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Antioxidant Activity of *Nigella sativa* Essential Oil

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The essential oil of black cumin seeds, *Nigella sativa* L., was tested for a possible antioxidant activity. A rapid evaluation for antioxidants, using two TLC screening methods, showed that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging property. These four constituents and the essential oil possessed variable antioxidant activity when tested in the diphenylpicrylhydracyl assay for non-specific hydrogen atom or electron donating activity. They were also effective ·OH radical scavenging agents in the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay.

GC-MS analysis of the essential oil obtained from six different samples of *Nigella sativa* seeds and from a commercial fixed oil showed that the qualitative composition of the volatile compounds was almost identical. Differences were mainly restricted to the quantitative composition. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Nigella sativa L.; essential oil; antioxidant activity; DPPH assay; ·OH radicals; GC-MS-analysis.

INTRODUCTION

The seeds of *Nigella sativa* L., an annual Ranunculaceae herbaceous plant, have been used traditionally for centuries in the Middle East, Northern Africa and India for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza, eczema, as a diuretic, lactagogue and vermifuge. Furthermore, black cumin seeds are of importance as a carminative and spice, often they are used as a condiment in bread and other dishes (Lautenbacher, 1997; Merfort *et al.*, 1997; Eschborn, 1997; Aboutabl *et al.*, 1986).

The black, angular seeds, in Arabia known as ‘Habbah Sauda’, ‘Habbet el Baraka’, ‘Kamun-aswad’ and ‘Shunez’ (Houghton *et al.*, 1995), contain 36%–38% fixed oil (‘Al Amin’, ‘Al-Khaial’), proteins, alkaloids, saponins and 0.4%–2.5% essential oil (Lautenbacher, 1997).

The generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Gutteridge and Halliwell, 1994; Maxwell, 1995; Sies, 1991). Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases such as atherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS (Halliwell and Gutteridge, 1989). Since *Nigella* seeds are used for the treatment of inflammatory diseases it was reasonable to investigate the ability of the volatile oil to act as a radical scavenger. Various pharmacological tests were carried out to investigate different compounds of black cumin seeds (El-Dakhakhny, 1965; Mahfouz *et al.*, 1965; Marozzi *et al.*, 1970; Akhtar *et al.*, 1996; Khanna *et al.*, 1993). However, little is known about the activity of the volatile oil of *N. sativa* (Mahmoud and Shaheen, 1996; El-Kamali *et al.*, 1998). Preliminary experiments (Houghton *et al.*, 1995) showed that both the fixed oil and thymoquinone, the main compound of the essential oil, inhibit non-enzymatic lipid peroxidation in liposomes. The aim of the present study was to evaluate the antioxidant properties of the essential oil of black cumin seeds and to find out which components contribute to this effect. In addition the main compounds of several seed samples were examined by GC-MC analysis.

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MATERIALS AND METHODS

Plant material. The material used in studies for antioxidant activity were commercial seeds from Ritzberger (Linz, Austria). For GC-analysis three samples of seeds were bought from wholesalers (Ritzberger, Linz and Kottas, Vienna), and three specimens of *N. sativa* were purchased from markets in Graz and Vienna. Voucher specimens are deposited at the herbarium of the Institute of Pharmacognosy, University of Graz. The fixed oil of black cumin seeds was bought from Gall-Pharma (Judenburg, Austria).

Chemicals. Chemicals were purchased from Fluka (Buchs, Switzerland). Diphenylpicrylhydrazyl, thymoquinone and bovine brain phospholipids were bought from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Carvacrol, 4-terpineol and t-anethole were bought from Dragoco (Vienna, Austria) and quercetin was from Roth (Karlsruhe, Germany). TLC was carried out on silica gel F_{254} aluminium sheets (Merck, Darmstadt, Germany).

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Extraction of the oil. Crushed seeds were continuously extracted with light petroleum (BP 40°–60 °C) using a Soxhlet apparatus. The solvent was removed under vacuum and the brownish residue was steam distilled. Extraction of the aqueous distillate with *n*-hexane and removal of the solvent gave the volatile oil.

In comparison the oil was also isolated through hydrodistillation using the apparatus of the Austrian pharmacopoea (Clevinger apparatus). Through this process only a very small amount of essential oil was obtained, but the main disadvantage was that the percentage content of thymoquinone was only 3% instead of 48% obtained using Soxhlet extraction. For this reason the volatile oil for the experiments was isolated by the procedure described above and not by hydrodistillation.

From the commercial fixed oil a volatile fraction was obtained by steam distillation.

GC-MS analysis. In the present work both volatile oil samples from six different samples of seeds and the volatile fraction obtained from a commercial fixed oil were analysed and their qualitative and quantitative composition compared.

Analyses were carried out on a Hewlett-Packard 5890 Series II Plus gas chromatograph interfaced to a Hewlett Packard 5989B mass spectrometer. Separations were performed on Ultra 1 (49 m × 0.20 mm I.D., 0.11 µm, Hewlett-Packard) and DB-Wax (60 m × 0.25 mm I.D., 0.25 µm, J&W Scientific) capillary columns. Helium was used as a carrier gas (1.0 mL/min C.F.) and the oven temperature was programmed as 70° to 230 °C with a heating rate of 2 °C/min. Injector and interface temperatures were 230 °C and 250 °C, respectively. EI mass spectra were recorded at 70 eV ionization voltage over the mass range 40–400 u. Samples (0.5 µL of oil solutions 1:10 in hexane) were injected by split injection (1:33).

Temperature programmed retention indices of the compounds were determined relative to the retention times of a series of *n*-alkanes.

Identification of the compounds was accomplished using a mass spectra library (Wiley 138K and laboratory own database) as well as retention indices as an auxiliary confirmation tool (Bucar and Schweiger, 1998).

Rapid screening for antioxidants. The diluted volatile oil (5 µL, 1:10 in hexane) was spotted on silica gel sheets and developed in toluene–ethylacetate (97:3 v/v). Spraying with β-carotene–linoleic acid reagent (Pratt and Miller, 1984) and a 0.2% solution of the stable radical diphenylpicrylhydrazyl, DPPH (Cuendet *et al.*, 1997; Kirby and Schmidt, 1997), on another plate detected four potential radical scavenging compounds.

DPPH assay. The DPPH test was carried out as described before (Cuendet *et al.*, 1997; Kirby and Schmidt, 1997). 50 µL of various dilutions of the essential oil and its active main compounds were mixed with 5 mL of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm using a Jasco 7800 spectrophotometer. Butylhydroxytoluene (BHT), quercetin and ascorbic acid were used as positive controls.

Assay for non-enzymatic lipid peroxidation in liposomes. The experiment was carried out as described by Houghton *et al.* (1995) with small modifications. Phospholipid liposomes were prepared from Type VII Folch bovine brain extract (Sigma) by mixing with KH_2PO_4–K_2HPO_4 buffer (5 mg/mL) and storing at 4 °C for 2 days. Before the test it was sonicated under cooling with ice until a milky solution was obtained. The reaction mixture contained in a final volume of 1.0 mL 500 µL of this suspension, 300 µL buffer, containing the compounds tested (dissolved in buffer/Tween 80, which was used in a maximum concentration of 1.5% of the reaction mixture), 100 µL of $FeCl_3$ (1 mM) and 100 µL ascorbic acid (1 mM) to start peroxidation. Samples were incubated at 37 °C for 1 h, after that liposome lipid peroxidation was measured using the reaction with thiobarbituric acid, TBA (Houghton *et al.*, 1995; Aruoma *et al.*, 1989). 1.0 mL of TBA (1% in 50 mM NaOH), 1.0 mL trichloracetic acid, TCA, (2.8%) and 100 µL BHT (2% in ethanol) were added and tubes were heated at 100 °C for 20 min. After cooling 2.5 mL of *n*-butanol was added and reaction mixtures were centrifuged at 3500 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. All reactions were carried out in triplicate. Quercetin was used as a positive control. All reagents were prepared freshly.

Inhibition (I) of lipid peroxidation in percent was calculated by following equation:

$$I(\%) = 100\times\ (A_0 - A_1/A_0)$$

where A_0 was the absorbance of the control reaction (= full reaction, containing no test compound) and A_1 was the absorbance in the presence of the inhibitor.

Deoxyribose assay. The assay was performed as described by Halliwell *et al.* (1987) with minor changes. All solutions were prepared freshly. 1.0 mL of the reaction mixture contained 100 µL of 28 mM 2-deoxy-2-ribose (Fluka, dissolved in KH_2PO_4–K_2HPO_4 buffer pH 7.4), 500 µL solution of various concentrations of the oil or the other four compounds (in buffer and Tween 80), 200 µL of 200 µM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 µL H_2O_2 (1.0 mM) and 100 µL ascorbic acid (1.0 mM). After an incubation period of 1 h at 37 °C the extent of deoxyribose degradation was measured by the TBA reaction. 1.0 mL of TBA (1% in 50 mM NaOH) and 1.0 mL of TCA were added to the reaction mixture and the tubes were heated at 100 °C for 20 min. After cooling the absorbance was read at 532 nm against a blank (containing only buffer and deoxyribose).

The absorbance (A_1) read at the end of the experiment was used for the calculation of the percentage inhibition of deoxyribose degradation by the test compound. Calculations were done as mentioned before.

The IC_{50} value represented the concentration of the compounds, that caused 50% inhibition. All experiments were carried out in triplicate. Quercetin was used as a positive control.

Assay for site-specific reactions. This assay presented only another feature of the deoxyribose method. To assess site-specific actions (Halliwell *et al.*, 1987; Aruoma, 1996a; Aruoma *et al.*, 1997) it was adapted in the following three ways:

1. The deoxyribose assay was carried out as mentioned before, except that 100 µL of $FeCl_3$ instead of 200 µL of Fe^{3+}-EDTA-solution was added.

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2. The test was performed without ascorbic acid as a starter for oxidation.
3. Deoxyribose itself was omitted from the assay. The lacking volume was always completed with buffer.

RESULTS

GC-MS analysis

The isolation of the essential oil by Soxhlet extraction and hydrodistillation gave a yield of 0.41%–0.44%. The percentage content of thymoquinone was between 28% and 57%. Through distillation with the Clevinger apparatus 0.18% volatile oil was obtained and its thymoquinone content was only 3%. Only oil samples obtained by Soxhlet extraction/steam distillation were used for the *in vitro* tests.

Seven samples of essential oil of *N. sativa*, which were all obtained by Soxhlet extraction and steam distillation, were investigated by GC-MS. 32 compounds were identified. As shown in Table 1 the main fraction of every volatile oil examined consisted of a mixture of monoterpenes. The main compounds were thymoquinone (30%–48%), p-cymene (7%–15%), carvacrol (6%–12%), 4-terpineol (2%–7%), t-anethole (1%–4%) and the sesquiterpene longifolene (1%–8%).

Traces of the esters of certain saturated and unsaturated fatty acids were also present in the volatile oils. No disulphides, which were detected in the essential oil of black cumin seeds by Aboutabl *et al.* (1986) could be found.

The seven analysed volatile fractions had an almost identical qualitative composition. Considerable variations in the quantitative composition could be found (see Table 1).

TLC-screening for antioxidant compounds

The volatile oil of the seeds was tested for its antioxidant activity starting with a rapid TLC screening.

In the first test the TLC sheet with the eluted essential oil was sprayed with β-carotene–linoleic acid reagent. The chromatogram was exposed to daylight until the background colour was bleached (45 min after spraying). Zones in which a yellow colour persisted possessed antioxidant activity. Because of the orange colour of the

Table 1. GC-MS analysis of seven samples of *Nigella sativa* essential oil on ultra 1 column

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
α-Pinene	0.85	0.30	0.94	2.00	0.43	0.53	0.20
Camphene	0.05	$\leq$0.01	$\leq$0.01	$\leq$0.01	$\leq$0.01	$\leq$0.01	—
β-Pinene	0.52	0.10	1.80	0.29	0.23	0.49	0.15
Sabinene	0.33	0.09	1.20	0.42	0.18	0.28	0.08
β-Myrcene	0.05	0.16	$\leq$0.01	0.40	0.12	0.15	—
α-Terpinene	0.12	0.11	$\leq$0.01	0.05	0.07	0.02	0.05
Limonene	1.12	0.29	3.30	1.17	0.80	1.10	0.37
β-Phellandrene	$\leq$0.01	0.08	$\leq$0.01	0.01	0.02	$\leq$0.01	—
1,8-Cineole	0.12	0.04	0.02	0.09	0.07	0.05	0.19
γ-Terpinene	0.31	0.12	1.10	0.45	0.02	0.02	—
p-Cymene	15.53	12.0	14.2	11.0	13.9	15.20	7.07
α-Terpinolene	$\leq$0.01	$\leq$0.01	$\leq$0.01	0.05	0.02	0.01	$\leq$0.01
2-Heptanal	$\leq$0.01	—	0.04	$\leq$0.01	—	0.12	0.38
Thujone	0.03	0.07	0.10	0.08	0.06	0.20	0.15
Trans-Sabinenehydrate	0.16	0.12	—	0.05	0.21	0.20	0.12
Longipinene	1.37	0.21	1.06	1.68	1.25	1.68	0.20
Camphor	0.06	0.06	0.05	0.07	0.07	0.10	0.05
Linalool	0.19	0.25	0.13	0.35	0.22	0.38	0.44
cis-Sabinenehydrate	0.06	0.09	—	—	0.08	0.16	—
Longifolene	7.20	1.26	4.30	7.37	5.00	8.00	1.31
Bornylacetate	0.46	0.28	0.41	0.47	0.41	0.82	0.49
2-Undecanone	0.12	—	—	0.47	0.11	0.14	—
4-Terpineol	3.10	4.02	3.10	2.16	1.98	6.59	3.43
Borneol	0.12	$\leq$0.01	—	0.47	0.25	0.25	0.24
Carvone	1.05	0.88	—	0.93	0.34	0.93	0.13
Thymoquinone	45.30	46.60	43.90	48.30	57.0	27.8	35.30
2-Tridecanone	0.32	0.11	—	0.29	0.38	0.13	0.22
t-Anethole	1.97	1.30	2.3	4.28	0.25	0.80	1.28
p-Cymene-8-ol	0.39	0.78	—	0.25	0.45	0.41	—
p-Anisaldehyde	0.07	—	0.02	$\leq$0.01	0.06	$\leq$0.01	—
Thymol	0.15	0.25	0.09	0.11	0.11	0.24	0.20
Carvacrol	7.20	11.6	5.80	7.42	8.41	10.77	8.50
Unknown peak[a]	4.04	5.57	11.0	4.9	4.9	10.53	6.17
Total	92.14	86.77	94.91	95.61	98.3	88.13	66.73[b]

Samples 1, 3 and 4 were purchased from wholesalers, seeds 2, 5 and 6 were bought from markets in Graz and Vienna; sample 7 was obtained from extraction of commercial black cumin fixed oil.
[a] m/z (% rel.int.): 168 (3), 153 (98), 136 (48), 125 (100), 121 (47).
[b] The fatty acid content of the essential oil was 16.3% (after steam distillation of the fixed oil).

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Table 2. Effect of the test compounds in the DPPH assay

Test compound[a]	IC_{50}[b]
Essential oil	460.0
Thymoquinone	211.0
Carvacrol	28.8
Quercetin	1.31
Butylhydroxytoluene	12.12
Ascorbic acid	3.76

[a] 4-terpineol and t-anethole could not inhibit 50% of the reaction under test conditions. The limiting factor was the volume of the test samples of 50 μL, which was added to the DPPH reagent.
[b] Concentration (μg/mL) for a 50% inhibition.

essential oil and its main compound thymoquinone, differences in colour between the background and the spots themselves were not easy to examine. To overcome this disadvantage a second spraying reagent was used.

With the DPPH reagent the active compounds were detected as yellow spots on a violet background. Only zones where the colour turned from purple to yellow within the first 30 min (after spraying) were taken as positive results. Two spots appeared immediately after spraying the chromatogram. They were identified by means of reference substances as carvacrol and thymoquinone. Two other zones (t-anethole and 4-terpineol) changed their colour 15 min later.

All four compounds were subjected to further testing.

DPPH assay

In the DPPH test the ability of a compound to act as donor for hydrogen atoms or electrons was measured spectrophotometrically.

Both the oil and the pure compounds were able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. The strongest effect was measured for the monoterpene, carvacrol, with an IC_{50} of 28.8 μg/mL. The results are shown in Table 2.

Non-enzymatic lipid peroxidation

Ox brain phospholipid liposomes undergo rapid non-enzymatic peroxidation when incubated with $FeCl_3$ and ascorbic acid. The use of Fe(III) in the presence of a reducing agent such as ascorbate produces ·OH (Aruoma, 1996b). A widely used test for analysing the extent of lipid peroxidation is the measurement of the pink pigment, absorbing at 532 nm, produced through reaction of 2-thiobarbituric acid and oxidation products, not only with malondialdehyde but also with other aldehydes (Kosugi *et al.*, 1987). Dilutions of the oil, thymoquinone, carvacrol, t-anethole and 4-terpineol were examined for their ability to act as ·OH radical scavenging agents. As demonstrated in Fig. 1 the volatile oil exhibited a very strong antioxidant effect. 0.0011 μg of it could inhibit 50% of lipid damage. The IC_{50} for thymoquinone was 1.84 μg/mL and 9.3 μg/mL for carvacrol. The weakest inhibition was measured for 4-terpineol.

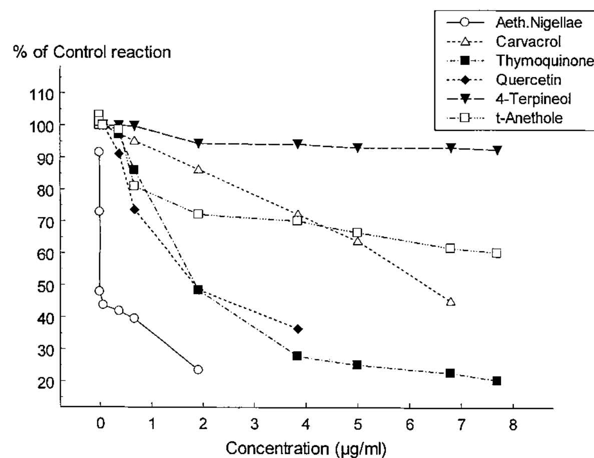

Figure 1. Inhibition of the non-enzymatic lipid peroxidation by the essential oil of *N. sativa*, thymoquinone, carvacrol, 4-terpineol, t-anethole and quercetin (positive control).

Deoxyribose degradation

Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA at low pH form a pink chromogen (Halliwell *et al.*, 1987; Aruoma *et al.*, 1989). When *Nigella* essential oil or its active components were added to the reaction mixture they removed hydroxyl radicals from the sugar and prevented the degradation. The results are shown in Fig. 2. Carvacrol was the most effective inhibitor of the free radical sugar degradation with an IC_{50} of 21 μg/mL.

Assay for site-specific actions

The deoxyribose test was adopted to assess site-specific reactions.

When iron is added to the mixture as ferric chloride instead of chelated iron some of the Fe^{3+} ions bind to deoxyribose. The damage to the carbohydrate becomes

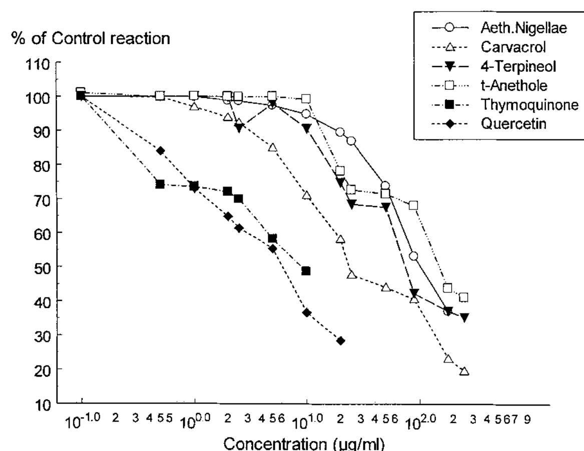

Figure 2. Inhibition of the radical degradation of 2-deoxy-2-ribose by the essential oil, thymoquinone, 4-terpineol, t-anethole, carvacrol and quercetin (positive control).

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Table 3. Results of the assay for site-specific reactions

Test compound	μg/mL	Omit EDTA[a]	Omit ascorbic acid[b]	Omit deoxyribose[c]
None		0.482	0.149	0.168
Essential oil	88.6 (1.0 μL/ml)	0.499	0.059	0.078
	88.6 (10.0 μL/ml)	0.679[d]	0.285	0.189
Thymoquinone	1.0	0.307	−0.006	0.089
	10.0	0.620[d]	0.012	0.089
Carvacrol	1.0	0.490	0.106	0.092
	10.0	0.540	0.034	0.112
t-Anethole	1.0	0.460	0.113	0.101
	10.0	0.444	0.080	0.106
4-Terpineol	1.0	0.476	0.110	0.112
	10.0	0.456	0.045	0.102
BHT	1.0	0.479	0.032	0.089
	10.0	0.540	0.029	0.090
Quercetin	1.0	0.494	0.057	0.095
	10.0	0.355	0.105	0.093

[a] Absorbance of the control reaction plus EDTA: 0.772.
[b] Absorbance of the control reaction plus ascorbic acid: 0.821.
[c] Absorbance of the control reaction plus deoxyribose: 0.999.
[d] Disturbing colour development, probably caused by thymoquinone.

site-specific such that the ·OH radicals which are formed by bound iron ions immediately attack the sugar (Aruoma *et al.*, 1987). Only components that are able to interfere with site-specific-Fenton chemistry (Aruoma, 1991; Halliwell, 1990) inhibit the deoxyribose degradation under these conditions. Neither thymoquinone nor any of the three other components could inhibit this reaction (Table 3). In a concentration of 1.0 μl/mL the volatile oil interfered with colour development during this $FeCl_3$-assay. When tested at a lower concentration no interference occurred.

If ascorbate is omitted from the reaction mixture the ability of a substance to reduce ferric-EDTA can be examined. In the absence of ascorbic acid a slow rate of hydroxyl generation results. If an agent possesses prooxidative activity the deoxyribose degradation is stimulated, more fragments are produced and the absorbance increases. None of the tested compounds stimulated peroxidation under these conditions.

Finally it was checked whether the compounds under examination themselves could form degradation products, like aldehydes, that react with TBA to give coloured products. By omission of deoxyribose from the reaction mixture, the oil and the pure components, respectively, were the only agents to react with ·OH radicals in the way mentioned above. None of the tested agents was able to form thiobarbituric acid reactive material, TBARS (Aruoma and Cuppett, 1997). The results of this experiment are also shown in Table 3.

DISCUSSION

In a series of *in vitro* tests the essential oil exhibited antioxidant activity. It acted as a donating agent in the DPPH assay and possessed hydroxyl radical scavenging properties in both the assay for non-enzymatic lipid peroxidation and the deoxyribose test. This was not obvious, as antioxidants that protect lipids against free radical damage may actually accelerate damage to other molecules, such as carbohydrates, under certain conditions (Aruoma, 1996a; Gutteridge and Halliwell, 1994; Aruoma *et al.*, 1997). So it is always important to examine suspected antioxidant activity in different assays involving lipids and carbohydrates.

In the site-specific assays neither the essential oil nor the compounds thymoquinone, carvacrol, t-anethole or 4-terpineol, which contribute to the radical scavenging effect of the volatile fraction in a different strong manner, exhibited prooxidant activities. Further studies will be needed to confirm these results by checking the oil and its active components in assays involving DNA damage, such as the bleomycin assay (Halliwell, 1993; Gutteridge *et al.*, 1981; Aruoma, 1991).

Looking at the effects of the essential oil and the pure compounds in the different assays and comparing the results, it seems that the three monoterpenes and t-anethole are not the only radical scavenging compounds in the oil. At least in the lipid peroxidation test the essential oil possessed a strong antioxidative effect that can not just be achieved by summarizing the activities of the four compounds. It is possible that there are synergistic effects, as they were demonstrated to exist in drugs used for the cure of gastric diseases and plants with radical scavenging properties (Beckstrom-Sternberg and Duke, 1994), but there might also be another active principle not yet identified in the oil.

Analysing the mechanism(s) involved in the radical scavenging behaviour of *N. sativa* volatile oil it is obvious that there are no agents with iron-complexing ability, as the test compounds failed to inhibit deoxyribose degradation when iron was added as $FeCl_3$ instead of Fe^{3+}-EDTA. Only compounds that complex iron ions can prevent oxidation under these conditions (Gutteridge, 1984; Aruoma and Halliwell, 1988; Aruoma *et al.*, 1987).

The results of the present study, which demonstrate the radical scavenging activity of *N. sativa*, indicate that the use of black cumin seeds for the treatment of various inflammatory diseases seems quite useful and reasonable.

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REFERENCES

Aboutabl, E. A., El-Azzouny, A. A., and Hammerschmidt, F. J. (1986). Aroma volatiles of *Nigella sativa* L. seeds. *Progress in Essential Oil Research*, pp. 49–55. Walter de Gruyter & Co, Berlin, New York.

Akhtar, A. H., Ahmed, K. D., Gilani, S. N., and Nazir, A. (1996). Antiulcer effects of aqueous extract of *Nigella sativa* and *Pongamia pinnata* in rats. *Fitoterapia* **3**, 195–199.

Aruoma, O. I., Grootveld, M., and Halliwell, B. (1987). The role

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of iron in ascorbate-dependant deoxyribose degradation. *J. Inorg. Biochem.* **29**, 289–299.

Aruoma, O. I., and Halliwell, B. (1988). The iron-binding and hydroxyl radical scavenging of anti-inflammatory drugs. *Xenobiotica* **18**, 459–470.

Aruoma, O. I., Laughton, M. J., and Halliwell, B. (1989). Carnosine, homocarnosine and anserine: could they act as antioxidants *in vivo? Biochem. J.* **264**, 863–869.

Aruoma, O. I. (1991). *Free Radicals and Food Additives*, pp. 18–19. Taylor & Francis, London.

Aruoma, O. I. (1996a). Assessement of potential prooxidant and antioxidant actions. *J. Am. Oil Chem.Soc.* **73**, 1617–1625.

Aruoma, O. I. (1996b). Characterization of drugs as antioxidant prophylactics. *Free Rad. Biol. Med.* **20**(5), 675–705.

Aruoma, O. I., and Cuppett, S. L. (1997). *Antioxidant Methodology—In Vivo and In Vitro Concepts*, p. 10. AOCS Press, Champaign, IL.

Aruoma, O. I., Spencer, J. P. E., Warren, D., Jenner, P., Butler, J. and Halliwell, B. (1997). Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem.* **60**(2), 149–156.

Beckstrom-Sternberg, S. M., and Duke, J. A. (1994). Potential for synergistic action of phytochemicals in spices. *Spices, Herbs and Edible Fungi*, pp. 201–223. Elsevier Science, Oxford.

Bucar, F., and Schweiger, U. (1998). Phytochemical and pharmacological investigations of *Aegopodium podagraria* L., Institute of Pharmacognosy, University of Graz, In, *Essential Oils: Basic and Applied Research, Proceedings of the 27th International Symposium on Essential Oils*, ed. by Ch. Franz, A. Mathe and G. Buchbauer, Allured Publishing Corporation, Carol Stream, IL.

Cuendet, M., Hostettmann, K., and Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei. Helv. Chim. Acta* **80**, 1144–1152.

El-Dakhakhny, M. (1965). Studies on the Egyptian *Nigella sativa* L. *Arzneimittelforschung* **15**, 1227–1229.

El-Kamali, H. H., Ahmed, A. H., Mohammed, A. S., Yahia, A. A. M., El-Tayeb, I. H., and Ali, A. A. (1998). Antibacterial properties of essential oils from *Nigella sativa* seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts. *Fitoter apia* **1**, 77–78.

Eschborn, M. I. (1997). Prüfung von Schwarzkümmelöl. *Pharmazeut. Ztg.* **142**, 46–48.

Gutteridge, J. M. C., Rowley, D. A., and Halliwell, B. (1981). Superoxide dependant formation of hydroxyl radicals in the presence of iron salts. *Biochem. J.* **199**, 263–265.

Gutteridge, J. M. C. (1984). Reactivity of hydroxyl and hydroxyl-like radical discriminated by release of thiobarbituric acid-reactive material from deoxyribose, nucleosides and benzoates. *Biochem. J.* **224**, 761–767.

Gutteridge, J. M. C., and Halliwell, B. (1994). Oxidative stress. In, *Antioxidants in Nutrition, Health and Disease*, pp. 90–102. Oxford University Press, Oxford.

Halliwell, B., Gutteridge, J. M. C., and Aruoma, O. I. (1987). The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. *Anal. Biochem.* **165**, 215–219.

Halliwell, B., and Gutteridge, J. M. C. (1989). *Free Radicals in Biology and Medicine*, 2nd ed, pp. 416–494. Clarendon Press, Oxford.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Rad. Res. Commun.* **9**, 13–14.

Halliwell, B. (1993). *DNA and Free Radicals*, pp. 315–327. Horwood, New York.

Houghton, P. J., Zarka, R., de la Heras, B., and Hoult, J. R. S. (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* **61**, 33–36.

Khanna, T., Zaidi, F. A., and Dandiya, P. C. (1993). CNS and analgesic studies on *Nigella sativa*. *Fitoterapia* **5**, 407–410.

Kirby, A. J., and Schmidt, R. J. (1997). The antioxidant activity of chinese herbs for eczema and of placebo herbs—I. *J. Ethnopharmacol.* **56**, 103–108.

Kosugi, H., Kato, T., and Kikugava, K. (1987). Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem.* **165**, 456–464.

Lautenbacher, L. M. (1997). Schwarzkümmelöl. *Dtsch. Apoth. Ztg.* **137**, 68–69.

Mahfouz, M., Abdel-Maquid, R., and El-Dakhakhny, M. (1965). The effect of nigellone therapy on the histaminopexic power of the blood sera of asthmatic people. *Arzneimittelforsch.* **15**, 1230–1231.

Mahmoud, A., and Shaheen, R. (1996). Effects of the volatile oil of *Nigella sativa* seeds on the uterine smooth muscle of rat and guinea pig. *J. Ethnopharmacol.* **52**, 23–26.

Marozzi, F. J., Kocialski, A. B., and Malone, M. H. (1970). Studies on the antihistaminic effects of thymoquinone, thymohydroquinone and quercetin. *Drug Res.* **20**, 1574–1577.

Maxwell, S. R. J. (1995). Prospects for the use of antioxidant therapies. *Drugs* **49**, 345–361.

Merfort, I., Wray, V., Barakat, H. H., Hussein, S. A. M., Nawwar, M. A. M., and Willuhn, G. (1997). Flavonoid triglycerides from seeds of *Nigella sativa*. *Phytochemistry* **46**, 359–363.

Pratt, D. E., and Miller, E. E. (1984). A flavonoid antioxidant in Spanish peanuts. *J. Am. Oil Chem. Soc.* **61**, 1064–1067.

Sies, H. (1991). Oxidative stress: Introduction. *Oxidative Stress: Oxidants and Antioxidants*, Academic Press, London.

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