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Original Article

Levels of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds

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Abstract

The seeds of *Nigella sativa* are used commonly in the Middle East as a traditional medicine to treat a variety of health conditions. This paper examines the levels of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol in different brands of *N. sativa* seeds purchased from local markets in Riyadh, Saudi Arabia. Selenium was determined by the inductively coupled plasma spectrometry coupled with the hydride system. DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol were measured by high-performance liquid chromatography. The average mean concentrations (mg/kg fresh weight) of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol in all tested seeds were 0.17 ± 0.10, 9.02 ± 4.84, 5.42 ± 3.96, 0.27 ± 0.27, 2224.49 ± 1629.50 and 169.35 ± 100.12, respectively. The concentrations of these analytes were significantly affected by the country of origin of the *N. sativa*. It is concluded that *N. sativa* provides an important source of antioxidants.

Keywords: *Nigella sativa*; *Habbat Al-Baraka*; Thymoquinone; Thymol; All-*trans*-retinol; DL-α-tocopherol; DL-γ-tocopherol; Selenium

1. Introduction

Habbat Al-Baraka is the most famous Arabic name for *Nigella sativa* (an annual Ranunculaceae herbaceous plant). Its seeds and oil have been used for centuries in the Moslem World; in particular, as sacred and holistic medicine. It has been also used in India and other countries for similar purposes. The plant is also commonly known as black seeds, black cumin and black caraway seed. It constitutes a very popular additive to the traditional diet, and is often mixed with either bread or honey. Commercial use of these seeds has recently been extended to many products including shampoos, oils, soaps, etc.

The religious connection of the plant explains its historical persistence and wide cultural use in Saudi Arabia. It has been used to treat a variety of disorders such as hypertension, diabetes, respiratory problems, stomach and intestinal complaints, kidney and liver function, and circulatory and immune system support. Its value has been assessed in numerous studies (El-Daly, 1998; Zaoui et al., 2000, 2002a; El-Dakhakhny et al., 2000, 2002a, b; Medenica et al., 1997; Burits and Bucar, 2000; Salomi et al., 1992; Aqel and Shaheen, 1996; Morsi, 2000; Gilani et al., 2001; Meral et al., 2001; Hawsawi et al., 2001; Mahmoud et al., 2002; Swamy and Tan, 2000; Mabrouk et al., 2002; Aboul-Ela, 2002; Fararh et al., 2002; Hansen et al., 2003; Kanter et al., 2003; Khan et al., 2003; Al-Jishi and Abuo-Hozaifa, 2003; Hajhashemi et al., 2004).

The chemical composition of *N. sativa* is very rich and diverse. The main constituents of *N. sativa* are alkaloids, fixed and volatile oils. El-Alfy et al. (1975) found that *N. sativa* contains 0.4% volatile oil calculated on the basis of the dry weight of the seeds. A recent study by Nickavar et al. (2003), using gas chromatography with a mass spectrometer found that *N. sativa* contains 8 fatty acids and 32 compounds in the fixed and volatile oils. The fixed oil consists mainly of linoleic acid, oleic acid and palmitic

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acid, while *trans*-anethole, *p*-cymene, limonene and carvone were the major compounds in the volatile oil.

Thymoquinone is the most active constituents of *N. sativa*, representing 18.4–24% of the volatile oil (Canonica et al., 1963; El-Dakhakhny, 1963). There were a number of experimental studies demonstrating the pharmacological effects of thymoquinone on hepatotoxicity (Daba and Abdel-Rahman, 1998; Nagi et al., 1999; Mansour et al., 2001), cardiotoxicity (Al-Shabanah et al., 1998; Nagi and Mansour, 2000), diabetes mellitus (El-Mahmoudy et al., 2005), bronchial asthma (Al-Majed et al., 2001), nitric oxide production (El-Mahmoudy et al., 2002; Mahmood et al., 2003), nephrotoxicity (Badary et al., 1997, 2000; Badary, 1999), schistosomiasis (Aboul-Ela, 2002), methylcholanthrene-induced fibrosarcoma tumorigensis (Badary and Gamal El-Din, 2001), ifosfamide-induced Faconi syndrome (Badary, 1999), benzo(a)pyrene-induced forestomach (Badary et al., 1999), hyperglycemia (Hawsawi et al., 2001), lipid peroxidation (Houghton et al., 1995; Meral et al., 2001; Mansour et al., 2002; El-Saleh et al., 2004), multiple sclerosis (Mohamed et al., 2003), high blood lipids (Bamosa et al., 2002), acetic acid-induced colitis (Mahgoub, 2003) and gastric mucosal injury (El-Abhar et al., 2003).

In spite of the large number of experimental studies, there have been few studies on humans. Recent studies by Gali-Muhtasib et al. (2004) and Mansour and Tornhamre (2004) have shown the potential use of thymoquinone for the treatment of colon cancer and inflammatory and asthma diseases. Most of these studies were using thymoquinone in *N. sativa* seed oil. Although the content of the thymoquinone, dithymoquinone, thymohydroquinone and thymol in the oil of *N. sativa* has been reported (Houghton et al., 1995; Abou-Basha et al., 1995; Aboul-Enein and Abou-Basha, 1995; Ghosheh et al., 1999; Michelitsch and Rittmannsberger, 2003), no data about its content in the seeds is available. Burits and Bucar (2000) have shown that thymoquinone contents in *N. sativa* seeds and commercial oils were different. This could be due to the country of origin and manufacturing process. Such variation should be taken into account, because it might be reflected in the pharmacological properties.

There have been a number of studies supporting the antimicrobal properties of thymol extracted from *Thymus vulgaris* L. or other species (Manou et al., 1998; Mazino et al., 1999). This substance has been used in human medicine for topical treatment of skin disorders, for inhalation in respiratory disorders and in dental care. Results of Youdim and Deans (2000) highlight the potential benefit of thyme oil as a dietary antioxidant. In 1992, the Committee of Experts on flavouring substances of the Council of Europe, established that the upper limit for adding thymol should be not more than 50 mg/kg in food and 10 mg/kg in beverages. There have hardly been any studies on the antioxidant activity of thymol extracted from *N. sativa*.

Despite the extensive information on the antioxidant activity of *N. sativa* due to thymoquinone, the presence of other constituents that might have pharmacological properties such as selenium or vitamins A and E has not been studied. Ramadan and Mörsel (2002) measured a number of vitamins in a variety of seed oils. They concluded that fat-soluble vitamins comprised more than 0.2% of the total oil content. There were many reports on the role of antioxidants supplementation such as selenium, vitamin A and vitamin E in lowering the incidence of cancer, cardiovascular and other chronic health conditions.

Selenium is a nutritionally essential element to the life of humans and animals (Thomson, 2004; Goldhaber, 2003). A component of selenoproteins, some of which have important enzymatic functions. This element plays key roles in redox regulation and antioxidant function, membrane integrity, energy metabolism and protection against DNA damage. All these functions are mediated through more than 35 selenoproteins. which require adequate selenium intake for synthesis and expression. Known selenoproteins that carry out nutritional functions of selenium are glutathione peroxidase, thioredoxin reductase and iodothyronine 5′-deiodinase (Rayman, 2000; Daniels, 2004). These might help to prevent cellular damage from free radicals, regulate thyroid function and play a role in the immune system (Arthur, 1991; Corvilain et al., 1993; Levander, 1997; McKenzie et al., 1998). A large-scale cancer prevention trial in 1983 demonstrated that taking a daily supplement containing 200 μg of selenium per day could lower the risk of developing prostate, lung, and colorectal cancer (Clark et al., 1996). More recently, a number of epidemiological studies have more recently investigated the potential protective role of selenium in the prevention of many degenerative conditions including cancer, inflammatory diseases, thyroid function, cardiovascular disease (Yu et al., 1997; Yoshizawa et al., 1998; van den Brandt et al., 2003; Derumeaux et al., 2003; Gartner and Gasnier, 2003; Zhuo et al., 2004; Hercberg et al., 2004; Faure et al., 2004).

Vitamin A (retinol) and vitamin E (α-tocopherol) are lipid-soluble vitamins essential for human health. Both groups have free-radical-scavenging properties that allow them to function as physiologic antioxidants in protecting a number of chronic diseases such as cancer and cardiovascular disease (Rimm et al., 1993; Morris et al., 1994; Olmedilla et al., 2001; Schwenke, 2002; Bates, 1995; Van Poppel and van den Berg, 1997; Kaegi, 1998; Dawson, 2000; Evstigneeva et al., 1998; Elmadfa and Wagner, 2003; Hak et al., 2004; Wakai et al., 2005). Vitamin E comprises eight naturally occurring compounds (tocopherols and tocotrienols); of which DL-α-tocopherol is the predominant form in the circulation, although γ-tocopherol is the main form in the diet (Traber and Jialal, 2000). The parent compound of vitamin A family is all-*trans*-retinol. It is the most abundant dietary form of vitamin A, and occurs naturally in the form of fatty acid esters such as retinyl palmitate. There are other naturally occurring forms of vitamin A of minor dietary components such as retinal, retinoic acid, etc. (Bates, 1995).

This study was undertaken to determine the extent of variation in the levels of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol in five different brands of *N. sativa* seeds commonly consumed in Saudi Arabia.

2. Materials and methods

2.1. Materials

HPLC grade acetonitrile, methanol, ethyl acetate, methylene chloride and acetone (Fisher Scientific, Pittsburgh, USA) were used. A stock standard of DL-α-tocopherol, DL-γ-tocopherol, d-α-tocopherol acetate, all-*trans*-retinol, all-*trans*-retinol acetate and thymol were purchased from Sigma Chemical Company (Sigma, MO, USA). Thymoquinone was obtained from Aldrich Chemical Company (Aldrich, WI, USA). The selenium reference solution (1 PPM) was obtained from Fisher ChemAlert Guide, Fisher Scientific. Trace metal-free hydrochloric acid and "selectipur" nitric acid select were obtained from Fisher Scientific, Pittsburgh, USA, and E. Merck, Darmstaot, Germany, respectively.

2.2. Samples collection

In all, 115 samples of *N. sativa* seeds were purchased from several retail stores in Riyadh City, Saudi Arabia. The samples were classified according to their country of origin, and there were 25 locally grown samples from Al-Qassim. The others were imported: 30 from Ethiopia, 25 from India, 5 from Sudan and 30 from Syria. Random batches of each were bought from different shops, but the Sudanese batch was sold in one shop only. A composite sample of the *N. sativa* seeds from each batch was ground up.

2.3. Apparatus

An ATI Unicam 701-inductively coupled plasma emission spectrometer (ICP), equipped with the hydride generation system, also equipped with the hydride generation system (ATI UNICAM, Cambridge, UK), was used for the determination of selenium. Injection was carried out manually. An automated Digestion system 12,0019 with a 1012 Autostep Controller from Tecator AB, Hoganas, Sweden was utilized for acid digestion. Whatman filter paper no. 541 was obtained from Whatman International Ltd., Maidstone, England.

The Alliance Waters 2690 separations module equipped with 2487 dual λ-absorbance detector, Waters reagent manager, Waters temperature control module and column heater was used for DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymol and thymoquinone. This system was controlled by a Dell Optiplex GX1 computer and Millennium software. Stainless steel column (25 cm × 4.6 mm, i.d.), pre-column (5 cm × 4.6 mm, i.d.) packed with ODS Supelcosil LC 18, 5 μm particles as well as 500 mg C18 cartridges were purchased from SUPELCO Inc., Bellefonte PA, USA.

2.4. Analytical procedures

2.4.1. Selenium

We used a modified method of Al-Saleh and Al-Doush (1997) for determination of selenium in *N. sativa* seeds. A weighed sample of approximately 0.5 g seeds were reacted with 5 mL of concentrated "selectipur" nitric acid into a 100 mL reflux Pyrex digestion tubes. An automated Digestion system 12,0019 with a 1012 Autostep Controller was programmed as follows: 15 min ramp to 190 °C. When the digestion was complete, and the tubes cooled down for 2 h at room temperature, 5 mL of 5 M hydrochloric acid was added to each sample converting the selenium to the selenium (IV) state. The mixture was heated to 100 °C for minutes and held for 20 min. After cooling for 1 h at room temperature, tubes were kept overnight at 4 °C. Then, the digestate was filtered through a filter paper. The clear supernatant was transferred to polypropylene tubes and diluted to 10 mL with deionised water. The sample was then analyzed by the ICP, and the selenium contents were expressed as mg/kg fresh weight.

Working standard solutions were made up each day in the range 0.002–0.016 μg/mL using 5 M hydrochloric acid solution. A calibration curve of emission intensity versus concentration of selenium was drawn, and the concentrations of the unknown samples were read from the calibration graph.

2.4.2. DL-α-tocopherol, DL-γ-tocopherol and all-trans-retinol determination

A weighed sample of 0.1 g of *N. sativa* seeds was extracted with 10-mL methanol–acetone–ethyl acetate (2:2:1) and shaken vigorously for 4 min. Next, DL-α-tocopherol acetate (400 mg/kg) and retinol acetate (20 mg/kg) were added to each 0.1 g *N. sativa* seed sample, working standards and spiked samples as internal standards. Tubes were then placed in an ultrasonic bath for 20 min and centrifuged at 2500 rpm for 20 min at 20 °C. C18 cartridges were pre-conditioned with 2 mL iso-octane, 2 mL ethyl acetate, 2 mL methanol and 2 mL distilled water. Each supernatant was aspirated and diluted with 13 mL distilled water, then passed through the cartridges at a flow rate of 4–5 mL min^{-1}. The cartridges were washed with 2 mL deionized water and then with 2 mL 10% acetonitrile at a flow rate of 0.3 mL min^{-1} and dried by pulling air through the cartridges for 5 min. The DL-α-tocopherol and all-*trans*-retinol were eluted from the cartridges with 3 × 0.5 mL methylene chloride. The extract was evaporated to dryness under nitrogen stream and the dried residue was dissolved with 1.0 mL methanol.

Mobile phases of methanol: water (92:8) and 100% methanol were used for DL-α-tocopherol, DL-γ-tocopherol

and all-*trans*-retinol, respectively, at a flow rate of 1.0 mL/min. Wavelengths were set at 325 and 292 nm for all-*trans*-retinol and DL-α-tocopherol and DL-γ-tocopherol, respectively. The absorption unit full scale was 0.01. Individual stock solutions were prepared in ethanol. Injection volume was 20 μL. The exact concentrations of these solutions were determined spectrophotometrically using the extinction coefficient values: DL-α-tocopherol 75.8 at 292 nm, DL-γ-tocopherol 92.8 at 298 nm and DL-α-tocopherol-acetate, 43.4 at 285 nm. Four ranges of calibration curves of peak heights for retinol (0.025, 0.05, 0.075 and 0.15 μg/mL) and DL-α-tocopherol and DL-γ-tocopherol (0.5, 1.0, 1.5 and 3.0 μg/mL) were constructed by adding aliquots of all-*trans*-retinol, DL-α-tocopherol and DL-γ-tocopherol to 0.1 g of *N. sativa* seed sample. Linear calibration curves were generated. There was a good linear relation between peak areas and standard concentration of DL-α-tocopherol, DL-γ-tocopherol and all-*trans*-retinol.

2.4.3. Thymol and thymoquinone

A ground *N. sativa* seed sample of 0.01 g was extracted with 1 mL methanol, vortexed for 1 min and sonicated for 20 min. After that, it was left overnight in constant rotamix, vortexed for 1 min and centrifuged for 25 min at 1400 rpm. The supernatant was aspirated and an aliquot of 20 μL was injected into the HPLC with UV detector at a wavelength of 275 nm. The mobile phase utilized was methanol: water (72:25) at 1.0 mL/min flow rate. Calibration curves of peak area verses the concentrations of 2.0, 4.0, 8.0, 16.0 μg/mL for thymol and 20, 40, 80 and 160 μg/mL for thymoquinone were constructed.

2.5. Recovery

The efficiency of tested methods was evaluated by spiking *N. sativa* seed samples with selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol at various concentration levels. These spiked seed samples were run with the test samples and blanks using the same analytical procedure. The analytical recovery for spiked seeds samples with 0.05, 0.1, and 0.2 μg/mL selenium was 95.0% ± 11.46%, 90.7% ± 8.82% and 88.2% ± 5.11%, respectively, which is thought to be satisfactory. In the case of seeds spiked with 5.0, 10.0, 20.0 and 30.0 mg/kg DL-α-tocopherol and DL-γ-tocopherol, recoveries were, respectively, 101.6% ± 14.9%, 97.3% ± 8.3%, 107.4% ± 17.3% and 101.6% ± 5.9% for DL-α-tocopherol and 95.4% ± 9.8%, 92.3% ± 7.4%, 96.2% ± 9.6% and 91.2% ± 5.7% for DL-γ-tocopherol. The recoveries for spiked seeds with 2.0, 4.0, 8.0 and 12.0 mg/kg all-*trans*-retinol were 103.0% ± 8.1%, 104.7% ± 8.1%, 108.9% ± 6.4% and 112.4% ± 6.5%, respectively. Spiked seeds with 200, 400, 800 and 1600 mg/kg thymol gave recoveries of 89.2% ± 10.1%, 92.7% ± 7.2%, 101.3% ± 2.5% and 100.5% ± 9.0%, respectively, for thymol. In the case of thymoquinone, 2000, 4000, 8000 and 1600 mg/kg, the recoveries were 82.9% ± 6.8%, 92.5% ± 2.9%, 94.8% ± 3.0% and 95.8% ± 5.7%, respectively. The overall recovery results are thought to be satisfactory.

2.6. Statistical analyses

Because of the non-Gaussian distribution pattern of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol, a non-parameteric statistical method such as Kruskal–Wallis one-way analysis of variance was used to evaluate the analytical data. Statistical significance was based on the level of $p < 0.05$. All the analyses were performed using the statistical software Statgraphics Software (1999).

3. Results and discussion

Selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol were determined in five different brands of *N. sativa* seeds either locally grown locally or imported from India, Sudan, Syria and Ethiopia as shown in Table 1. These brands are commonly consumed in Saudi Arabia. Indian seeds had the highest contents of selenium, DL-α-tocopherol and all-*trans*-retinol. On the other hand, DL-γ-tocopherol, thymoquinone and thymol were the highest in the seeds imported from Ethiopia. The variation in the concentration of tested analytes among the five different brands was tested using the Kruskal–Wallis one-way analysis of variance after excluding data from Sudan due to the small sample size. Apart from selenium ($P > 0.05$), other analytes were significantly different among the four different brands of *N. sativa* seeds ($P < 0.001$). Apart from selenium, DL-γ-tocopherol and all-*trans*-retinol, the lowest average means for other analytes were in the seeds imported from Syria. In this study, oil content in tested seeds was not measured. However, it is expected to be different among the five brands. Ramadan and Mörsel (2003) tested the glycolipids contents in *N. sativa*, *Coriandrum sativum* and *Guizotia abyssinica* Cass. The three seed oils had different fatty acid patterns, which could reflect on its food value. Seed composition can be also influenced by geochemical and climatological conditions. The seed composition of soybeans, in particular the fatty acids, is affected by row spacing and irrigation (Boydak et al., 2002). A study by Britz and Kremer (2002) found elevated α-tocopherol levels in soybean seeds from weather conditions. Environmental growing conditions such as sowing date were reported to reduce isoflavone content of soybeans (Aussenac et al., 1998).

There is hardly any data published on the average daily intake of *N. sativa* seeds in Saudi Arabia. However, it seems to us based on general knowledge and local culture that it would be reasonable to assume that; in general, adults may consume 2 g *N. sativa* seeds per day (equivalent to 1 teaspoon), the calculated daily

Table 1
Means, standard deviations and ranges of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol concentrations (mg/kg) in five brands of *Nigella sativa* seeds

Analytes	Country of origin				
	Ethiopia	India	Saudi Arabia (Qassim)	Syria	Sudan
Selenium	0.18 ± 0.06 (0.114–0.330) $n = 24$	0.20 ± 0.05 (0.13–0.30) $n = 20$	0.16 ± 0.12 (0.02–0.33) $n = 20$	0.16 ± 0.15 (0.01–0.40) $n = 24$	0.13 ± 0.03 (0.10–0.16) $n = 4$
DL-α-tocopherol	10.41 ± 3.99 (3.11–16.75) $n = 28$	11.39 ± 4.71 (6.18–18.05) $n = 25$	9.52 ± 6.41 (4.01–22.4) $n = 25$	5.65 ± 1.98 (2.35–9.49) $n = 30$	7.04 ± 0.20 (6.79–7.30) $n = 5$
DL-γ-tocopherol	6.95 ± 2.43 (3.22–12.38) $n = 30$	6.47 ± 2.42 (2.25–9.26) $n = 25$	6.09 ± 6.55 (1.53–22.79) $n = 23$	2.76 ± 2.01 (0–5.39) $n = 27$	2.26 ± 0.20 (2.07–2.57) $n = 5$
all-*trans*-retinol	0.21 ± 0.03 (0.16–0.26) $n = 29$	0.27 ± 0.04 (0.21–0.36) $n = 25$	0.20 ± 0.06 (0.13–0.29) $n = 23$	0.42 ± 0.48 (0.16–1.57) $n = 30$	0.15 ± 0.005 (0.14–0.16) $n = 5$
Thymoquinone	3098.5 ± 1519.66 (1138–5983) $n = 30$	2362.68 ± 1320.29 (900–4256) $n = 25$	2250.56 ± 1923.55 (42–5424) $n = 25$	1371.9 ± 1381.5 (0–3916) $n = 30$	1274.6 ± 60.67 (1196–1344) $n = 5$
Thymol	230.63 ± 84.20 (76–336) $n = 30$	201.16 ± 127.33 (66–383) $n = 25$	133.88 ± 79.33 (29–258) $n = 25$	120.43 ± 69.47 (52–224) $n = 30$	113.40 ± 8.11 (104–123) $n = 5$

mean can be expressed expressed as μg/day for selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol intakes were 0.34 μg, 18.04 μg, 10.84 μg, 0.54 μg, 4448.98 μg and 338.70 μg, respectively. The daily intake of selenium, DL-α-tocopherol, DL-γ-tocopherol and all-*trans*-retinol is low compared to the Recommended Dietary Allowances (RDA) of the US National Academy of Sciences (1989) as indicated in Table 2.

All the different brands of *N. sativa* seeds analysed were rich in thymoquinone and thymol. Seeds imported from Ethiopia contained the highest levels of thymoquinone (3098.5 mg/kg) and thymol (230.6 mg/kg). The lowest levels of thymoquinone (1274.6 mg/kg) and thymol (113.4 mg/kg) were found in seeds imported from Sudan. In our study, the calculated daily intake from *N. sativa* was 4448.98 μg for thymoquinone (range, 0–11966 μg) and 338.70 μg for thymol (range, 58–766 μg). To our knowledge, there is no RDA for "thymoquinone and thymol", the most active constituent of the *N. sativa*. However, animal studies showed that treating mice with 8 mg/kg/day thymoquinone for 5 days protected them against cisplatin nephrotoxicity (Badary et al., 1997), carbon tetrachloride hepatotoxicity (Al-Gharably et al., 1997) and doxorubicin cardiotoxicity (Al-Shabanah et al., 1998). Mahgoub (2003) reported that a dose of 5 mg/kg thymoquinone taken for 3 days can give partial protection against colitis, while complete protection can be achieved at 10 mg/kg.

A significant effect of six different doses of thymoquinone (0.5, 1, 2, 4, 6 and 8 mg/kg/day) on blood lipids in rats was noted after 4 days by Bamosa et al. (2002), but a dose of 8 mg/kg was found to be toxic. Abuo-Hozaifa (2002) has concluded that the influence of thymoquinone on inhibition of platelet aggregation was effective only at a dose of 16 mg/kg. It seems that these results are controversial, and further research is needed to determine the therapeutic effective dose of thymoquinone in various diseases. The concentration of thymol in tested seeds (169.35 mg/kg with a range of 29.0–383.0 mg/kg) was much higher than the upper acceptable limit of thymol in food (50 mg/kg), which was set by the Committee of Experts on Flavouring Substances of the Council of Europe in 1992. Although the toxicological profile for thymol (oral LD50) is 980 mg/kg body weight in rats, 1800 mg/kg in mice, and 880 mg/kg in guinea pigs (EMEA, The European Agency for the Evaluation of Medicinal Products, 1996), the cumulative effects on human safety over a very long period of consumption has not been yet recorded.

In summary, our results support the view that *N. sativa* contains a number of antioxidants that are essential for health. There were differences in the concentrations of these antioxidants depending on their country of origin. Major constituents were thymoquinone and thymol. In spite of the low toxicity of *N. sativa* seeds as evidenced by the high LD50 value, key hepatic enzyme stability and organ integrity (Ali and Blunden, 2003), it is important to remember its hypoglycaemic effect, changes

Table 2
Calculated daily intake of selenium, DL-α-tocopherol, DL-γ-tocopherol, all-*trans*-retinol, thymoquinone and thymol (μg/day) from the five different brands of *Nigella sativa*

Country of origin	Selenium	DL-α-tocopherol	DL-γ-tocopherol	all-*trans*- retinol	Thymoquinone	Thymol
Ethiopia	0.36	20.82	13.90	0.42	6197.0	461.26
	$n = 24$	$n = 28$	$n = 30$	$n = 29$	$n = 30$	$n = 30$
India	0.40	22.78	12.94	0.54	4725.36	402.32
	$n = 20$	$n = 25$	$n = 25$	$n = 25$	$n = 25$	$n = 25$
Saudi Arabia-Qassim	0.32	19.04	12.18	0.40	4501.12	267.76
	$n = 20$	$n = 25$	$n = 23$	$n = 23$	$n = 25$	$n = 25$
Syria	0.32	11.31	5.52	0.84	2743.8	240.86
	$n = 24$	$n = 30$	$n = 27$	$n = 30$	$n = 30$	$n = 30$
Sudan	0.26	14.08	4.52	0.30	2549.2	226.8
	$n = 4$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$
All	0.34	18.04	10.84	0.54	4448.98	338.70
	$n = 92$	$n = 113$	$n = 110$	$n = 112$	$n = 115$	$n = 115$
RDA[a] (μg/day)						
Male (31–50 yrs)	70	10[a]		1000		
Female (31–50 yrs)	55	8[a]		800		
Children (4–8 yrs)	30	7[a]		700		
Infants (7–12 months	13	4[a]		375		

[a]In milligrams.

in haemoglobin metabolism and fall in leukocyte and platelet count when taken at higher doses (Zaoui et al., 2002b).

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