

Comparison of Physicochemical Analysis and Antioxidant Activities of *Nigella sativa* Seeds and Oils from Yemen, Iran and Malaysia

(Perbandingan Analisis Fizikokimia dan Aktiviti Antioksidan dalam Biji dan Minyak

Nigella sativa dari Yemen, Iran dan Malaysia)

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ABSTRACT

The study was aimed to analyze the physicochemical properties and antioxidant activities in five batches of seeds and oils of *Nigella sativa*, obtained from Malaysia, Iran and Yemen. Proximate analysis showed that the seeds contained 20.63-28.71% crude fat, 11.35-14.04% crude protein, 5.37-7.93% total moisture, 4.15-4.51% total ash contents and 48.69-57.18% total carbohydrate contents. Physicochemical analysis showed a refractive index of 1.4697-1.4730, specific gravity of 1.369-1.376 g/cm³, peroxide value of 3.33-21.33 meq O₂/kg, 184-220 mg/g in saponification number and unsaponifiable matter of 1.1-1.8% in the oil samples. The seeds showed high mineral content such as Ca (2242 mg/kg), K (6393 mg/kg) and Mg (2234 mg/kg). The oil sample from Kelantan, Malaysia contained the lowest saturated fatty acid (SFA) (1.42±0.29%) while Sudan, Yemen contained the highest content of polyunsaturated fatty acid (PUFA) (65.13±5.45%). Monounsaturated fatty acid (MUFA) were found the highest (20.45±2.61%) in the seed samples originated from Iran. Seeds from Iran showed the highest antioxidant activity (IC₅₀ = 1.49 mg/mL) and total phenolic content (30.84 mg GAE/g) while oil sample from Sudan, Yemen has the highest antioxidant activity (IC₅₀ = 4.48 mg/mL). Seeds from Iran have the highest quality among the seed samples while oil samples from Kelantan, Malaysia was the best among the oil samples in terms of low SFA, high PUFA, MUFA and antioxidant activities.

Keywords: Antioxidants; *Nigella sativa*; oil; physicochemical; seeds

ABSTRAK

Kajian ini bertujuan menganalisis ciri fizikokimia dan aktiviti antioksidan dalam lima sampel biji dan minyak *Nigella sativa* yang diperolehi dari Malaysia, Iran dan Yemen. Analisis proksimat menunjukkan biji *Nigella sativa* mengandungi 20.63-28.71% lemak kasar, 11.35-14.04% protein kasar, 5.37-7.93% air, 4.15-4.51% jumlah abu dan 48.69-57.18% jumlah karbohidrat. Analisis fizikokimia menunjukkan indeks refraktif adalah sebanyak 1.4697-1.4730, spesifik graviti sebanyak 1.369-1.376 g/cm³, nilai peroksida sebanyak 3.33-21.33 meq O₂/kg, 184-220 mg/g dalam nombor saponifikasi dan jirim tidak disaponifikasi sebanyak 1.1-1.8% di dalam sampel minyak. Biji menunjukkan kandungan mineral yang tinggi iaitu Ca (2242 mg/kg), K (6393 mg/kg) dan Mg (2234 mg/kg). Sampel minyak dari Kelantan, Malaysia mengandungi asid lemak tepu (SFA) yang terendah (1.42±0.29%) sementara Sudan, Yemen mengandungi kandungan asid lemak poli tak tepu (PUFA) yang tertinggi (65.13±5.45%). Asid lemak mono tak tepu (MUFA) didapati paling tinggi (20.45±2.61%) di dalam sampel biji yang berasal dari Iran. Biji dari Iran menunjukkan aktiviti antioksidan (IC₅₀ = 1.49 mg/mL) dan jumlah kandungan fenolik (30.84 mg GAE/g) yang tertinggi sementara sampel minyak dari Sudan, Yemen mempunyai kandungan aktiviti antioksidan yang tertinggi (IC₅₀ = 4.48 mg/mL). Biji dari Iran mempunyai kualiti yang paling tinggi antara sampel biji yang lain sementara sampel minyak dari Kelantan, Malaysia adalah yang terbaik antara sampel minyak yang lain dari segi rendah SFA, tinggi PUFA, MUFA dan aktiviti antioksidan.

Kata kunci: Antioksidan; biji; fizikokimia; minyak; *Nigella sativa*

INTRODUCTION

Nigella sativa L. or the common English name, black cumin is known for its seeds that has special healing properties as being made known in Quran (Al-Bukhari: 815) which states that black cumin seeds are used to treat all kinds of illnesses except death itself. Black cumin seeds have been used for centuries long in culinary due to the strong, hot peppery taste to it (Ramadan 2007). The seed oil or extract is found to have therapeutic properties

and is considered as one of the newer sources of edible oils (Cheikh-Rouhou et al. 2007). Both seeds and oils are often used as nutritional supplement due to its various health properties as they have been reported to possess antitumour activity (Ghosheh et al. 1998), antioxidant activity (Burits & Bucar 2000), anti-inflammatory activity (Houghton et al. 1995), antibacterial activity (Morsi 2000) and a stimulatory effect on the immune system (Salem & Hossain 2000).

Scientific investigations have reported its proximate results for moisture, oil, protein, ash and total carbohydrate to be in the range of 3.8-8.65%, 24.48-40.35%, 20.8-26.7%, 3.7-4.86% and 24.9-40.0%, respectively (Atta 2003; Cheikh-Rouhou et al. 2007; Takruri & Dameh 1998). The chemical properties of the oil reported were 0.9110-0.9210 g/cm³ for specific gravity, 1.46-1.47 for refractive index, 192-218 mg/g for saponification value and 4.35-18.1 meq O₂/kg for peroxide value (Abdel-Aal & Attia 1993; Atta 2003; Cheikh-Rouhou et al. 2007). Minerals that are found dominantly in the seeds are potassium, calcium, phosphorus and magnesium (Sultan et al. 2009). Both seeds and oils are abundant in oleic, linoleic and linolenic acids which are unsaturated fatty acids (Atta 2003). The vastness of medicinal properties of the *N. sativa* can be attributed to its phenolic compounds that contain high levels of antioxidant activity. It has been reported that black cumin seeds have a phenolic content that is higher than most edible oils except for olive oil (Salvador et al. 2001).

Even with the amount of work done on *N. sativa*, there is a lack of data on the nutrient composition of both seeds and oils, thus, preventing further comparisons to be done as to determine the effectiveness of either forms of *N. sativa*. Besides that, local varieties have not been studied and compared with those imported. It is of utmost importance to determine the composition of local varieties as black cumin seeds are often affected by geographical differences, climate, soil, harvest and storage. This study was carried out to determine and compare the physicochemical characteristics, nutrient profile and antioxidant activities found in both *Nigella sativa* seeds and oils that were obtained from Malaysia, Iran and Yemen.

MATERIALS AND METHODS

NIGELLA SATIVA SEED AND OIL SAMPLES

Five varieties of mature black cumin seeds were purchased from Yemen and Malaysia. The three varieties seeds from Yemen were reported to be from Sanaa, Sudan and Habsyah. While the other two varieties black cumin seeds purchased from Malaysia were reported to be originated from Kelantan, Malaysia and Iran. Five different batches of black cumin seed oils were also purchased from Yemen and Malaysia. The three different batches of oil that were obtained from Yemen were reported to be from Sanaa, Sudan and Habsyah. Meanwhile, the other two batches of oil samples were obtained from Malaysia (Kelantan and Shah Alam). All analytical determinations were performed in triplicates. *N. sativa* seeds were cleaned and crushed using an electric grinder for 1 min. Both oil and seed samples were analysed for its macronutrient, fatty acid, antioxidant activity and total phenolic contents. Physicochemical analyses were analysed on oil samples while mineral compositions was carried out on the seed samples only.

PROXIMATE ANALYSES FOR NIGELLA SATIVA SEEDS SAMPLES

Determination of moisture, crude fat, crude protein and total ash in seed samples was carried out using AOAC (1990) method. Total carbohydrates were determined by difference (100%-moisture-crude fat-crude protein-total ash).

MINERAL CONTENTS IN NIGELLA SATIVA SEED SAMPLES

Approximately 5 g of sample was ashed and followed by wet ashing method. The ashed samples were diluted to the volume and measured using Atomic Absorption Spectrophotometry (Perkin Elmer Analyst 400, US) as described by Osborne and Voost (1978). Commercial standards such as sodium (Na), potassium (K), Magnesium (Mg), Iron (Fe), Copper (Cu) and Zinc (Zn) were used.

PHYSICOCHEMICAL ANALYSES OF THE NIGELLA SATIVA OIL SAMPLES

The specific gravity and refractive index of the oils were determined using an Abbe refractometer according to Pearson (1991) method. The saponification value and percentage of unsaponifiable matter of the oils were determined according to AOCS (1997). The peroxide value was determined according to AOCS (1999) using titration techniques as described.

FATTY ACID PROFILE IN NIGELLA SATIVA SEEDS AND OIL SAMPLES

The crude oils and processed oils were methylated according to Folch (1957) method and thereby, the fatty acid methyl esters (FAMES) were analysed using a Shimadzu GC-2010 Gas Chromatography according to Atta (2003), using capillary column (30 m, 0.25 mm) (Supelco 2380) equipped with FID detector. Identification of FAMES was based on comparison of retention times of unknown peaks with authentic fatty acid methyl esters. Fatty acid composition was expressed as weight percent (%) of total fatty acid methyl ester.

SAMPLE EXTRACTION FOR ANTIOXIDANT ANALYSES

About 20 g of seeds were dried in the oven at 40°C for three days until a constant weight was reached. The seeds were then blended in an electric blender until powdery. The powdered seeds were extracted using 80 mL of methanol, using a sonicator for 30 min. The extract was then filtered with a filter paper and concentrated in a vacuum rotary evaporator at 40°C. The thick paste was then dried in the oven at 40°C for another three days before storing in the freezer for further analysis. For the oil samples, 1 g of black cumin seed oil was extracted with 3 mL of methanol (1 mL × 3 times) according to Parry et al. (2006). The three methanol extracts were combined and kept in the dark at ambient temperature until further analysis of total phenolic content and antioxidant capacity.

1,1 – DIPHENYL-2-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITY IN *NIGELLA SATIVA* SEED AND OIL SAMPLES

The DPPH test done was according to Sahgal et al. (2009). About 0.1 g of extract was dissolved in 1 mL of pure methanol to prepare the stock solution. Dilution was done from the stock solution of a concentration of 2 to 0.031 mg/mL. About 5 mL of 0.004% of DPPH solution was added to 50 µL of extracts in different concentration. The mixture was incubated in the dark at room temperature for 30 min. Absorbance was read at 517 nm using a spectrophotometer. Methanol and DPPH were used as control. IC₅₀ which is the concentration of antioxidant needed for 50% of DPPH radical scavenging was calculated by using a calibration curve on a linear range, plotting the extract concentration versus the absorbance. Calculation is as follows:

$$\text{Scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100\%$$

where A₀ is the absorbance of control reaction and A₁ is the absorbance of sample extract.

β-CAROTENE-LINOLEIC ACID ASSAY IN *NIGELLA SATIVA* SEED AND OIL SAMPLES

The antioxidant activity was analyzed according to Mariod (2009). About 2 mg of β-carotene was dissolved in 10 mL of chloroform. Next, 2 mL of this solution was pipetted into a 100 mL round bottom flask. Chloroform was then eliminated under vacuum using a rotary evaporator at 40°C. About 40 mg of pure linoleic acid, 400 mg of Tween 40 and 100 mL of distilled water were added into the flask and stirred vigorously. An aliquot of 4.8 mL from the emulsion was transferred into tubes containing 200 µL of the extract. The total volume of the system was adjusted to 5 mL with methanol. As soon as the emulsion was added to each tube, the absorbance was read at 470 nm using a spectrophotometer. Further readings were recorded in a 2 h span of a 20 min interval by keeping the samples in a water bath of 50°C. Blank samples, devoid of β-carotene were prepared for background subtraction.

TOTAL PHENOLIC CONTENT IN *NIGELLA SATIVA* SEED AND OIL SAMPLES

The total phenolic content was measured with a Folin-Ciocalteu reagent according to Yu et al. (2002). In brief, the reaction mixture contained 100 µL of extract, 500 µL Folin-Ciocalteu reagent, 1.5 mL 0.2 g/mL sodium carbonate and 1.5 mL pure water. Absorbance of the samples were read at 765 nm after 2 h of reaction at ambient temperature and was calculated as the total phenolic content using gallic acid as the standard. The results were expressed as gallic acid equivalents (GAE).

STATISTICAL ANALYSIS

The results were reported as mean and standard deviation. Analysis of variance (ANOVA) and Kruskal-Wallis

significant difference tests were conducted (SPSS for Windows, Version 17.0) to determine the difference among means. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

PROXIMATE AND MINERAL COMPOSITION OF THE *NIGELLA SATIVA* SEEDS

The average composition of five different *N. sativa* seed samples are shown in Table 1. The results of proximate analysis showed that the seeds contained a range of 20.63-28.71% crude fat, 11.35-14.04% crude protein, 5.37-7.93% total moisture content, 4.15-4.51% total ash and 48.69-57.18% total carbohydrate contents. The difference obtained was due to the variations in plantation areas, storage conditions and maturity of seeds (Atta 2003). It could be attributed to the different climatic changes, geographical conditions in plantation farms and also the variance in genetic make-up of the seeds themselves (Cheikh-Rouhou et al. 2007). Seed samples from Sanaa have a higher proximate composition compared to the other four samples in terms of crude oil, crude protein and total ash. Even so, a higher amount of crude oil does not signify seed quality. The lower proximate composition of the five seed samples in this study as compared to previous studies (Atta 2003; Cheikh-Rouhou et al. 2007; Sultan et al. 2009) is due to the dry weight versus wet weight basis of analysis. Analysis based on dry weight would produce a higher composition of nutrients. However, a wet weight analysis was undertaken in this study to provide a more accurate proximate analysis especially for raw samples.

In terms of mineral composition, the study showed that mineral content is abundant as can be seen in the range of its Ca (2158-2894 mg/kg), Fe (8.61-30.04 mg/kg), K (4842-7275 mg/kg), Na (98.4-178.7 mg/kg), Mg (2118-2452 mg/kg), Cu (9.45-13.43 mg/kg) and Zn (40.32-47.60 mg/kg). The dominant minerals in this study were potassium, calcium and magnesium. This was in accordance to the previous studies which also reported the most abundant mineral was potassium followed by calcium and magnesium (Atta 2003; Cheikh-Rouhou et al. 2007; Sultan et al. 2009; Takruri & Dameh 1998). Other minerals like iron, copper and zinc were relatively high.

PHYSICOCHEMICAL PROPERTIES OF THE *NIGELLA SATIVA* OILS

The physicochemical characteristics of the five batches of black cumin seed oils are shown in Table 2. The physicochemical analysis showed a refractive index of 1.4697-1.4730, specific gravity of 1.369-1.376 g/cm³, peroxide value of 3.33-21.33 meq O₂/kg, 184-220 mg/g in saponification number and unsaponifiable matter of 1.1-1.8% in the oil samples. The values of refractive index are normally associated with the purity of oils and thus, seed oil samples from Kelantan showed the highest purity of oil extracted from *N. sativa* seeds. This could be

TABLE 1. Proximate and mineral composition of black cumin seeds

Component	Sudan	Iran	Kelantan	Sanaa	Habsyah
Crude oil (%)	26.23±1.26	27.50±1.12	20.63±4.91	28.17±0.32	26.76±0.15
Crude protein (%)	12.81±0.50	12.05±1.36	12.66±0.60	14.04±0.59	11.35±0.65
Total moisture (%)	7.93±0.08 ^a	7.39±0.03 ^{bce}	5.37±0.41 ^{bcd}	5.70±0.18 ^{cde}	5.69±0.32 ^{bcd}
Total ash (%)	4.34±0.06	4.36±0.03	4.15±0.16	4.51±0.04	4.28±0.04
Total carbohydrate (%)	48.69±1.78	48.71±2.21	57.18±4.71	47.59±0.14	51.92±0.44
Calcium*	2249 ± 11.62 ^b	2158 ± 20.99 ^b	2894 ± 15.11 ^a	2296 ± 266.62 ^b	2512 ± 37.54 ^{ab}
Iron*	30.04 ± 6.02 ^a	8.61 ± 6.12 ^b	11.18 ± 2.98 ^b	16.57 ± 4.22 ^c	24.67 ± 1.73 ^{ac}
Potassium*	6886 ± 29.32 ^{bc}	6787 ± 12.57 ^{bc}	4842 ± 41.76 ^a	6179 ± 30.80 ^c	7275 ± 32.77 ^b
Sodium*	178.7 ± 0.99	132.6 ± 1.07	98.4 ± 1.08	109.7 ± 0.83	112.4 ± 0.64
Magnesium*	2118 ± 9.33	2145 ± 11.20	2180 ± 7.34	2452 ± 10.62	2276 ± 9.40
Copper*	12.67 ± 0.03 ^b	11.97 ± 0.13 ^b	12.08 ± 0.05 ^b	9.45 ± 0.02 ^a	13.43 ± 0.07 ^b
Zinc*	45.91 ± 0.22	40.32 ± 0.07	47.60 ± 0.36	42.15 ± 0.19	46.96 ± 0.10

All values given are means of three determinations.

* In mg/kg of dry matter.

^aDifferent alphabets in a row indicates significant difference ($p<0.05$)

TABLE 2. Physicochemical properties of black cumin seed oils

Parameter	Sudan	Kelantan	Sanaa	Habsyah	Shah Alam
RI	1.4717±0.0000 ^a	1.4706±0.0001 ^b	1.4727±0.0002 ^c	1.4730±0.0001 ^c	1.4697±0.0000 ^d
SG (g/cm ³)	1.372±0.000 ^a	1.369±0.000 ^b	1.375±0.000 ^c	1.376±0.000 ^c	1.367±0.000 ^d
PV (meq O ₂ /kg)	21.33±2.49 ^b	3.33±0.94 ^a	13.33±4.11 ^b	16.67±2.49 ^b	15.33±1.89 ^b
SN	186±6.71	207±6.31	220±5.16	184±16.57	201±10.33
USM (%)	1.2±0.1 ^b	1.2±0.1 ^b	1.5±0.2 ^{ab}	1.8±0.1 ^a	1.1±0.2 ^b

All values given are means of three determinations.

^aDifferent alphabets in a row indicates significant difference ($p<0.05$)

largely due to the extraction process using supercritical fluid extraction which give a higher purity as compared to other seed oil extraction (Patel et al. 2008). Refractive index and specific gravity gives a quantitative estimation of oxidative stability of fats and oils (Sultan et al. 2009). The peroxide value obtained in this study coincides with previous values in the literatures (4.35-18.1 meq O₂/kg) (Abdel-Aal & Attia 1993; Atta 2003; Cheikh-Rouhou et al. 2007; Sultan et al. 2009). According to Kirk and Sawyer (1991), new processed oils would have a peroxide value of less than 10 meq O₂/kg while rancidity starts when the oil has a peroxide value of 20 to 40 meq O₂/kg. With reference to this range, it is clear that oil sample from Kelantan was still brand new while the one from Sudan was probably processed much earlier. An increase in peroxide value is influenced by storage conditions, temperature and time as stated by Ramadan and Morsel (2004). The saponification value and percentage of unsaponifiable matter in this study was in the range of values described by previous studies (Atta 2003; Cheikh-Rouhou et al. 2007; Sultan et al. 2009). The saponification value in the oil sample from Sanaa showed a lower acidity as compared to other samples. Low acidity in oils indicates a longer shelf life and that the oil is still edible (Cheikh-Rouhou et al. 2007). Unsaponifiable matter usually correlates with natural antioxidants present in the oil (Malecka 2002). The differences obtained were influenced by the extraction processes. By using solvent extraction, larger amounts of unsaponifiable matter can be

extracted due to the ability of the solvent to extract most lipid components such as phospholipid, sterol, lipid-soluble vitamins, hydrocarbons and pigments (Bastic et al. 1978; Durkee 1971; Salunkhe et al. 1992).

FATTY ACID COMPOSITION IN THE *NIGELLA SATIVA* SEEDS AND OILS

The major saturated fatty acids found in both *N. sativa* seeds and oils were arachidic acid (C20:0), undecanoic acid (C11:0), lauric acid (C12:0) and behenic acid (C22:0). Whereas the major polyunsaturated fatty acids found in both the seeds and oils were linoleic acid (C18:2n6t), linoleic acid (C18:2n6c) and linolenic acid (C18:3n6). Major monounsaturated fatty acids in this study were palmitoleic acid (C16:1) and oleic acid (C18:1n9c). Previous studies showed that major saturated fatty acids were myristic, palmitic, stearic, behenic and arachidic while polyunsaturated fatty acids were linoleic acid. Monounsaturated fatty acids that were dominant in previous studies were similar to the ones reported in this study (Atta 2003; Cheikh-Rouhou et al. 2007; Lutterodt et al. 2010; Sultan et al. 2009).

For the seed samples, Iran showed the lowest amount (22.46±15.41%) of saturated fatty acid, the highest polyunsaturated fatty acid (16.48±7.13%) and monounsaturated fatty acid (20.45±2.61%) compared with other seed samples. Oil samples from Kelantan showed the lowest amount of saturated fatty acid (1.42±0.29%)

while oil from Sudan showed the highest polyunsaturated fatty acid (65.13±5.45%) and Shah Alam oil's contained the highest monounsaturated fatty acid (14.05±1.46%). Comparing the fatty acids in *N. sativa* seeds and oils, oils that were already extracted showed better fatty acid composition than seeds. This may be due to the other nutrient composition found in seeds such as protein, ash, moisture and carbohydrate that make up most of the seed composition (Table 3).

Saturated fatty acids and polyunsaturated fatty acids found in this study were analyzed and showed a significant difference ($p<0.05$) in their association with one another. Both variables displayed a strong inversal relationship with a Pearson correlation coefficient of -0.83. Arici et al. (2007) reported that radiation processes done on *N. sativa* seeds increased the saturated fatty acids but decreased the unsaturated fatty acids. The depletion of unsaturated fatty acids was caused by the radiation that changes the molecular structure of the fatty acid by breaking the double bonds and thereby, creating more trans fatty acids.

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN *NIGELLA SATIVA* SEEDS AND OILS

The DPPH radical-scavenging assay was done on ten *N. sativa* seed and oil samples and the results are shown in Table 4. This assay was done to evaluate the antioxidant activity in black cumin seeds and oils. As the DPPH radicals were quenched, the faded purple colour of the solution indicates the ability of the sample's antioxidants to scavenge the radicals formed. The IC₅₀ showed that seed sample from Iran had the highest antioxidant activity with a value of 1.49 mg/mL while the IC₅₀ in oil sample showed Sudan with the highest scavenging activity with the IC₅₀ of 4.48 mg/mL. The previous studies done on the antioxidant activity of *N. sativa* seeds were in the range of IC₅₀ 2.26-28.8 mg/mL (Burits & Bucar 2000; Mariod et al. 2009). The IC₅₀ obtained in this study was lower than that of previous studies and thereby, indicating a higher antioxidant activity in the seed samples. The variance in the values could be due to different geographical regions and the extraction process of the antioxidants

TABLE 3. Fatty acid composition in *Nigella sativa* seeds and oils

Samples	SFA (%)	PUFA (%)	MUFA (%)
Seed samples			
Sudan	25.11 ± 4.83	16.32 ± 2.23	15.88 ± 8.81 ^a
Iran	22.46 ± 15.41	16.48 ± 7.13	20.45 ± 2.61 ^{ab}
Kelantan	26.59 ± 8.62	12.91 ± 7.75	11.84 ± 4.14 ^a
Sanaa	37.81 ± 3.98	9.89 ± 2.22	9.56 ± 7.33 ^a
Habsyah	42.43 ± 1.24	8.70 ± 0.70	2.55 ± 0.59 ^{ac}
Oil samples			
Sudan	1.62 ± 0.66 ^a	65.13 ± 5.45 ^a	6.37 ± 0.60 ^a
Kelantan	1.42 ± 0.29 ^{ac}	56.45 ± 3.49 ^{ac}	7.39 ± 0.50 ^{ab}
Sanaa	13.12 ± 20.35 ^a	38.96 ± 26.67 ^{ab}	9.73 ± 0.88 ^{ab}
Habsyah	38.30 ± 26.66 ^a	23.06 ± 22.66 ^{ab}	9.61 ± 7.83 ^{ab}
Shah Alam	57.85 ± 8.49 ^b	15.20 ± 1.91 ^b	14.05 ± 1.46 ^b

All values given are means of three determinations.

^a Different alphabets in a column indicates significant difference ($p<0.05$)

TABLE 4. Antioxidant activity and total phenolic content in *Nigella sativa* seeds and oils

Samples	IC ₅₀ (mg/ml)	Inhibition Activity (%)	Total Phenolic Content (mg GAE/g)
Seed Samples			
Sudan	7.50	85.12 ± 3.25 ^{bc}	24.27
Iran	1.49	63.99 ± 0.04 ^a	30.84
Kelantan	1.79	96.95 ± 1.29 ^b	27.32
Sanaa	2.94	90.89 ± 0.88 ^c	16.19
Habsyah	1.86	87.21 ± 0.61 ^c	22.83
Oil Samples			
Sudan	4.48	85.01 ± 0.04 ^a	1.87
Shah Alam	8.17	97.71 ± 0.50 ^{bc}	6.48
Kelantan	7.19	97.86 ± 0.24 ^c	7.60
Sanaa	12.79	88.94 ± 0.49 ^d	0.96
Habsyah	8.52	94.85 ± 0.87 ^{ce}	1.79

All values given are means of three determinations.

^a Different alphabets in a column indicates significant difference ($p<0.05$)

from the seeds. The results from Mariod et al. (2009) showed similarities with the IC_{50} values in this study. Isolation of individual antioxidants component would decrease the effectiveness of scavenging activity but the synergism of various antioxidants would produce an opposite effect. This is shown by Burits and Bucar (2000) who showed a huge difference in IC_{50} values in this study due to the isolation of antioxidant components into thymoquinone, carvacrol, t-anethole and 4-terpineol. Singh et al. (2005) suggested that the antioxidant activity found in *N. sativa* is comparable to the commercial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PA). The seeds in this study had an overall better antioxidant activity than the oil. This is probably due to the lower scavenging activity exhibited by the oil as a result of processing, refining and purification of the oil from crude oil. Processes such as these exposed the oil to the environmental conditions, high temperature and pressure which will indirectly destroy the natural antioxidants found in the extracted oil whilst increasing oil oxidation (Lutterodt et al. 2010). The IC_{50} values of the DPPH assay showed a significant association ($p < 0.05$) with polyunsaturated fatty acid of the samples. This delineates the roles of antioxidants as a protector of polyunsaturated fatty acid from oxidation and destruction.

In the β -carotene-linoleic acid assay, the highest inhibition activity of the oxidation of β -carotene was found in both the seed and oil samples from Kelantan with an inhibition activity of $96.95 \pm 1.29\%$ and $97.86 \pm 0.24\%$, respectively. Both the DPPH radical-scavenging assay and β -carotene-linoleic acid assay were done to assess the antioxidant activity of the *N. sativa* seeds and oils. However, both these assays were investigated on different aspects of the antioxidants. The DPPH assay concentrated on the scavenging aspect of the antioxidants while the β -carotene-linoleic acid assay focused on the inhibition of oxidation by free radicals. One is the hunter while the other offers protection. Therefore, the *N. sativa*

antioxidants complemented one another in providing a full-proof fight against free radicals. Even so, the β -carotene-linoleic acid assay is more suited to determine lipophilic antioxidants. This would explain the higher inhibition activity found in the oil samples as compared with the seed samples. The oil samples only have lipophilic compounds but the seed samples have both the lipophilic and the hydrophilic compounds. Hence, the hydrophilic antioxidants in the seed samples were not measured and in this manner causing the inhibition activity in seeds to be lower.

The total phenolic content in the *N. sativa* seeds and oils were analyzed and the highest phenolic content was found in the seed samples from Iran with a value of 30.84 mg GAE/g and oil samples from Kelantan with a value of 7.60 mg GAE/g. Phenolic content is important because most antioxidants are phenolics themselves. Razali et al. (2008) reported that most phenolics showed high levels of antioxidant activity. The linear calibration curve for the seed samples showed the R^2 of 0.985 (Figure 1) while the R^2 for the oil samples were 0.957 (Figure 2). The previous studies reported the total phenolic content in *N. sativa* seeds to be in a range of 12.1-78.8 mg GAE/g (Erkan et al. 2008; Mariod et al. 2009) while the total phenolic content in *N. sativa* oils were in a range of 1.02-1.40 mg GAE/g (Lutterodt et al. 2010). Both phenolic content of seeds and oils in this study were in the range of those reported. Lutterodt et al. (2010) reported that the total phenolic content in *N. sativa* contribute to the stability of the oil under accelerated oxidation conditions.

Local oil samples such as those from Shah Alam and Kelantan showed better antioxidant activity than their foreign counterparts as the antioxidants in the oils were affected by the extraction process. In Malaysia, *N. sativa* oil was extracted from the seeds using supercritical fluid extraction method while those in foreign countries were usually extracted using cold press method. Alhaj et al. (2008) reported that the supercritical fluid extraction method was able to produce a higher concentration

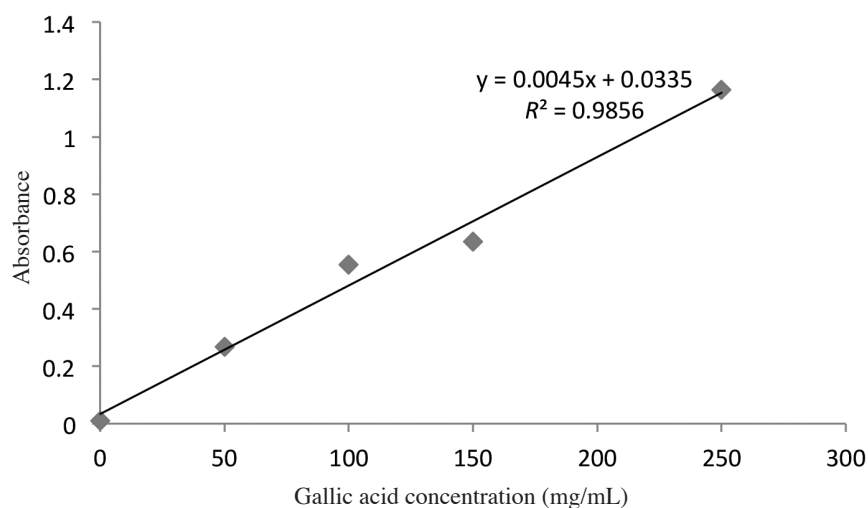


FIGURE 1. Linear calibration curve of gallic acid for *Nigella sativa* seeds

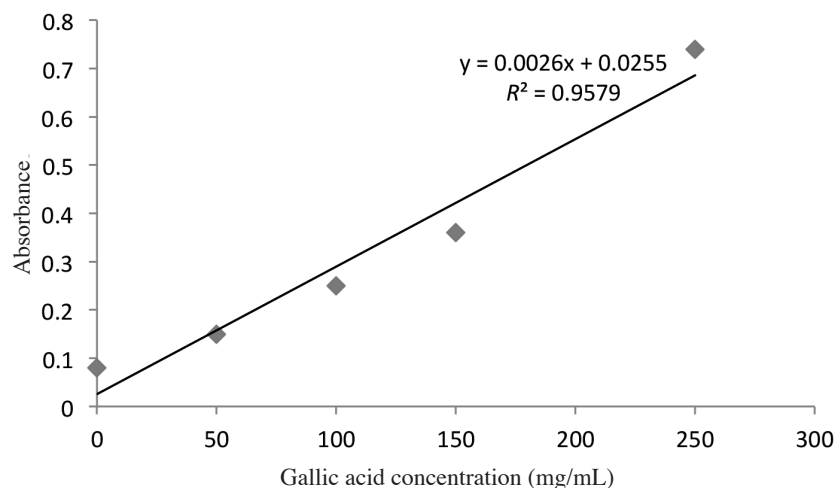


FIGURE 2. Linear calibration curve of gallic acid for *Nigella sativa* oils

of the main antioxidant found in *N. sativa* which is thymoquinone. Lutterodt et al. (2010) reported that black cumin seed oils that were cold pressed showed good oxidative stability in the oils but a low phenolic content was obtained. This implies that oxidative stability does not need high concentrations of phenolic content.

CONCLUSION

Both *N. sativa* seeds and oils are abundant in nutrient and antioxidants. The oil sample from Kelantan contained the lowest amount of saturated fatty acids ($1.42 \pm 0.29\%$) while oil sample from Sudan contained the highest content of polyunsaturated fatty acid ($65.13 \pm 5.45\%$). Monounsaturated fatty acids ($20.45 \pm 2.61\%$) were found the highest in the seed samples of Iranian origins. Antioxidant activities showed Iran has the most antioxidants in seed samples while the oil sample from Kelantan is the best in antioxidants compared with other oil samples. Seed samples generally have better quality of antioxidants compared to oil samples while oil samples made locally were better than those bought in Yemen due to a higher technology of extraction using supercritical carbon dioxide. It is recommended that future animal studies be done on local Malaysian *N. sativa* seeds and oils as the analysis done in this study have showed potential for clinical treatment.

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