Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 76 (2010) 210–216

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Bioactivities of black cumin essential oil and its main terpenes from Tunisia

S. Bourgou [a,*], A. Pichette [b], B. Marzouk [a], J. Legault [b]

[a] *Unité des Plantes Aromatiques et Médicinales, Centre de Biotechnologie à la Technopole de Borj-Cedria, 901, Hammam-Lif, Tunisia*

[b] *Laboratoire d'Analyse et de Séparation des Essences Végétales, Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada G7H 2B1*

Received 27 May 2009; received in revised form 23 October 2009; accepted 26 October 2009

Abstract

Ex vivo antioxidant, anti-inflammatory, anticancer and antibacterial activities of the essential oil from Tunisian *Nigella sativa* seeds and its main terpenes (*p*-cymene, γ-terpinene, thymoquinone, β-pinene, carvacrol, terpinen-4-ol and longifolene) were determined. The essential oil exhibited strong *ex vivo* antioxidant activity, inhibiting DCFH oxidation with an IC_{50} of 1.0 μg/ml, and high anti-inflammatory activity, inhibiting NO radical excretion with an IC_{50} value of 6.3 μg/ml. Thymoquinone was found to be the most active to decrease DCFH oxidation and NO excretion. The oil was found to significantly inhibit the growth of A-549 and DLD-1 cancer cell lines (IC_{50} values of 43.0 and 46.0 μg/ml, respectively) and to exert antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with IC_{50} values of 12.0 and 62.0 μg/ml. The anticancer and antibacterial activities could be mainly due to the action of thymoquinone and longifolene.

Keywords: Antibacterial; Anticancer; Anti-inflammatory; Antioxidant; Essential oil; *Nigella sativa* L.; Terpenes

1. Introduction

Nigella sativa L. (Ranunculaceae), commonly called black cumin and "sinouj" in Tunisia, is an annual herbaceous plant cultivated in different parts of the world, mainly in countries bordering the Mediterranean Sea. The seeds are used extensively for flavouring and medicinal purposes. They are used in the preparation of a traditional sweet dish and eaten with honey and syrup and for sprinkling on bread, flavouring of foods (as a spice), especially bakery products and cheese (Takruri and Dameh, 1998). The seeds are also used in traditional medicine as a natural remedy for several illnesses that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza, and as a carminative, diuretic, lactagogue and vermifuge (Ali and Blunden, 2003). Extensive studies have been conducted on the pharmacological properties of solvent extracts of *N. sativa* seeds including ethanol (Swamy and Tan, 2000; Kumara and Huat, 2001), methanol (Al-Naggar et al., 2003; Thippeswamy and Naidu, 2005), aqueous (Al-Ghamdi, 2001; Thabrew et al., 2005) and diethyl ether extracts (Hanafy and Hatem, 1991; Aljabre et al., 2005), while there are some reports on the biological activities of the essential oil (Burits and Bucar, 2000; Islam et al., 2004; Nair et al., 2005; Mbarek et al., 2007).

The biological properties of the essential oils have been found to be directly linked to their chemical compositions, which are influenced by the origin of the plants (Celiktas et al., 2007). A variety of chemotypes have been described in the literature. An Iranian *N. sativa* essential oil was found to be dominated by phenylpropanoid components and displayed a *trans*-anethole chemotype (Nickavar et al., 2003) while a chemotype with 33% *p*-cymene and 26.8% thymol and the preponderance of monoterpenes was reported for *N. sativa* essential oil from Morocco (Moretti et al., 2004). In addition, Benkaci-Ali et al. (2007) demonstrated a considerable difference in the chemical composition of the Algerian *N. sativa* seed essential oils obtained from specimens growing in two localities; the oil being dominated by both alcohols and acids or by monoterpene hydrocarbons, according to the origin.

The objective of this study was to investigate, for the first time, the *ex vivo* antioxidant activity as well as the anticancer, anti-inflammatory and antibacterial activities of Tunisian *N. sativa* essential oil. Moreover, in order to identify bioactive

* Corresponding author.
E-mail address: soumaya_swiss@yahoo.ca (S. Bourgou).

0254-6299/$ - see front matter © 2009 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2009.10.009

volatile components, the biological activities of the main, commercially available volatiles present in the oil, were simultaneously evaluated.

2. Materials and methods

2.1. Plant material and essential oil extraction

Seeds of *N. sativa* were collected at maturity, in July 2006, from cultivated plants from the region of Menzel Temime (Northeastern Tunisia). The specimen was authenticated by Dr. Abedrrazak Smawy at Biotechnologic Center, Technopark of Borj-Cédria, and the voucher specimen "RNS18" was deposited in the herbarium of the Unit of Aromatic and Medicinal Plants at the Biotechnologic Center. The essential oil was obtained from seeds (100 g) by hydrodistillation for 90 min in a Clevenger type apparatus. The oil was then dried with anhydrous sodium sulphate and stored at −20 °C in darkness until used.

2.2. Chemicals

The terpenes, *p*-cymene, β-pinene, thymoquinone and terpinen-4-ol were purchased from Sigma-Aldrich (purity > 95%), while γ-terpinene, longifolene and carvacrol were purchased from Fluka (purity ≥ 97%).

2.3. Analysis conditions

The essential oil composition was determined using gas chromatography with flame ionisation detection (GC-FID) and verified using GC with mass spectrometry (GC-MS) detection. For GC-FID, an Agilent 6890 GC, equipped with a polar Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm) and an apolar DB-5 column with the same dimensions, was used. Helium was the carrier gas at a flow rate of 1 ml/min. A split ratio of 50:1 and injector and detector temperatures of 200 °C and 260 °C, respectively, were used. Initially, the oven temperature was set at 40 °C for 2 min, then raised by 2 °C/min to a final temperature of 210 °C (held for 33 min). A Hewlett-Packard (Model HP5890) gas chromatograph coupled to a Model HP5972 mass spectrometer was employed for GC-MS analysis. Separation was achieved on the same DB-5 column, using identical oven temperature conditions. The ionisation energy was set at 70 ev. Conditions used for the carrier gas and the split ratio were the same as for GC-FID. However, the injector and detector temperatures were set at 225 °C and 280 °C, respectively. Identification of volatile constituents was made on the basis of their retention indices on both columns (Kovats, 1965) and their mass spectra, which were compared with reference data (Adams, 2007).

2.4. Cell culture

The human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221), normal skin fibroblast (WS-1) and murine macrophage RAW 264.7 (ATCC #TIB-71) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). Cell lines were grown in Minimum Essential Medium with Earle's salts. The medium was supplemented with 10% fetal bovine serum (Hyclone, Logan, USA), a solution of vitamins (1×), sodium pyruvate (1×), non-essential amino acids (1×), penicillin (100 IU) and streptomycin (100 μg/ml) (Mediatech Cellgro®). Cells were cultured in a humidified atmosphere at 37 °C under 5% CO_2.

2.5. Antioxidant activity

Antioxidant activity was evaluated using the 2′,7′-dichlorofluorescin-diacetate (DCFH-DA) assay as described by Girard-Lalancette et al. (2009), with some modifications. Briefly, WS-1 cells were plated in 96-wells microplates at 10,000 cells per well and incubated for 24 h at 37 °C under 5% CO_2. The cells were washed with 150 μl Hank's balanced salt solution (HBSS) at pH 7.4 and incubated for 30 min with 100 μl HBSS (pH 7.4) containing 5 μM DCFH-DA. The cells were then washed again with 150 μl HBSS. To assess the antioxidant activity, the cells were incubated either with increasing concentrations of essential oil or increasing concentrations of pure compounds in DMSO, in the absence or the presence of 200 μM *tert*-butylhydroperoxide (*t*-BuOOH). Fluorescence was measured immediately after *t*-BuOOH administration and again 90 min later, using an automated 96-well Fluoroskan Ascent Fl™ plate reader (Fluoroskan Ascent Fl, Thermo-Labsystems) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.6. Anti-inflammatory activity

To investigate the anti-inflammatory activity of *N. sativa* seed essential oil, nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells was examined. Exponentially growing macrophages were plated in 24-well microplates (BD Falcon) at a density of 2×10^5 cells per well in 400 μl of culture medium and were allowed to adhere for 24 h at 37 °C under 5% CO_2. Cells were then treated with increasing concentrations of essential oil and pure compounds dissolved in DMSO. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cells were then stimulated with 100 μg/ml lipopolysaccharide (LPS) and incubated at 37 °C under 5% CO_2. After 24 h, cell-free supernatants were collected and nitrite production was measured using the modified method of Green et al. (1990). Griess reagent (50 μl of 1% sulphanilamide and 50 μl of 0.1% *N*-1-naphtylethylenediamine dihydrochloride in 2.5% H_3PO_4) was added in equal volume (100 μl) to cell supernatant and incubated at room temperature for 30 min. N(G)-nitro-L-arginine methyl ester (L-NAME) was used as a positive control. The absorbance at 540 nm was then measured using an automated 96-well Varioskan Ascent plate reader (Thermo Electron) and nitrite was quantified by comparison with a $NaNO_2$ standard curve.

2.7. Anticancer activity

Exponentially growing cells were plated at a density of 5×10^3 cells per well, in 96-well microplates (Costar, Corning

Inc.) into 100 µl of culture medium and were allowed to adhere for 24 h at 37 °C under 5% CO_2 before treatment. Then, 100 µl of increasing concentrations of essential oil or pure compounds in DMSO were added. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. The cells were incubated for 48 h in the presence or absence of essential oil or compounds. The cytotoxicity was assessed using the resazurin reduction test as described by O'Brien et al. (2000). Fluorescence was measured using an automated 96-well Fluoroskan Ascent Fl™ plate reader (Thermo-Labsystems) at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxic activity was expressed as the concentration of essential oil or compound inhibiting cell growth by 50% (IC_{50}).

2.8. Antibacterial activity

Antibacterial activity was assessed according to the micro-dilution method described by Banfi et al. (2003). Briefly, exponentially growing bacteria were plated in 96-well round bottom microplates (Costar, Corning Inc.) at a density of 25×10^3 gram-positive *Staphylococcus aureus* (ATCC 25923) or 5×10^3 gram-negative *Escherichia coli* (ATCC 25922) per well in 50 µl nutrient broth (Difco). Then, 100 µl of increasing concentrations of essential oil or pure compounds were added per well. The final concentration of solvent in the culture medium was maintained at 0.1% (v/v) to avoid solvent toxicity. Antibacterial activity was assessed by adding 50 µl of 4% resazurin to each well; the microplates were then incubated at 37 °C. Fluorescence was measured after 6 h on an automated 96-well Fluoroskan Ascent Fl™ plate reader (Thermo-Labsystems) using excitation and emission wavelengths of 530 nm and 590 nm, respectively. Antibacterial activity was expressed as the concentration of essential oil or compound inhibiting bacterial growth by 50% (IC_{50}).

3. Results and discussion

3.1. Chemical composition of the essential oil

The essential oil of mature Tunisian *N. sativa* seeds obtained using hydrodistillation was isolated in high yield (0.5%). Results of GC and GC-MS analysis of the essential oil (Table 1) indicate that the essential oil was characterized mainly by monoterpenes, with a relative total concentration of 86.5%. Sesquiterpenes represented only 0.9% of the oil. The major constituent of the oil was the hydrocarbon monoterpene *p*-cymene, with a relative concentration of 60.5%. The Tunisian population sampled therefore displayed a different volatile profile when compared with that of *N. sativa* from Iran, Morocco and Algeria (Nickavar et al., 2003; Moretti et al., 2004; Benkaci-Ali et al., 2007). It has been reported that the chemical compositions of the essential oil are highly influenced by climatic conditions and geographical factors (Sangwan et al., 2002; Burt, 2004). The high level of *p*-cymene in the essential oil could contribute to the valorization of Tunisian *N. sativa* species, since this monoterpene is of great importance in industry as intermediate for synthesis of fragrances, pharmaceuticals and herbicides (Martin-Luengo et al., 2008).

Table 1
Relative percentage chemical composition of *Nigella sativa* seed essential oil.

Components	RI DB-5 [a]	RI Supelcowax-10 [b]	Percentage
α-Thujene	936	1028	6.9 [c]
α-Pinene	942	1021	1.7 [c]
Sabinene	978	1125	0.9 [c]
β-Pinene	980	1109	2.4 [c]
Myrcene	992	–	0.1 [c]
α-Terpinene	1019	1179	1.0 [c]
p-Cymene	1028	1278	60.5 [c]
Limonene	1033	1196	1.4 [c]
1,8-Cineole	–	1204	0.1 [d]
γ-Terpinene	1068	1250	3.5 [c]
Unidentified A [e]	1108	1260	1.1 [c]
Unidentified B [f]	1130	1306	7.0 [c]
Unidentified C [g]	1171	1516	0.5 [c]
Terpinen-4-ol	1180	1598	2.1 [c]
p-Cymen-8-ol	1186	1849	0.2 [c]
Unidentified D [h]	1202	1553	1.0 [c]
Thymoquinone	1254	1751	3.0 [c]
Carvacrol	1310	2205	2.4 [c]
Longifolene	1408	1561	0.9 [c]
Thymohydroquinone	1557	–	0.4 [c]
Total			96.9

[a] Retention indices on apolar DB-5 column.
[b] Retention indices on polar Supelcowax 10 column.
[c] Percentage composition determined on DB-5 column.
[d] Percentage composition determined on Supelcowax column.
[e] m/z (relative intensity) 93 (100),125 (95), 85 (87), 72 (74), 43 (64), 41 (63), 55 (51), 81 (46), 100 (41), 91 (34), 153 (35), 79 (34), 69 (32), 53 (26), 67(24), 154 (4).
[f] m/z (relative intensity) 72 (100), 85 (96), 93 (85), 125 (76), 43 (70), 81 (63), 55 (61), 153 (59), 79 (38), 69 (37), 77 (36), 121 (33), 91 (32), 42 (31), 67 (30), 53 (28), 95 (26), 154 (7).
[g] m/z (relative intensity) 43 (100), 67 (46), 109 (45), 81 (30), 41 (29), 79 (20), 91 (19), 55 (18), 93(15), 53 (13), 65 (9), 69 (8), 134 (5).
[h] m/z (relative intensity) 43 (100), 41 (74), 67(71), 79(66), 109(65), 81 (57), 93 (48), 119 (46), 91 (44), 137 (42), 55 (39), 77 (33), 55 (31), 35 (30), 152 (28), 69 (22), 65 (15), 107 (14), 152 (3).

The results indicated that the essential oil was characterized by the presence of appreciable levels of α-thujene (6.9%), γ-terpinene (3.5%), thymoquinone (3.0%), β-pinene (2.4%), carvacrol (2.4%) and terpinen-4-ol (2.1%). An unidentified compound made up 7.0% of the oil was also present.

3.2. Antioxidant activity

Reactive oxygen species (ROS), including oxygen radicals and their reaction products, are known to react with biological molecules, leading to cell and tissue damage. *In vitro* methodologies have been widely used to assess antioxidant properties of medicinal plant extracts, however, these assays are often very specific for a particular mode of action and do not necessarily reflect the normal biological context in which they react (Girard-Lalancette et al., 2009). Recently, the cell-based assay using DCFH-DA, a useful indicator of reactive oxygen species (ROS), has been developed as a new sensitive test which allows detection of both the anti- and pro-oxidant properties (Girard-Lalancette et al., 2009). The results, presented in Table 2,

Table 2
Antioxidant activities of *Nigella sativa* seed essential oil and its main constituents quercetin was used as positive control.

Tested compounds	Inhibition of DCFH oxidation
N. sativa essential oil	1.0±1.0 [a]
p-Cymene	>200 [b]
γ-Terpinene	>200 [b]
Thymoquinone	1.0±0.8 [b]
β-Pinene	>200 [b]
Carvacrol	190.0±60.0 [b]
Terpinen-4-ol	>200 [b]
Longifolene	>200 [b]
Quercetin	0.1±0.1 [b]

Values are mean±S.D. of three replications.
[a] IC_{50} values (μg/ml).
[b] IC_{50} values (μM).

demonstrated that the essential oil strongly inhibited *t*-BuOOH induced DCFH oxidation with an IC_{50} value of 1.0 μg/ml. This result indicates that *N. sativa* essential oil significantly inhibits ROS production and thus exhibits the ability to protect cells from oxidative stress. The antioxidant activity of the main components present in *N. sativa* seed essential oil revealed that thymoquinone possesses a strong *ex vivo* antioxidant activity with an IC_{50} value of 1.0 μM (0.2 μg/ml), while the activities of other terpenoids were weak. Surprisingly, the direct activity of carvacrol, which is a well known *in vitro* phenolic antioxidant compound (Ruberto and Baratta, 2000), was found to be low with an IC_{50} of 190.0 μM (28.5 μg/ml). In comparison, the positive control, quercetin, exhibited an IC_{50} value of 0.1 μM, which corresponds to 0.04 μg/ml. The monoterpene hydrocarbons, *p*-cymene, γ-terpinene and β-pinene were inactive (Table 2), despite previous reports of their *in vitro* antioxidant activities (Ruberto and Baratta, 2000). The antioxidant activities of these volatiles in cellular assays have not been previously reported and the results could be explained by the fact that *in vitro* tests do not take the physiological conditions of the cell, bioavailability of the antioxidant molecule, as well as general cellular metabolism into account (Girard-Lalancette et al., 2009). Carvacrol and thymoquinone have been reported to contribute to the *in vitro* antioxidant activity of *N. sativa* essential oil (Burits and Bucar, 2000). However, our results indicated that the *ex vivo* antioxidant activity of the essential oil is mainly due to the action of thymoquinone.

3.3. Anti-inflammatory activity

The anti-inflammatory activities of *N. sativa* seed essential oil and its major constituents were evaluated by measuring their capacity to inhibit cellular NO generation. Nitric oxide is an endogenous free radical species that is synthesized from L-arginine by nitric oxide synthase (NOS) in various tissues. This radical is an important regulator of physical homeostasis, whereas large amounts have been closely correlated with the pathophysiology of a variety of diseases and inflammations (Marletta, 1993). Therefore, the inhibition of NO production may be a useful strategy for the treatment of various inflammatory disorders (Choi et al., 2007).

The anti-inflammatory activity of *N. sativa* seed essential oil was evaluated on RAW 264.7 macrophages which were stimulated to induce an overproduction of NO. As presented in Fig. 1, the essential oil showed a strong inhibitory effect on LPS-induced NO secretion with 90.0% inhibition observed at 25.0 μg/ml and an IC_{50} value of 6.3 μg/ml. Comparatively, the L-NAME, used as positive control inhibited NO release by 45.7% at 250.0 μM (67.4 μg/ml). The cytotoxicity of the essential oil towards RAW 264.7 cells was also evaluated. The results revealed that doses up to 25.0 μg/ml produced no significant cytotoxic effect and the cells remain viable (data not shown).

To understand the relationship between the activity of the essential oil and its composition, the main volatiles present in *N. sativa* seeds were tested for their anti-inflammatory properties at non-cytotoxic concentrations. Thymoquinone was found to be the most active compound, inhibiting NO production by 95.0% at 25.0 μM (4.1 μg/ml). Literature data indicate that thymoquinone mediates its inhibitory effect on NO production *via* the reduction of inducible NOS mRNA and protein expressions (El-Mahmoudy et al., 2002). However, ours results (Table 2; Fig. 1) suggest that the anti-inflammatory capacity of thymoquinone could be mediated, at least in part, by its strong direct antioxidant activity as an effective ROS scavenger.

Nitric oxide inhibition was also demonstrated at 25.0 μM by longifolene (40.0%), carvacrol (35.1%), β-pinene (33.8%), *p*-cymene (32.3%), γ-terpinene (28.5%) and terpinen-4-ol (20.8%). Our results are concomitant with literature data indicating the potent anti-inflammatory activity of the oxygenated terpenes, thymoquinone and terpinen-4-ol (Hart et al., 2000; El-Mahmoudy et al., 2002). However, this study proved for the first time the activity of other terpenes including carvacrol, β-pinene, *p*-cymene and γ-terpinene. Although some sesquiterpenoids such as α-humulene and *β*-caryophyllene have been demonstrated to possess anti-inflammatory properties (Fernandes et al., 2007), the anti-inflammatory activity of the sesquiterpene longifolene is reported for the first time.

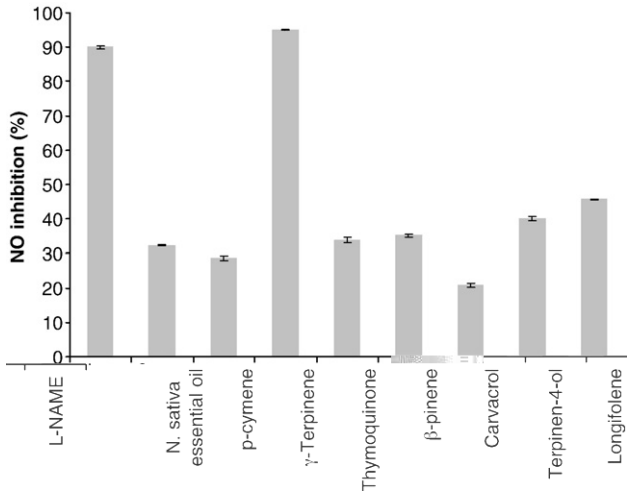

Fig. 1. Effects of *Nigella. sativa* seed essential oil (25.0 μg/ml) and its main constituents (25.0 μM) on NO production in LPS-stimulated RAW-264.7 macrophages. Values are mean±S.D. of three replications.

A relationship between the inhibition of cancer cell proliferation and inflammatory mediator production by extracts has been hypothesized. Indeed, inflammatory mediators, such as NO and cytokines, have been reported to contribute to mutagenesis (Marletta, 1993). It has been shown that the activity of the enzyme NOS is consistent in human cancer and its selective modulation has been suggested as a potential strategy for chemoprevention and reduction of cancer cell proliferation (Ahmad et al., 1997; Nishikawa et al., 2004). In this study, the effect of

In conclusion, these data indicate that the essential oil extracted from Tunisian black cumin seeds exhibit potent biological activities, which support their use in traditional medicine. In addition, the essential oil might be useful for therapeutic purposes to prevent ROS disorders, to treat chronic inflammatory pathologies associated with overproduction of nitric oxide and for use as an anticancer and antibacterial agent. Moreover, results regarding the bioactivities of the main volatile components suggest that the observed activities of the essential oil are connected to its chemical composition, where thymoquinone and longifolene has been found to be the most active compounds. Furthermore, this study proved for the first time, the potential of some terpenes as direct antioxidant (thymoquinone), anti-inflammatory (carvacrol, *p*-cymene, β-pinene, γ-terpinene and longifolene), anticancer and antibacterial (longifolene) principles with a potential use in pharmaceuticals.

Acknowledgements

The authors would like to thank Helene Gagnon, Serge Lavoie and Vakhtang Mshvildadze for their help and suggestions. We are also grateful to Catherine Dussault and Karl Lalancette for their help in biological assays.

References

Adams, R.P., 2007. Identification of essential oil components by Gas Chromatography/Mass Spectrometry, 4th ed. Allured Publishing Corporation.

Ahmad, N., Srivastava, R.C., Agarwal, R., Mukhtar, H., 1997. Nitric oxide synthase and skin tumor promotion. Biochemical and Biophysical Research Communications 232, 328–331.

Al-Ghamdi, M.S., 2001. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Journal of Ethnopharmacology 76, 45–48.

Ali, B.H., Blunden, G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytotherapy Research 17, 299–305.

Aljabre, S.H.M., Randhawa, M.A., Akhtar, N., Alakloby, O.M., Alqurashi, A.M., Aldossary, A., 2005. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. Journal of Ethnopharmacology 101, 116–119.

Al-Naggar, T.B., Gómez-Serranillos, M.P., Carretero, M.E., Villar, A.M., 2003. Neuropharmacological activity of *Nigella sativa* L. extracts. Journal of Ethnopharmacology 88, 63–68.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2007. Biological effects of essential oils. Food and Chemical Toxicology 46, 446–475.

Banfi, E., Scialino, G., Monti-Bragadin, C., 2003. Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. Journal of Antimicrobiology and Chemotherapy 52, 796–800.

Benkaci-Ali, F., Baaliouamer, A., Meklati, B.Y., Chemat, F., 2007. Chemical composition of seed essential oils from Algerian *Nigella sativa* extracted by microwave and hydrodistillation. Flavour and Fragrance Journal 22, 148–153.

Burits, M., Bucar, F., 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research 14, 323–328.

Burt, S., 2004. Essential oil: their antibacterial properties and potential applications in foods. International Journal of Food Microbiology 94, 223–253.

Celiktas, O.Y., Kocabas, E.E.H., Bedir, E., Sukan, F.V., Ozek, T., Baser, K.H.C., 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry 100, 553–559.

Choi, C.Y., Park, K.R., Lee, J.H., Jeon, Y.J., Liu, K.H., Oh, S., Kim, D.E., Yea, S.S., 2007. Isoeugenol suppression of inducible nitric oxide synthase expression is mediated by down-regulation of NF-κB, ERK1/2, and p38 kinase. European Journal of Pharmacology 576, 151–159.

El-Mahmoudy, A., Matsuyama, H., Borgan, M.A., Shimizu, Y., El-Sayed, M.G., Minamoto, N., Takewaki, T., 2002. Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. International Immunopharmacology 2, 1603–1611.

Fernandes, E.S., Passos, G.F., Medeiros, R., Dacunha, F.M., Ferreira, J., Campos, M.M., Pianowski, L.F., Calixto, J.B., 2007. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. European Journal of Pharmacology 569, 228–236.

Girard-Lalancette, K., Pichette, A., Legault, J., 2009. Sensitive cell-based assay using DCFH oxidation for the determination of pro- and antioxidant properties of compounds and mixtures: analysis of fruit and vegetable juices. Food Chemistry 115, 720–726.

Green, S.J., Meltzer, M.S., Hibbs, J.B., Nacy, C.A., 1990. Activated macrophages destroy intracellular Leishmania major amastigotes by an L-arginine-dependent killing mechanism. Journal of Immunology 144, 278–283.

Hanafy, M.S.M., Hatem, M.E., 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). Journal of Ethnopharmacology 34, 215–278.

Hart, P.H., Brand, C., Carson, C.F., Riley, T.V., Prager, R.H., Finlay-Jones, J.J., 2000. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. Inflammatory Research 49, 619–626.

Inouye, S., Uchida, K., Takizawa, T., Yamaguchi, H., Abe, S., 2006. Evaluation of the effect of terpenoid quinones on *Trichophyton mentagrophytes* by solution and vapor contact. Journal of Infection and Chemotherapy 12, 100–104.

Islam, S.N., Begum, P., Ahsan, T., Huque, S., Ahsan, M., 2004. Immunosuppressive and cytotoxic properties of *Nigella sativa*. Phytotherapy Research 18, 395–398.

Kovats, E., 1965. Gas chromatographic characterization of organic substances in the retention index system. Advances in Chromatography 1, 229–247.

Kumara, S.S., Huat, B.T., 2001. Extraction, isolation and characterisation of antitumor principle, alpha hederin, from the seeds of *Nigella sativa*. Planta Medica 67, 29–32.

Lampronti, I., Saab, A.M., Gambari, R., 2006. Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. International Journal of Oncology 29, 989–995.

Marletta, M.A., 1993. Nitric oxide synthase structure and mechanism. Journal of Biological Chemistry 17, 12231–12234.

Martin-Luengo, M.A., Yates, M., Domingo, M.M.J., Casal, B., Iglesias, M., Esteban, M., Ruiz-Hitzky, E., 2008. Synthesis of *p*-cymene from limonene, a renewable feedstock. Applied Catalysis. B, Environmental 81, 218–224.

Mbarek, L.A., Mouse, H.A., Elabbadi, N., Bensalah, M., Gamouh, A., Aboufatima, R., Benharref, A., Chait, A., Kamal, M., Dalal, A., Zyad, A., 2007. Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. Brazilian Journal of Medical and Biological Research 40, 839–847.

Moretti, A., D'Antuno, F.L., Elementi, S., 2004. Essential oil of *Nigella sativa* L. and *Nigella damascene* L., seed. Journal of Essential Oil Research 16, 182–183.

Nair, M.K.M., Vasudevan, P., Venkitanarayanan, K., 2005. Antibacterial effect of black seed oil on *Listeria monocytogenes*. Food Control 16, 395–398.

Nickavar, B., Mojab, F., Javidinia, K., Amoli, M.A.R., 2003. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Zeitschrift Fur Naturforschung 58, 629–631.

Nishikawa, M., Chang, B.J., Inoue, M., 2004. Inducible NO synthase inhibits the growth of free tumor cells, but enhances the growth of solid tumors. Carcinogenesis 25, 2101–2105.

O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. European Journal of Biochemistry 267, 5421–5426.

Rooney, S., Ryan, M.F., 2005. Effects of alpha-hederin and thymoquinone, constituents of *Nigella sativa*, on human cancer cell lines. Anticancer Research 25, 2199–2204.

Ruberto, G., Baratta, M.T., 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry 69, 167–174.

Sangwan, N.S., Farooqi, A.H.A., Shabih, F.R., Sangwan, S., 2002. Regulation of essential oil production in plants. Plant Growth Regulation 34, 3–21.

Swamy, S.M.K., Tan, B.K.H., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sa*tiva L. seeds. Journal of Ethnopharmacology 70, 1–7.

Takruri, H.R.H., Dameh, M.A.F., 1998. Study of the nutritional value of black cumin seeds (*Nigella sativa* L). Journal of Science of Food and Agriculture 76, 404–410.

Thabrew, M.I., Mitry, R.R., Morsy, M.A., Hughes, R.D., 2005. Cytotoxic effects of a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. Life Science 77, 1319–1330.

Thippeswamy, N.B., Naidu, A.K., 2005. Antioxidant potency of cumin varieties cumin, black cumin and bitter cumin on antioxidant systems. European Food Research Technology 220, 472–476.

Ultee, A., Slump, R.A., Steging, G., Smid, E.J., 2000. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. Journal of Food Protection 63, 620–624.

Edited by AM Viljoen