.77% at 20 μL, and a similar trend has been observed for oleoresin (**Figure 6**). However, the chelating ability of EDTA was 95.26% at 20 μL. Apparently, the essential oil and oleoresin could chelate ferrous ions but were not as effective chelators as EDTA. Because ferrous ions are the most effective pro-oxidants in food systems (*26*), the

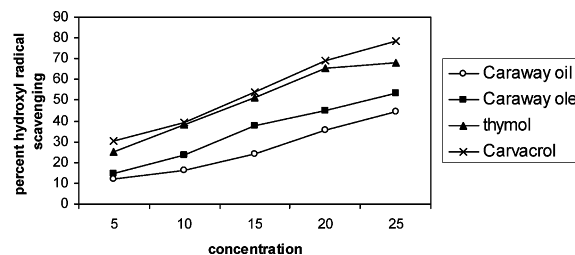

Figure 6. Chelating ability of essential oil and oleoresin.

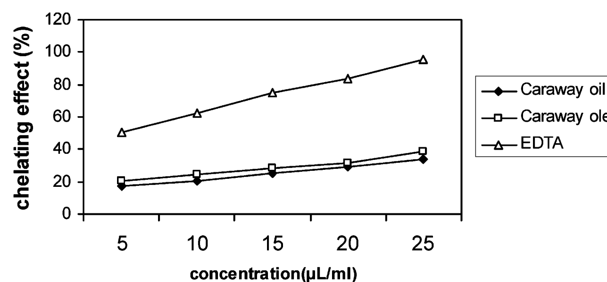

Figure 7. Hydroxyl radical scavenging effect of essential oil and oleoresin in comparison with selected components.

moderate to high chelating effects of caraway essential oil and oleoresin would be beneficial. The hydrogen peroxide scavenging activity of the essential oil and oleoresin is presented in **Figure 7**. The essential oil and oleoresin exerted a concentration-dependent hydroxide radical scavenging. Scavenging activities of essential oil and oleoresin at 10 μL were 16.1 and 23.76%, respectively. The concentration of hydrogen peroxide in water may vary according to the phenolic compounds. Phenolic compounds present in the oleoresin may be good electron donors, and they may accelerate the conversion of H_2O_2 to H_2O (*27*). Phenolic compounds tested in this study were able to form complexes with Fe^{3+} according to the EDTA assay of deoxyribose degradation.

It has been stated that phenolic groups play an important role in antioxidant activity (*28*). The presence of phenolic compounds such as thymol (19.22%), *p*-cresol (2.43%), and carvacrol (2.19%) could be responsible for the strong antioxidant activity of caraway oleoresin (**Table 2**). It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defense system against free radical attack (*28*).

Antimicrobial Studies. The results obtained for antibacterial activity of essential oil, oleoresin, and selected components are reported in **Table 5**. Caraway essential oil exhibited complete inhibition against *B. cereus* and *P. aeruginosa* at 2000 and 3000 ppm, respectively. Both oil and oleoresin have shown good to

Table 5. Antibacterial Activity of Caraway Essential Oil and Oleoresin by Agar Well Diffusion Method

		inhibition zone[a] (mm)					
		Gram-positive bacteria			Gram-negative bacteria		
test	concentration[b] (ppm)	BS	SA	BC	EC	ST	PA
caraway essential oil	1000	16.1 ± 0.4	26.1 ± 1.5	70.4 ± 1.9	12.0 ± 0.2	12.5 ± 0.8	61.7 ± 0.6
	2000	20.0 ± 0.6	34.9 ± 1.3	+	18.2 ± 1.1	14.6 ± 1.1	+
	3000	21.6 ± 1.2	48.7 ± 0.5	+	21.8 ± 0.5	17.9 ± 0.2	+
caraway oleoresin	1000	20.6 ± 1.2	27.1 ± 0.1	64.5 ± 0.6	25.4 ± 0.6	53.6 ± 1.3	40.5 ± 0.1
	2000	38.0 ± 0.2	38.9 ± 0.2	80.4 ± 1.1	31.1 ± 0.7	73.8 ± 0.5	51.4 ± 0.8
	3000	49.4 ± 0.8	49.3 ± 2.2	+	38.5 ± 1.1	78.1 ± 0.8	60.8 ± 0.1
cloxacillin	1000	–	22.7 ± 0.6	12.1 ± 0.2	–	–	–
	2000	–	26.4 ± 1.1	13.2 ± 1.1	–	12.4 ± 0.8	–
	3000	–	30.2 ± 1.0	15.4 ± 0.5	–	16.4 ± 0.4	–
thymol	1000	25.9 ± 1.2	60.8 ± 1.9	62.3 ± 0.5	–	60.2 ± 1.2	–
	2000	33.5 ± 0.1	+	78.5 ± 1.0	12.5 ± 0.2	66.2 ± 0.1	–
	3000	45.2 ± 0.3	+	86.3 ± 1.4	17.8 ± 0.4	74.6 ± 0.0	17.9 ± 0.7
p-cymene	1000	–	30.2 ± 0.6	45.7 ± i.2	–	17.9 ± 0.3	60.2 ± 0.5
	2000	–	39.8 ± 1.2	51.3 ± 1.3	–	25.8 ± 0.6	64.8 ± 1.4
	3000	16.9 ± 0.2	51.2 ± 0.3	57.1 ± 0.1	11.6 ± 0.1	32.2 ± 1.7	72.1 ± 1.5

[a] Average of three replicates. BS, *Bacillus subtilis*; SA, *Staphylococcus aureus*; BC, *Bacillus cereus*; EC, *Escherichia coli*; ST, *Salmonella typhimurium*; PA, *Pseudomonas aeruginosa*. "+" indicates complete inhibition, and "–" indicates no inhibition. [b] DMSO was for dilution.

Table 6. Antifungal Effect of Caraway Essential Oil and Oleoresin against Different Foodborne Pathogenic Fungi by Poison Food Medium Method

		% zone inhibition[a]					
test	concentration[b] (ppm)	2479	1786	3003	341	2007	1884
caraway essential oil	1000	58.8 ± 1.4	85.1 ± 0.2	50.4 ± 1.1	62.5 ± 0.1	40.7 ± 0.2	60.1 ± 1.2
	2000	67.5 ± 0.1	90.2 ± 1.2	56.3 ± 0.4	81.3 ± 1.2	53.1 ± 1.2	73.1 ± 2.1
	3000	75.3 ± 0.2	100.5 ± 1.3	68.8 ± 0.7	100.0 ± 0.5	62.5 ± 2.2	79.8 ± 1.9
caraway oleoresin	1000	17.6 ± 1.2	80.9 ± 0.3	37.5 ± 0.5	62.5 ± 0.5	70.2 ± 1.3	70.2 ± 0.1
	2000	29.4 ± 1.2	84.8 ± 0.2	62.6 ± 1.1	82.5 ± 1.9	86.1 ± 0.1	51.4 ± 0.8
	3000	38.9 ± 0.2		16.2 ± 1.1	100.2 ± 2.1	100.2 ± 2.1	81.9 ± 2.1
carbendazim[c]	1000	8.2 ± 0.6		24.2 ± 0.2	30.2 ± 2.1		
	2000	12.4 ± 2.2		31.2 ± 0.1	36.4 ± 0.3	12.6 ± 0.5	16.7 ± 1.2
	4000	16.5 ± 0.3	30.2 ± 1.0	15.4 ± 0.5	43.1 ± 0.5	17.4 ± 0.3	21.8 ± 1.1
thymol	1000	25.1 ± 0.1		62.3 ± 0.5	20.2 ± 0.7	60.2 ± 1.2	60.2 ± 0.3
	2000	56.5 ± 1.1	11.2 ± 0.2	12.5 ± 1.0	46.8 ± 0.1		74.2 ± 0.7
	4000	86.2 ± 0.7	17.1 ± 0.9	31.4 ± 0.5	65.3 ± 0.3		92.4 ± 1.6
p-cymene	1000	60.1 ± 1.2	16.6 ± 0.5		31.2 ± 1.1	40.1 ± 0.2	
	2000	72.9 ± 0.3	25.7 ± 1.1		42.7 ± 0.5	47.2 ± 0.5	16.7 ± 2.1
	4000	84.1 ± 0.2	37.2 ± 0.4	37.2 ± 2.1	55.4 ± 0.2	59.1 ± 2.2	21.8 ± 0.9

[a] Average of three replicates. 2479, *Aspergillus niger*; 1786, *Penicillium purpurogenum*; 3003, *Penicillium madriti*; 341, *Acrophialophora fusispora*; 2007, *Penicillium viridicatum*; 1884, *Aspergillus flavus*. [b] DMSO was used for dilation. [c] Aqueous solution was used.

Table 7. Antifungal Effect of Caraway Essential Oil and Its Oleoresin against Different Foodborne Pathogenic Fungi by Inverted Petri Dish Method

		% zone inhibition[a]					
test	concentration[b] (ppm)	2479	1786	3003	341	2007	1884
caraway essential oil	1000	46.7 ± 1.1	50.4 ± 0.3	30.7 ± 0.1	14.3 ± 0.1	37.5 ± 0.5	12.5 ± 0.9
	2000	66.6 ± 0.2	56.3 ± 1.3	45.8 ± 0.9	27.1 ± 2.2	43.7 ± 0.2	37.5 ± 1.1
	3000	100.2 ± 0.2	62.9 ± 0.3	69.3 ± 0.7	34.4 ± 2.1	68.7 ± 2.1	62.5 ± 1.2
caraway oleoresin	1000	8.8 ± 1.0	52.5 ± 1.9	12.5 ± 0.5	14.3 ± 0.5	37.5 ± 1.3	62.5 ± 0.8
	2000	26.7 ± 0.2	57.1 ± 0.2	17.8 ± 1.7	21.4 ± 0.9	50.0 ± 1.1	78.9 ± 0.8
	3000	34.7 ± 0.6	60.4 ± 2.1	21.2 ± 0.6	40.1 ± 2.0	63.8 ± 2.0	100.1 ± 2.0

[a] Average of three replicates. 2479, *Aspergillus niger*; 1786, *Penicillium purpurogenum*; 3003, *Penicillium madriti*; 341, *Acrophialophora fusispora*; 2007, *Penicillium viridicatum*; 1884, *Aspergillus flavus*. [b] DMSO was used for dilation.

moderate activity against other tested bacterial strains. The selected component thymol has given complete inhibition at 2000 and 3000 ppm. *p*-Cymene has shown strong antibacterial activity against *P. aeruginosa*.

The antifungal activity results for both essential oil and oleoresin obtained by the poison food and inverted Petri dish methods are reported in **Tables 6** and **7**. The essential oil has shown 100% mycelial zone inhibition against *P. purpurogenum* and *A. fusispora* at 3000 ppm concentration in the poison food method (**Table 6**). Caraway oleoresin has also exhibited 100% clear zone inhibition at 3000 ppm against *A. fusispora* and *P. viridicatum*. Both oil and oleoresin have shown moderate to strong antifungal activity for other tested fungal isolates. The selected components thymol and *p*-cymene have shown strong

antifungal activity against *A. niger* at 3000 ppm. The essential oil has shown 100% activity for *A. niger* and *A. flavus* at 3000 ppm in the inverted Petri dish method (**Table 7**). In general, the poison food method gave the best results for both oil and oleoresin in comparison with the inverted Petri dish method.

Antifungal activity of the essential oil and oleoresin against the present studied fungal isolates is reported for the first time. Most of the antimicrobial activity in essential oils derived from spices and culinary herbs derives from phenolic compounds (*29*), whereas other constituents are believed to contribute little to the antimicrobial effects (*30*). It is clear that oleoresin (**Table 2**) contains considerable amounts of phenolic compounds (thymol, carvacrol, and *p*-cresol), and hence the antimicrobial activity could be due to them. The lower efficacy of this essential oil and oleoresin against some microorganisms in the present study might have been due to the low activity of their main constituents against particular fungi or bacteria. It is likely that antifungal effects of the essential oil and oleoresin result from the synergistic action of all their components (*31*). Such synergistic or antagonistic action probably occurred with this essential oil or oleoresin. The strength of inhibition and the spectrum of antimicrobial activity of the caraway essential oil and oleoresin suggest that complex interactions between individual components led to the overall activity. It is not completely clear why Gram-negative bacteria should be less susceptible, but it may be associated with the outer membrane of Gram-negative bacteria that endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (*32*). In this respect, we believe that the methods used to evaluate antioxidant and antimicrobial activity would provide additional information to assess the antimicrobial properties of caraway essential oil and oleoresin.

The results of the present work indicate that the caraway essential oil and oleoresin possess high antioxidant activity and free radical scavenging activity. These assays are useful for establishing the ability of phenolics to chelate and reduce Fe^{3+} and have important applications for the pharmaceutical and food industries. The essential oil and oleoresin have also shown a broad spectrum of antimicrobial activity against tested fungal and bacterial isolates. However, further investigation of individual phenolic and other components, in vivo, and the antioxidant activity mechanism is warranted. These studies can be useful as a starting point for further applications of caraway essential oil and oleoresin and their constituents in food and pharmaceutical preparations.

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