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The Use of Thymoquinone as a Standard for Quality Assessment of the Seeds of *Nigella sativa* and Investigation of Their Antioxidant Activity

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Abstract

In this investigation, the quality of consumer-grade seeds of *Nigella sativa* was assessed using TQ (TQ) as a standard compound. Assessment of the seeds was based on the amount of TQ present in the seeds and on the antioxidant, activity exhibited by the crude oil extracted from the seeds. Seeds were purchased from Natco Foods Ltd (UK). Hexane extract from crushed seeds was prepared using rotavapor and filtered to produce crude oil. TQ in the crude oil of *N. sativa* was successfully identified using gas chromatography-mass spectrometry. TQ was then quantified by high-performance liquid chromatography and compared to a commercially available cold-pressed oil. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out to test the antioxidant activity of pure TQ and *N. sativa* crude oil using quercetin as a reference antioxidant compound.

Keywords

Antioxidant, *Nigella sativa*, TQ, Seeds, Phytochemical, Oil

Introduction

Natural products derived from plants have been used for a long time. Plant roots, leaves and extracts have been known for various functions, e.g., cold relief, anti-inflammatory, immune system improvements and many other functions. During the last century, many of the existing medicines originated from herbal substances, for example morphine and codeine from opium poppy; scopolamine, which blocks the neurotransmitter acetylcholine, was derived from *Datura stramonium*; paclitaxel, which is currently used for cancer therapy, originated from *Taxus brevifolia*. More than 25% of the currently used drugs have been directly derived from plants [1]. Natural substances have acted as a structural template for the synthesis of synthetic drugs, for example the analgesic pethidine was developed using morphine [2]. Drugs extracted from natural products have become a challenge for synthetic chemistry because some of the plant species are threatened due to the over-collection from nature.

Herbal medicines are widely researched for many functions, for example cancer cytotoxicity, analgesic properties, anti-inflammatory and antioxidant activity functions. This field is widely researched because of the prevalence of various diseases that raise public health concerns. Considering that 25% of current medications have been derived from plants [1], a strong hypothesis can be made that other plants contain active substances that could potentially relieve, or even cure various conditions.

Considering the various marketing claims associated with natural products, for example "organic", "healthy" or "natural" that are made by the companies producing them, the public has developed a tendency or rather a lifestyle that exalts natural products, therefore forming the basis for the consumer market and re-

search funding. It is more convenient to research existing substances than developing new medicines because at least some hypothesis can be made using the knowledge of herbalists and people who have reported potential anti-disease activity of a specific plant rather than developing new medications that have a weak hypothesis. It is more costly to develop a new medicine for a specific condition because it requires multi-disciplinary research, i.e. it requires more funding because new medications must be approved by the government, tested on animals, and then undergo fierce scrutiny during the clinical trials before they can be used. Various studies demonstrate that natural products have positive biological function in animal and human studies, without causing major side effects; therefore, it is sensible to suggest that active compounds from natural products should have investigative preference over the development of new synthetic medications.

The layman sometimes may hold a perception that natural products are harmless and prefer them to the synthetic products. However, such view is superficial; although some of the products may be harmless, however, many of them, of course, are harmful in high concentrations. Some antioxidants, if taken at high doses could damage body function. Researchers at Kansas State University have demonstrated, in an animal study, that high antioxidant intake in animals impaired muscle function [3, 4]. Acute antioxidant supplementation reduced skeletal blood flow [3] and reduced oxygen delivery to muscles in rats [4].

It is essential that plant extracts are pure and standardised i.e., contain a known amount of the active substance. If the product is standardised, the consumer may decide to pay more for such a product. Standardisation also allows manufacturers to assess the quality of their products.

It is also very useful to know the amount of a bioactive compound present in a natural product to form a consensus amongst bioactivity claims, i.e., it is essential to relate the possible health benefits to a certain concentration of a bioactive compound. For example, a phytochemical showing bioactivity may not show bioactivity at lower concentrations or it may be even producing a toxic effect if consumed in high concentrations. Thus, it is very important to relate its potential bioactivity to its concentration. If the amount of a bioactive compound in a plant is unknown, there is no scientific basis to justify its bioactivity. The assessment of the quality of compounds can be done using high performance liquid chromatography (HPLC) [5], gas chromatography–mass spectrometry (GC–MS) or thin layer chromatography (TLC), all of which require a standard to correlate. Different botanical processes of standardisation of plant extracts may influence the properties of antioxidants, for example, using one solvent to make a crude extract from a plant might yield less of an active compound or affect its structure compared to using another solvent. Food-grade extracts are usually prepared by the cold-press technique or using a food-grade solvent, such as ethanol.

Antioxidants are natural compounds that are widely distributed in the plant kingdom. They are secondary plant metabolites, i.e., they are generated via specific metabolic pathways. Antioxidants derived from plants are phytochemicals, i.e., compounds that usually possess one or more hydroxylated aromatic or phenolic rings [6]. The human body produces some anti-oxidising agents, for example the hormone melatonin and uric acid.

A considerable amount of research has been done in support of the prophylactic effect of antioxidants. Antioxidants are known to prevent cholesterol-related and oxidation-induced disease [6]. Antioxidants neutralise oxidising agents that are generated by the human body. For example, macrophages, or immunity cells produce nitric oxide to kill pathogens. Sometimes, oxidising agents become harmful to the host and therefore induce oxidising stress that may lead to the loss of cellular membrane integrity and in more severe cases to oxidising stress-induced disease. If the cell membrane integrity is lost, cellular constituents will come out, producing even more oxidising stress to surrounding tissues due to various oxidising enzymes that are present in the cell.

A good example of an antioxidant is quercetin. Quercetin is found in fruits and vegetables. It is known to be a very powerful free-radical scavenger [7]. As such, quercetin is widely used as a reference to test other potential antioxidants [7].

N. sativa

N. sativa, also called black seed or black cumin, is a seed-producing plant native to Southeast Asia. It is used in the continental food making, i.e., meat preparation, and as an herbal medicine. It has been known for its medicinal uses for centuries. It is widely mentioned in the Middle Eastern folklore.

There are many different claims associated with *N. sativa* extract, ranging from *in vitro* anti-cancer activity [8] to asthma treatment [9]. The active ingredients in *N. sativa* that are claimed to have bioactivity are: TQ, thymohydroquinone, diTQ, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and alpha-hederin [10].

TQ (2-isopropyl-5-methylbenzo-1,4-quinone) is found in *N. sativa* [11, 12]. TQ is classified as a phytochemical compound. The major constituent of *N. sativa* seeds is oil (Table 1).

Chemical analysis of *N. sativa* revealed the presence of both fixed oils and volatile oils. Linoleic acid is the major constituent of *N. sativa* oil (Table 2).

In the last two decades, scientists have shown a lot of interest in the pharmacological actions of the volatile oil of the *N. sativa* seeds and its major constituent, TQ. It has been reported that it has a lot of potential for further cytotoxicity in *in vivo* tests [13].

Table 1: The general chemical composition of *N. sativa* seeds [13].

Constituent	% Range (w/w)
Oil	31 - 35.5
Protein	16 - 19.9
Carbohydrate	33 - 34
Fiber	4.5 - 6.5
Ash	3.7 - 7
Saponins	0.013
Moisture	5 - 7

Table 2: The chemical composition of *N. sativa* oil [13].

Constituent	% Range (w/w)
Linoleic acid	44.7 - 56
Oleic acid	20.7 - 24.6
Linolenic acid	0.6 - 1.8
Arachidic acid	2 - 3
Palmitoleic acid	3
Eicosadienoic acid	2 - 2.5
Palmitic acid	12 - 14.3
Stearic acid	2.7 - 3
Myristic acid	0.16
Sterols	0.5

It has been reported that volatile oil from *N. sativa* has the following biological activities: decreases arterial blood pressure in dogs and rats at 4-32 µl/kg [13], reduces temperature in rats [14], protects rats from alcohol-induced ulcers [15], decreases glucose levels in rabbits and rats [16], concentrations of 120-380 µl/ml downregulate rat neutrophils *in vitro* [17] and has an immunosuppressant effect on rats [17]. Furthermore, it has been reported that the volatile oil has anti-fungal and anti-bacterial properties. *Salmonella*, *Shingella shigae*, *Bacillus cereus*, *Vibrio cholera* and *Pseudomonas aeruginosa* were reported to be susceptible to the volatile oil's anti-bacterial activities [18-20].

In more recent and novel studies by Wirries et al. [21], TQ was shown to accelerate the differentiation of osteoblasts, i.e., enhanced bone formation.

Another major study was undertaken by researchers at the American University of Beirut, Lebanon [22], which showed that TQ can induce apoptosis (programmed cell death) in leukaemia HTLV-1 negative cells. It is interesting to note that TQ induced apoptosis by increasing reactive oxygen species in leukaemia cells.

A study at the Yambian University, China [23] showed that TQ is hepatoprotective. Bai et al. [23] demonstrated that TQ can decrease the viability of tumor hepatic stellate cells It is also reported that TQ inhibits anti-apoptotic enzyme phosphoinositide 3-kinase [23], which is involved in cellular growth and differentiation. Overexpression of phosphoinositide 3-kinase is known to be related to many cancers. In the same study, TQ was shown to attenuate toll-like receptors as well. Overexpression of toll-like receptors is known to be related to various cancers and arthritis [24]. Recent studies suggest that TQ can be a potential cancer therapeutic agent.

It is also notable that several patents have been granted in the US for the medical use of TQ, one of them was granted in 2001 for anti-neoplastic and cytotoxic use (US patent number: US6218434 B1).

Materials and Methods

N. sativa extract preparation

25 g of commercially available food-grade *N. sativa* seeds (Natco Foods Ltd, batch number: 121106-BN7866, produced November 2012, exp. November 2014) were weighed using an analytical balance. The seeds were crushed into a fine powder using a mortar and pestle (Figure 1).



Figure 1: *N. sativa* seeds were crushed using a mortar and pestle.

The powder was then divided into two 1 L beakers. Thereafter, 500 ml of 99.5% reagent grade hexane (Fisher Scientific, Loughborough, UK) was poured into each beaker. The beakers were covered with foil and then left for 2 h, stirring each solution for 30 s at 20 min intervals with a glass rod. Solutions were then filtered under vacuum using a Büchner apparatus. The filtered solutions were then transferred into a round-bottom flask and attached to a Buchi Rotavapor R-205. A small amount of grease was applied to the flask connector to reduce the friction. The water bath was set at 40 ˚C, the round-bottom flask was then lowered into the bath. The evaporator's pressure was varied (120-200 millibars) according to the sample boiling properties. The sample was then transferred into a smaller (100 ml) round-bottom flask (56.9 g) and attached to the evaporator again until the solvent evaporated. The extract was then collected stored in a refrigerator at 4 ˚C.

Cold-pressed oil of *N. sativa* was purchased from Zamzam international for analytical and comparative purposes.

TLC analysis

Three silica-coated aluminum plates were prepared. An origin line was drawn 1.5 cm above the base of the plate. The solvent front line was drawn 1 cm from the top of the silica plate. Three equally distributed points were marked for each analyte: E (sedimentary part of the extract), O (crude oil) and TQ (TQ standard). The mobile phase was prepared in the system toluene:acetone:acetic acid (88 ml:10 ml). A small volume of the mobile phase was then transferred into a beaker, making sure it was below the line of origin drawn on the silica plate. Using glass capillaries, each of the three pure samples, including TQ standard, was spotted to the corresponding points on the line of origin. The silica plate with three sample spots was then immersed in the beaker with the mobile phase. After approximately 25 min, samples were taken out and observed under UV light.

GC-MS analysis

GC–MS was performed for the TQ standard, the extract (sedimentary part of the extract) and for the clear oil samples (whole extract sample). Samples were run by phase separation laboratory technician, Mr. Osman Erkek, Science Centre, London Metropolitan University. Chromatograms were produced for all three samples.

HPLC analysis

An HPLC (PerkinElmer, UK) system was used to quantify TQ. Reverse phase-HPLC was carried out using an isocratic system with a flow rate of 1 ml/min. The mobile phase was methanol:0.5% aqueous phosphoric acid (60:40). The detection wavelength was 254 nm. All analyses were performed at ambient temperature. Both solvents were degassed in an ultrasound bath. ACE 5 C18 column, serial ID: A92742 (250 mm x 4.6 mm) was used with the PerkinElmer HPLC system. Data acquisition and chromatographic analysis were done with TotalChrom software. To quantify TQ in the crude oil, a series of standard solution dilutions (Table 3) was prepared using 1.04 mg/ml standard concentration TQ (99%, Sigma Aldrich, Batch no: MKBL2542V) solution and run using HPLC.

All seven solutions were individually run on an HPLC, set at 254 nm, 1 ml/min, seven chromatograms were generated.

100 µl of filtered crude oil from *N. sativa* preparation was transferred into a volumetric 10 ml flask; methanol was added to dilute it. The dilution factor was 1:100, thus 1 ml of this solution had 10 µl of *N. sativa* crude oil. Because a 5 µl injection loop was used for all samples, theoretically 5 µl of injected sample had 0.05 µl of *N. sativa* crude oil. Additionally, once the correct peak for TQ was identified, it was spiked with the standard to make sure that it is the correct peak by mixing 1 ml of 1.04 µl/ml TQ stock solution was added to 1 ml of crude oil solution diluted in methanol (1:100).

Antioxidant activity test

DPPH (Sigma-Aldrich, Poole, Dorset, UK), 99% TQ (Sigma-Aldrich, Poole, Dorset, UK) and quercetin (Sigma-Aldrich, Poole, Dorset, UK) were used in this investigation.

Stock solutions were made as follows: 60 µg/ml of DPPH (0.0060 g of DPPH transferred into a 100 ml volumetric flask and volume made up with methanol), 1 mg/ml quercetin (0.0100 g of quercetin transferred into a volumetric 10 ml flask and volume made up with methanol), 1 mg/ml TQ (0.0100 g of TQ transferred into a 10 ml volumetric flask and volume made up with methanol), and 0.9 g/ml of *N. sativa* oil (1 ml of oil transferred into a 10 ml volumetric flask, volume made up with methanol). All stock solutions were mixed on a vortex mixer for 1 min. Three sets of 12 spectrophotometric cuvettes were prepared for all three solutions. Three sets of 12 tubes were prepared for both phytochemicals and for *N. sativa* oil. 0.5 ml of stock DPPH was added to all 12 cuvettes, for all sets. Twelve twofold serial dilutions were performed for each antioxidant solution. Contents from quercetin two-fold serial dilution tubes were transferred into corresponding cuvettes, i.e., tube 1 (1:2) to cuvette 1, tube 2 (1:4) to cuvette 2, etc. This was repeated for TQ and *N. sativa* oil dilutions. All three sets were left for 30 min at room temperature. In the meantime, a Jenway 7315 spectrophotometer was set to read at 517 nm wavelength and blanked with methanol. The absorbance was read and recorded for each sample, for all sets.

Table 3: Standard solution dilutions for HPLC analysis.

Tube	Dilution	Concentration (µg/ml)
1	1:1	1004
2	1:2	502
3	1:4	251
4	1:8	125.5
5	1:16	62.75
6	1:32	31.375
7	1:64	15.688

Results

Extract preparation

A flask with the sample was weighed (65.4879 g) using an analytical balance. The sample weight was calculated as 8.488 g (sample + flask)-flask = sample weight. The sample (seed extract) was then covered with aluminum foil and stored in a refrigerator. The remaining powder after the filtration process was covered with foil and stored at room temperature.

TLC analysis

Undiluted clear and sediment parts of the extract did not result in clear bands. Diluted extract showed a clear band for TQ in both, crude oil, and the sediment bottom solution. Some peak tailing can be observed, which can be attributed to different compounds, impurities and trailing due to slight inhomogeneities in the silica plate (Figure 2).

GC-MS analysis

The retention time for the TQ standard upon GC–MS analysis was 13.641 min (Figure 3A). TQ in the crude oil run was identified as the peak with a retention time of 13.561 min (Figure 3B). The TQ peak in the sedimentary part of the extract was identified as the peak with a retention time of 13.556 min (Figure 3C). The peaks for TQ in the extract samples had largely the same retention time with a slight mean deviation of 0.0825 min from the TQ standard peak. Thus, it was assumed that these peaks are for TQ. It was then confirmed by the analysis of GC–MS NIST database that all peaks are for TQ.

Quantification of TQ in *N. sativa*: HPLC

The peak height for TQ from the 1.04 mg/ml standard TQ solution run (Figure 4) was 932759.49 µV. Retention time and peak area for TQ were 13.940 min and 16642081.69 µV x s, respectively.

The peak height for TQ in the Zamzam Black Seed oil run (Figure 5) was 310954.39 µV. The TQ retention time was 14.110 min. The peak area was 5462995.72 µV x s.



Figure 2: TLC plates (E - sediment part of the crude oil; O - clear part of the crude oil and TQ - thymoquinone standard) using three dilutions for optimal signal.

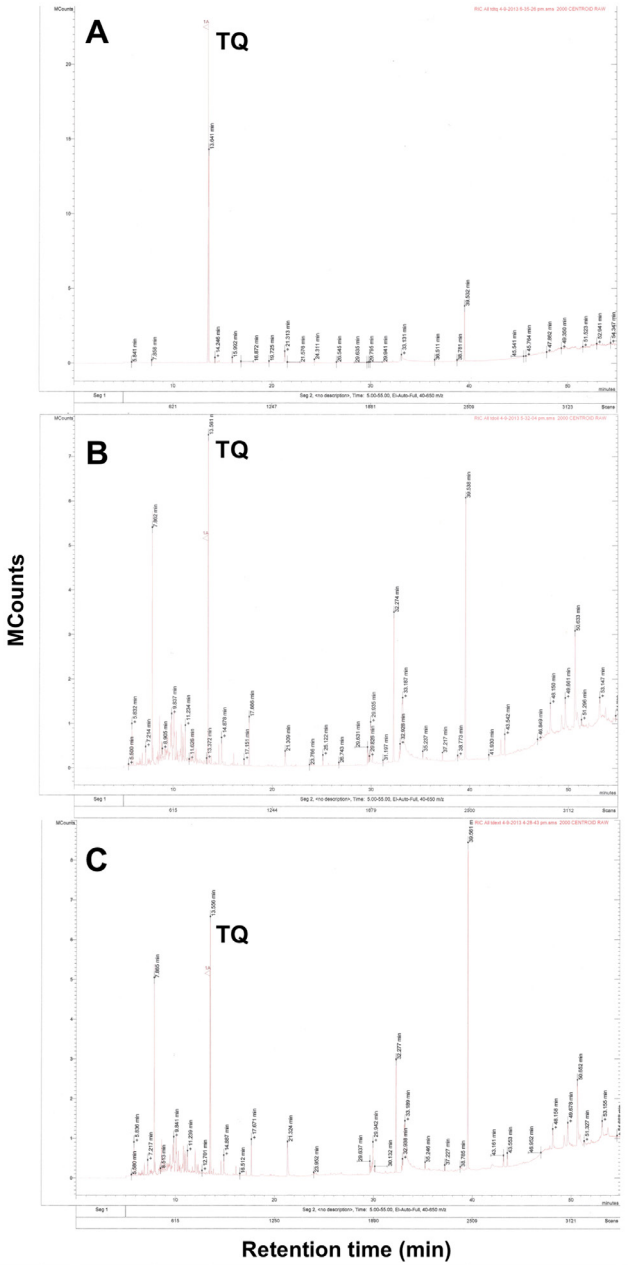

Figure 3: GC-MS spectra of **(A)** TQ standard, **(B)** Hexanoic crude oil (*N. sativa*), and **(C)** Sediment (*N. sativa*).

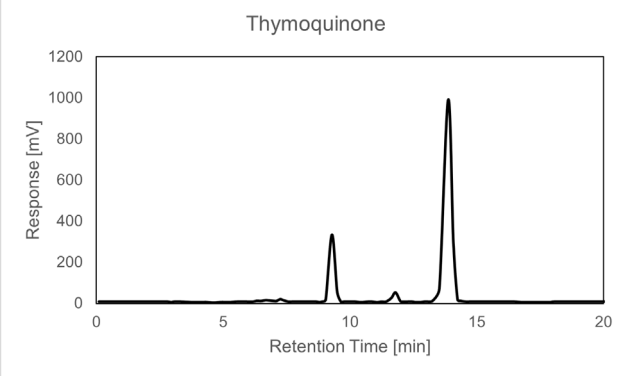

Figure 4: HPLC of TQ standard.

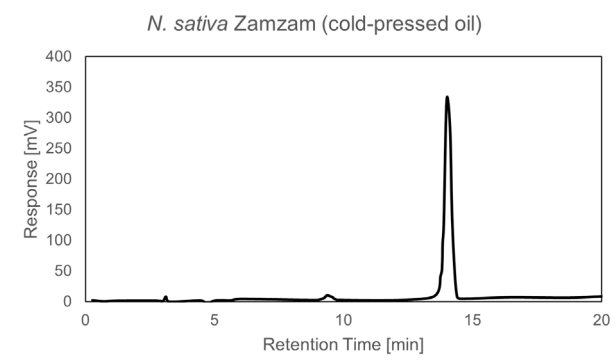

Figure 5: HPLC of cold-pressed Zamzam Black Seed oil.

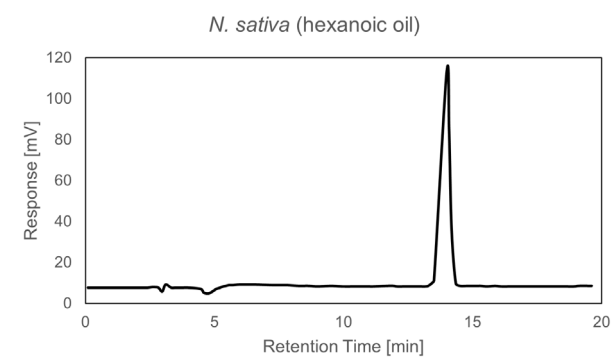

Figure 6: HPLC of hexanoic extract of *N. sativa* seeds.

The peak height for TQ in the extracted crude oil run (Figure 6) was 107132.79 µV. The TQ retention time was 14.00 min. The peak area was calculated as 1962512.66 µV x s.

Using the equation for a straight line, the following amount of TQ per 1 ml of *N. sativa* crude oil was found:

$y = mx + c$

$y = 0.00006x + 27.583$

$y = (0.00006 \times 1962512.66) + 27.583$

$y = 145.334$

Multiplied by the dilution factor:

$145.334 \times 100 = 14533.376$

Therefore, it was calculated that 1 ml of *N. sativa* oil has 14533.376 µg of TQ (14.533 mg/ml) (Figure 7).

The amount of TQ in cold-pressed *N. sativa* oil from Zamzam International was also calculated using the same formula. It was found that cold-press oil has 35.54 mg/ml of TQ (Table 4).

The weight of 1 ml of crude oil from *N. sativa* was 0.9424 g, therefore because 8.488 g of *N. sativa* crude oil was made from 25 g of seeds, it was calculated that 25 g of *N. sativa* seeds from Natco Foods Ltd could yield:

$0.9424 = 14.533$

$8.488 = x$

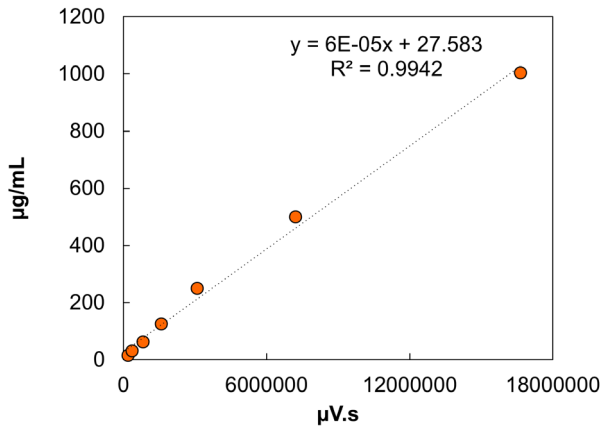

Figure 7: Calibration curve for HPLC quantification of TQ.

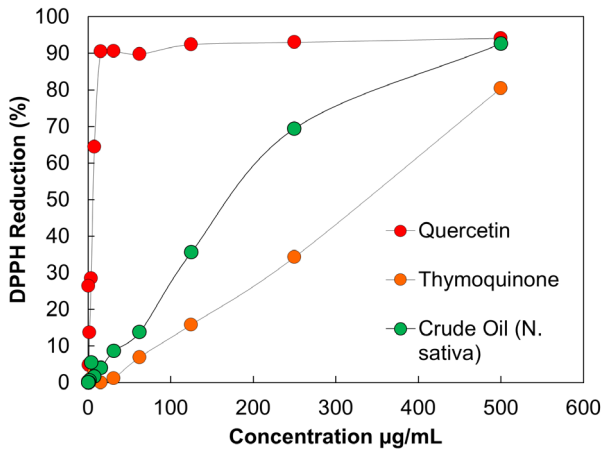

Figure 8: DPPH reduction: quercetin vs TQ.

Table 4: TQ concentrations in oils derived from HPLC standards.

Hexane-extracted from food-grade seeds	Cold-pressed oil (Zamzam international)
14.53 mg/ml	35.54 mg/ml

$$x = \frac{8.488}{0.942} \text{ x } 14.533$$

$$x = 130.951$$

Thus, 25 g of food-grade *N. sativa* seeds can yield 130.951 mg (0.13 g) of TQ.

Antioxidant activity tests

The following graph (Figure 8) was produced to compare quercetin's and TQ's antioxidant activities in reducing DPPH. It can be observed that the curve for TQ is more linear and shows lower reduction of DPPH (%). It's counterpart hexanoic extraction of *N. sativa* shows slightly higher reduction of DPPH.

The following graph (Figure 9) was produced to compare antioxidant activities of crude oil of *N. sativa* in reducing DPPH. It can be observed that the curve for TQ and the curve for the crude oil are similar in shape and scale.

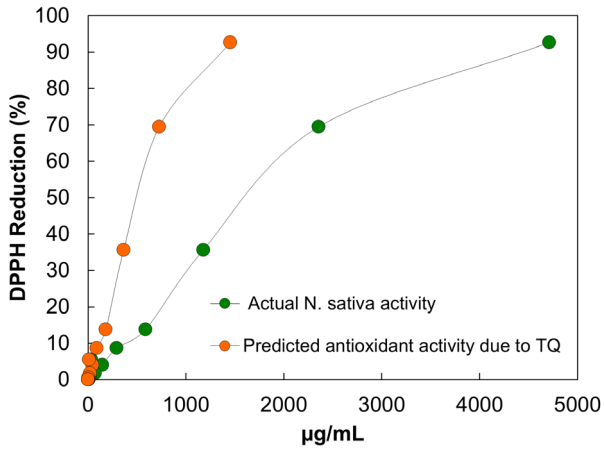

Figure 9: DPPH reduction: *N. sativa* crude oil vs it's TQ content.

Discussion

Based on the previous literature, *N. sativa* seeds contain from 31% to 35.5% oil [13]. Assuming no solvent was left in the final sample after evaporation, 25 g of *N. sativa* seeds can yield 8.488 g of crude oil. Thus, it can be calculated how much oil was extracted from the seeds using the following equation:

$$\frac{8.488}{25} \text{ x } 100 = 33.95\%$$

Therefore, it can be concluded that *N. sativa* seeds from Natco Foods Ltd have 33.95% oil, which falls within the range 31–35.5% published by El-Tahir and Bakeet [13]. Nevertheless, because the amount of oil in the seeds falls within the published range, it is indicative that the extraction materials and methods were appropriate for the isolation of oils from the plant. It is also notable that a negligible black sediment was observed after the extract from *N. sativa* seeds was left overnight, which means that there were small traces of protein and carbohydrate that were not fully filtered, which can contribute to the weight of the extract. Because of the relatively insignificant amount of sediment present in the extract, the sediment was not considered to contribute to the total weight of the extract for quantitative TQ analysis.

Hexane solvent is a non-polar solvent that is used to isolate non-polar organic substances, therefore the extract contained various oils (Table 2).

GC–MS revealed the presence of TQ in both samples, the sedimentary part of the extract and the clear part of the extract. The TQ standard peak on the resultant chromatogram was used as a reference. The TQ peak was checked against the GC–MS library. A match factor above 800 was used for TQ identification. The highest peak in figure 3A (TQ standard GC–MS chromatogram) was checked against the GC–MS library's database (NIST Libraries for Spectrum). The peak at 13.561 min (Figure 3B, crude oil run) and the peak at 13.556 min (Figure 3C, extract/sedimentary part) were close to the highest peak observed in the TQ standard chromatogram (Figure 3A) at 13.641 min. Upon NIST Libraries for Spectrum chromatographic analysis, considering all compounds

above match factor 800, it was found that the highest peak in the TQ GC–MS run (Figure 3A) was matched to 2,5-cyclohexadiene-1,4-dione-2-methyl-5-(1-methylethyl), with the empirical formula of TQ ($C_{10}H_{12}O_2$). Thus, this peak was considered a peak for TQ. Various peaks were found in the crude oil and the extract analyses (Figure 3). Other peaks checked against the NIST Libraries for Spectrum databases in both chromatograms revealed the presence of thymol, carvacrol, other phenols and benzene compounds, as well as plant steroids and oils.

TQ was quantified from *N. sativa* crude oil using a calibration curve (Figure 7). Seven TQ standards were prepared in a series of two-fold dilutions and then were run on the HPLC system using 40:60 (deionised water:methanol) ratio. Because water is subject to H_3O^+ and OH^- ions because of polar interactions between water molecules, phosphoric acid had to be used to buffer the ions. So, on the assumption that these ions would have otherwise reacted with TQ because of its radical-scavenging activity, 0.5% aqueous phosphoric acid was used. If TQ would scavenge these radicals, it might not be detected at 254 nm wavelength in the HPLC detector because of molecular changes that would affect its λ_{max}. TQ peaks were very well eluted; therefore the solvent system (isocratic, water:methanol, 40:60 ratio) was selected as the most appropriate for TQ quantification. Several other methods were used, including isocratic water:methanol (20:80), gradient elution water:methanol (20:80) and others. Because methanol is slightly less polar than water, it was used as the main eluent. As shown in figures 4, figures 5, and figures 6, no peak tailing is observable. The HPLC method used in these quantitative analyses allowed the quantification of TQ, however, other compounds that were expected to elute in the given run time were thymol and carvacrol. There were no peaks of thymol or carvacrol on any of the chromatograms. An assumption can be made that the run time must be increased for thymol and carvacrol to elute. As the method in this investigation was very similar to the method published by Hadad et al. [5], except for the different flow rate (in this investigation 1 ml/min was used, Hadad et al. [5] used 1.5 ml/min flow rate) and the addition of phosphoric acid to water in the solvent system in this investigation, it can be concluded that either upon the extension of the solute run time or an increase in the flow rate of the eluent, both solutes would be eluted.

Antioxidant activity tests for quercetin, TQ and crude oil from *N. sativa* revealed that quercetin has the highest antioxidant activity because of the numerous phenol groups in the structure of quercetin. The method in antioxidant activity measurements relied on the hydrogen-atom donating activity that reduces DPPH. DPPH is a stable free radical that is purple in colour. When DPPH is reduced, a color changes from purple to yellow is observed. In this antioxidant activity test, TQ was found to have very little antioxidant activity. As shown in figure 8, 500 µg/ml of TQ is required to reduce 80.38% of DPPH, whereas only 15.625 µg/ml quercetin reduced 90.5% of DPPH. Considering these findings, it can be said that quercetin shows approximately 32 times higher antioxidant activity than TQ (500/15.625). Quercetin's cut-offs point concentration is 15.625 µg/ml, which is the smallest concentration of quercetin to still exhibit above 90% reduction of DPPH. Quercetin concentrations below this show a sharp decrease in antioxidant activity. It is reasonable to conclude that the TQ cut-off point concentration to show strong antioxidant activity is at its highest concentration, 500 µg/ml.

Crude oil from *N. sativa* shows slightly stronger antioxidant activity than TQ (Figure 8). It is also notable that quantified TQ can be included in the consideration of the antioxidant activities of crude oil from *N. sativa*. It is notable that the crude oil and TQ standard had initial concentrations of 1 mg/ml and 1.453 mg/ml, respectively, (based on the quantitative HPLC analysis), so the crude oil showing a negligible increase in the antioxidant activity may be purely based on slightly higher TQ concentration in the oil than in the standard TQ solution. Therefore, TQ is the major, and probably the only phytochemical in the crude oil to show any antioxidant activity (Figure 9). Other antioxidants may also be present in different extractions of the seeds.

Conclusion

It was found that 100 g (25 g) of *N. sativa* seeds from Natco Foods Ltd could yield 0.52 g (0.13 g) of TQ. It is evident that cold-pressed oil from *N. sativa* has more than double the amount of TQ than in the hexane extract of *N. sativa* (crude oil). Since TQ was identified in the relatively small sedimentary part of the extract, the filtrate subjected to further hexane recovery may yield slightly higher amount of TQ. It can be concluded that neither TQ nor *N. sativa* crude oil show strong antioxidant activity when compared to quercetin, thus its biological function remains uncertain and open to further investigations.

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Conflict of Interest

The author declares that there is no conflict of interest.

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