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Fatty Acid Profile, α-Tocopherol Content and Total Antioxidant Activity of Oil Extracted from *Nigella sativa* Seeds

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Abstract: *Nigella sativa* (*N. sativa*) is popularly known as the black seed, a herb that has traditionally been used for centuries in many parts of the world. It has gained popularity due to its potential health benefits. However, more scientific data is needed to support the various health claims. This study was carried out to determine the fatty acid profile, α-tocopherol content and to evaluate the antioxidant activity of seed oil samples from three different regions in Yemen namely Marib, Sadah and Taiz. *N. sativa* seeds oil was extracted using three different solvents (n-hexane, petroleum ether and chloroform: methanol 2:1 v/v) and the fatty acids composition was analyzed using gas chromatography, while the α-tocopherol was determined using HPLC. Ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods were used to evaluate the antioxidant activity of the seeds oil. Results indicated that *N. sativa* seeds contain high amount of oil (30-48%) and the major unsaturated fatty acids were linoleic acid (57.96, 58.04 and 57.04%) followed by oleic acid (21.49, 20.87 and 20.60%), while the main saturated fatty acids were palmitic (11.56, 11.23 and 11.22%), followed by stearic and myristic acids in Marib, Taiz and Sadah samples respectively. Oil extracts exhibited strong antioxidant properties when compared to α-tocopherol with 78-82% inhibition in the FTC method and 70-80% in the TBA assays. The oil extracts were found to be rich in α-tocopherol content 290±1.5, 170±0.40 and 120±0.15 mg/100 g, in Marib, Sadah and Taiz samples, respectively. Present results suggest that *N. sativa* seeds contain high amount of antioxidants that are essential for health and preventing numerous diseases.

Key words: *Nigella sativa*, fatty acids profile, total antioxidant activity, α-tocopherol

INTRODUCTION

Plant seeds are important source of oil of nutritional, industrial and pharmaceutical importance. No oil from any single source has been found to be suitable for all purposes because oil from different sources generally differ in their fatty acid composition. Plant seeds oil is also important source of lipid soluble antioxidants as phenols, where they have many health benefits. This necessitates the search for new sources of novel oil (Ramadan and Morsel, 2002a).

Seeds of *Nigella sativa* L., an annual spice, also known as black cumin or black caraway, are a member of the Ranonculaceae family and native to some parts of the Mediterranean region (El-Dakhakhani *et al*., 2000). The seeds have traditionally been used in middle Eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years. *N. sativa* L. is cultivated in Yemen mainly in the areas of Marib, Sadah and Taiz, where Marib is in the East, Sadah is in the West and Taiz is in the South of Yemen. The seeds are used for edible and medicinal purposes and are sold in the markets to be used as a spice, condiment, additive on bread, for cheese and as a native medicine.

Recently, researchers have taken interest in the *N. sativa* seeds in different forms: the seed extract, its oil and its volatile substances. Several studies on black cumin seeds (Nagi and Mansour, 2000) shoots and roots (Bourgou *et al*., 2008) have been reported. The seeds have been subjected to have a range of pharmacological,

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phytochemical and nutritional investigations. Human studies and laboratory studies on the seeds and oil have provided scientific support for the traditional uses of the seed and its oil for the treatment of rheumatism, immune stimulation (Haq *et al*., 1999), diabetic, cancer and related inflammatory disease (Ramadan and Morsel, 2002b) as well as parasitic diseases (EL Shenawy *et al*., 2008). It has been shown that *N. sativa* seeds contain 32 to 53% (w/w) of fixed oil with 85% of total unsaturated fatty acid (Houghton *et al*., 1995). Fatty acid composition of *N. sativa* oil has been analyzed using gas chromatography and was expressed as weight percent of total fatty acid methyl ester. It has been found that the major fatty acids in the seed oil were myristic (C14:0), palmitic (C16:0) and stearic (C18:0) as saturated fatty acids, whereas oleic (C18: 1), linoleic (C18: 2) and linolenic (C18: 3) were the main unsaturated fatty acids. High-Performance Liquid Chromatograph (HPLC) was used to determine α, β and γ tocopherols content in *N. sativa* seed oil (Nergiz and Otles, 1993).

In vitro studies on *N. sativa* were showed that seeds extract protects erythrocytes against lipid peroxidation, protein degradation, loss of deformability and increased osmotic fragility caused by H_2O_2. The crude *N. sativa* oil and its fractions (neutral lipids, glycolipids and phospholipids) showed potent *in vitro* radical scavenging activity that is correlated well with their total content of polyunsaturated fatty acids, unsaponifiables and phospholipids, as well as the initial peroxide values of crude oil (Suboh *et al*., 2004). The antioxidant activity of 80% methanolic extracts of *N. sativa* from Yemen was studied by Alzoreky and Nakahara (2001) and expressed in Trolox Equivalent Antioxidant Capacity (TEAC), their results showed that 80% methanolic extracts of *N. sativa* had 1.1 mM TEAC per g dry weight and total phenolics of 0.1 mg g^{-1}.

However, there have been limited studies on *N. sativa* of which describe the oil content of the seeds grown in Yemen and α-tocopherol content. Hence, the principal objective of this study was to determine the fatty acids profile, α-tocopherol content and the antioxidative activity of *N. sativa* seeds oil grown in Yemen.

MATERIALS AND METHODS

Samples: *N. sativa* seeds were collected from three different regions in Yemen, namely Marib, Sadah and Taiz. Three batches from each region were purchased, mixed, cleaned, divided into plastic bags and covered with aluminum foil. The packed seeds were kept at 4°C in tissue culture laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia until further use. The seeds analysis has been carried out between 2005-2007 at the Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, Malaysia.

Chemicals: Standard fatty acid methyl esters were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Missouri, USA), Petroleum ether, n-hexane, sodium hydroxide and Boric acid were purchased from Fisher (Fisher Scientific Co Ltd., Ottawa, Canada). Isopropanol, chloroform and methanol were purchased from BDH (BH15 ITD2, England). Thiobarbituric acid was purchased from GmbH (Daramstadt, Germany). Linoleic acid, butylated hydroxytoluene (BHT) and trichloroacitic acid, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, ferrous chloride and α tocopherol were purchased Sigma-Aldrich (St. Louis, MO). Ammonium thiocyanate was purchased from AJAX chemical (Australia).

Oil extraction, GC analysis of fatty acids and total antioxidant activity of *N. sativa* oil

Oil extraction: *N. sativa* seeds were finely ground using an electrical grinder (Waring Blender, Tokyo, Japan) and was passed through a 35 mm (42 mesh) sieve. Homogenized and ground samples (100 g) were soaked overnight in three different solvents 1:5 (w/v): n-hexane, petroleum ether and chloroform-methanol (2:1 v/v) at room temperature. The mixture of samples and solvent was covered with aluminum foil, kept overnight at room temperature. The mixture was filtered through a filter paper (Whattman No. 2). The extraction procedure was repeated twice and the solvent was removed using a rotary evaporator (Rikakika Co. Tokyo, Japan) at 50°C and 90 rpm. The extracted oil was transferred into glass sealed amber dark bottles and then stored in a freezer (-20°C) for subsequent analysis.

GC analysis of fatty acids: Fatty acids composition of the oil was determined using gas chromatography according to the International Union of Pure and Applied Chemistry (IUPAC, 1979) method. Fatty acid methyl ester was prepared as the following: the oil was mixed with 0.95 mL hexane in 2 mL screw capped vial and was vortexed. 0.05 mL of 1 M-sodium methoxide (prepared by dissolving 1.15 g sodium in 50 mL MeOH) was added into the mixture and shaken again for 5 sec. The clear upper layer of the methyl ester was injected into a Hewlett- Packard GC apparatus (Model 439 USA), equipped with a hydrogen flame ionization detector. The carrier gas used was helium, at a flow rate of 3 mL min^{-1} and a glass column (DB 23 cis/trans (50% cyanopropyl)-methylpolisiloxane) was used as the stationary phase. The column was set at 90°C, injection and detection temperatures were 300°C and column temperature reached 400°C.

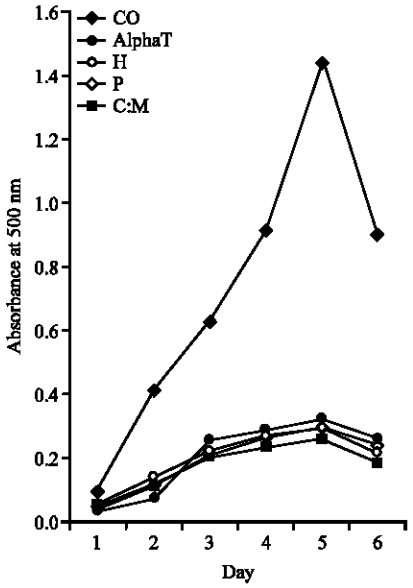

Fig. 1: Estimation of the amount of peroxides at the primary stage of linoleic acid peroxidation as measured by the FTC method. Each value represents the mean of three replicates±SD. CO: Control which was prepared as samples, alpha T: α-tocopherol, H: Oil extracted using n-hexane, C: M: oil extracted using chloroform: methanol (2:1) and P: Oil extracted using petroleum ether of samples from Marib using FTC method. The values were compared with absorbance values of control

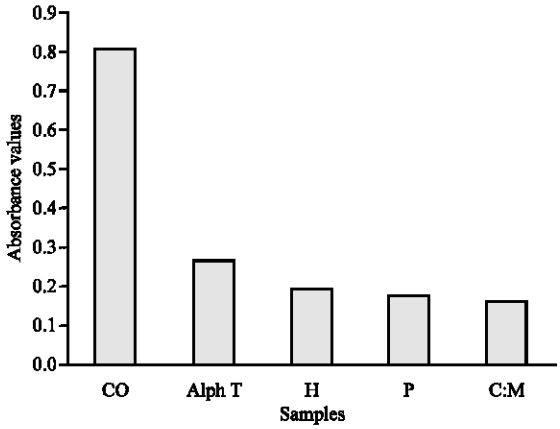

Fig. 2: Estimation of the amount of peroxides at the secondary stage of linoleic acid peroxidation as measured by the TBA method. Each value represents the mean of three replicates±SD. CO = control which was prepared as samples but without adding oil, alpha T = α-tocopherol, H = oil extracted using n-hexane, C: M = oil extracted using chloroform: methanol (2:1) and P = oil extracted using petroleum ether of samples from Sadah using TBA method. The values were compared with absorbance values of control

Total antioxidant activity of *N. sativa* oil

Ferric thiocyanate method: The method of Osawa and Namiki (1981) was used to study the antioxidant activity of *N. sativa* oil using the FTC method. The capacity of seeds oil or α-tocopherol to inhibit the lipid peroxides produced from peroxidation of linoleic acid is measured in comparison with a negative control. The absorbance was measured at 500 nm using spectrophotometer (Shimazu, Co., Ltd., Koyto, Japan) every 24 h until one day after the maximum absorbance of the control was reached.

Thiobarbituric acid method: Method of Ottolenghi (1959) was used to study the antioxidant activity of *N. sativa* oil using the TBA method. The amount of the lipid peroxides formed from the peroxidation of linoleic acid were measured in the presence of seeds oil and α-tocopherol and compared with the negative control. Absorbance of the supernatant was measured one day after absorbance of the control from FTC has reached the maximum at 532 nm. The lower absorbance value indicated higher antioxidant activity. Since, peroxide production was inhibited by the antioxidant (Fig. 1, 2).

Determination of α-tocopherol in *N. sativa* seed oil using HPLC: Alpha tocopherol content of *N. sativa* seed oil extracted using n-hexane and was determined according to the method of Katsanidis and Addis (1999). One gram of oil was mixed with 0.25 g ascorbic acid and 7.3 mL saponification solution (55% ethanol in distilled water with 11% potassium hydroxide). Then the mixture were incubated in a shaking water bath for 15 min at 80°C, cooled using tap water for 1 min and 4 mL hexane and 2 mL distilled water were then added into the tubes. The tubes were vortexed and the upper layer was injected into a silica column (Zorbax RX-SIL, 5 μm partial size, 4.6 mm Idx 25 cm). The mobile phase used in this experiment was hexane-isopropanol (99:1) with a flow rate of 1.3 mL min^{-1}. The wavelength was programmed at 295 nm and α-tocopherol was eluted within 12 min. Identification and quantification of α-tocopherol was accomplished by comparing the retention times of peaks with those of pure standards of α-tocopherol and analyzed under the same conditions.

Statistical analysis: Data were analyzed using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 11.0 for Windows. One-way Analysis of Variance, ANOVA was used to compare the means of

oil yield, α-tocopherol content of the oil and total antioxidant activity of the oil at level $p<0.05$ level of significant.

RESULTS

Crude oil content in *N. sativa* seeds: The oil yield extracted using chloroform: methanol was significantly higher (28%) compared to the yield extracted using hexane and while only (8%) higher than that of the petroleum ether extract (Table 1). Samples collected from Sadah (48.6±0.45) showed significantly higher (17%) oil yield compared to samples collected from Marib (41.2±0.31) and (11.6%) higher than samples collected from Taiz (43.3±0.50).

Fatty acid composition in *N. sativa* seed oil: Unsaturated fatty acids were the highest fatty acids content representing up to 80.9, 80.6 and 79.5% in Marib, Taiz and Sadah samples respectively. However, the major unsaturated fatty acids were oleic acid (21.5, 20.9 and 20.6%) and linolenic acid (58, 58 and 57%) in Marib, Taiz and Sadah samples, respectively. On the other hand, the major saturated fatty acids of *N. sativa* oil were palmitic acid (11.56, 11.23 and 11.22%) and stearic acid (2.75, 2.60 and 2.55%) in Marib, Taiz and Sadah samples, respectively. In addition arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) were detected as a saturated fatty acids as well as palmitoleic (C16: 1) and erucic (C22: 1n9) acids were detected as monounsaturated fatty acids in the three different samples (Table 2). The ratio of saturated to unsaturated fatty acids (S/U%) was found to be comparable in the three samples (19, 20 and 21% in Marib, Taiz and Sadah samples, respectively).

Antioxidants activity in *N. sativa* seed oil: The FTC method was used to measure the amount of peroxides at the primary stage of linoleic acid peroxidation (Fig. 1). Since, the concentration of the peroxide decreases as the antioxidant activity increases, the intensity of the reddish pigment will be reduced, leading to lower absorbance value. Absorbance values of the control as well as α-tocopherol and *N. sativa* seeds oil were increased until day 5 and then decreased on day 6. The control showed increase in the absorbance values at day 5 over that of day 3, reached maximum level on day 5 and finally dropped on day 6 due to the MDA formation from linoleic acid oxidation, where the peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment (Osawa and Namiiki, 1981). In comparing the total antioxidant activity of oil extracted using the three different solvents with α- tocopherol, the results were showed that total antioxidant activity content in the three samples were significantly higher ($p<0.05$) than α- tocopherol.

Table 1: Oil yield from three different samples of *N. sativa* seeds

	Oil yield (%)		
Samples	CCl4:MeOH	Hexane	Petroleum ether
Marib	41.2±0.31[a]	32.0±0.36[a]	38.1±0.41[a]
Sadah	48.6±0.45[b]	33.2±0.44[a]	38.8±0.35[a]
Taiz	43.3±0.50[a]	30.7±0.46[b]	36.9±0.57[b]

Each value represents the mean of three replications±SD. Within a column, values with the different superscript letter(s) are significantly different from each other at $p<0.05$ level of significant

Table 2: Fatty acid composition of three different samples of *N. sativa* oil

		FA profile in *N. sativa* oil (%)		
Fatty acid profile		Marib	Taiz	Sadah
C14	Myristic	0.18	0.17	0.17
C14: 1	Myristoleic	0.10	0.18	0.20
C15	Pentadecenonic	0.03	0.04	0.05
C15: 1	Cis-10-Pentadecenonic	-	0.01	-
C16	Palmitic	11.56	11.23	11.22
C17	Heptadecanoic	0.14	0.47	0.72
C18	Stearic	2.75	2.60	2.55
C18: 1n9c	Oleic	21.49	20.87	20.60
C18: 3n3A	α-linolenic	0.30	0.28	0.26
C20	Arachidic	0.16	0.15	0.15
C20: 1n9	Cis-11-Eicosenoic	0.34	0.36	0.36
C20: 2	Cis-8,11,14-Eicosadienoic	0.26	0.40	0.57
C203n3	Cis-11,14,17-Eicosatrienoic	-	-	-
C20: 4n6	Arachidonic	-	-	-
C22: 6n2	Cis-4,7,10,13,16 19-Docosahexaenoic-	-	-	
C23	Tricosanoic	0.51	0.23	0.29
TSFA	Total Saturated FA	15.54	16.06	17.02
TUFA	Total Unsatyrated FA	80.88	80.55	17.02
PUFA	Polyunsaturated FA	77.84	77.11	76.04
MUFA	Monounsaturated FA	3.41	3.44	3.42
S:U ratio	TSFA:TUFA	0.19	0.20	0.21

Each value represents the mean of two replications. TSFA: Total saturated fatty acids, TUSF: Total unsaturated fatty acids, PUFA: Polyunsaturated fatty acids, MUFA: Monounsaturated fatty acids, TUFA: TSFA: The ratio of total unsaturated fatty acids and total saturated fatty acids

The TBA method was used to measure the secondary stage of linoleic acid peroxidation. The absorbance values of oil extracted from the three samples using three different solvents were lower than the control and α-tocopherol, on day 6 of the FTC method due to the formation of MDA. Malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. Results obtained from the TBA method showed that total antioxidant activity of oil samples from the three samples using the three different solvents were significantly ($p<0.05$) higher compared to α-tocopherol. There were no significant differences ($p>0.05$) observed in total antioxidant activity of oil extracted using the three different solvents from the three different samples as shown in Table 3.

α-Tocopherol content in *N. sativa* seed oil: The contents of α-tocopherol were observed to be 290±1.5, 170±0.40

Table 3: Percentage of antioxidant activity of three different samples of *N. sativa* seed oil extracted using three different solvents (FTC and TBA method)

	Antioxidant activity of *N. sativa* seed oil (%) (α-tocopherol = 77.1±0.32)					
	Chloroform-Methanol		n-hexane		Petroleum ether	
Samples	FTC	TBA	FTC	TBA	FTC	TBA
Marib	82.8±0.75^{a}	80.9±0.50^{a}	81.6±1.5^{a}	77.9±1.0^{a}	81.9±0.30^{a}	79.2±0.42^{a}
Sadah	81.9±0.51^{b}	79.2±0.26^{a}	79.1±0.25^{b}	75.2±0.25^{b}	80.0±0.25^{b}	77.5±0.45^{b}
Taiz	79.9±0.36^{c}	74.4±0.45^{b}	78.5±0.78^{c}	70.7±0.4^{c}	79.1±0.78^{b}	72.7±0. 60^{a}

Oil was extracted using chloroform: methanol (2:1), n-hexane and petroleum ether at room temperature. Each value represents the mean of three replications±SD. Within a column, values with the same superscript letter(s) are not significantly different from each other

Table 4: α-Tocopherol content of three different samples of *N. sativa* seed oil

Samples	α-Tocopherol (mg/100 g)
Marib	290±1.50^{a}
Sadah	170±0.40^{b}
Taiz	120±0.15^{c}

Each value represents the mean of three replications±SD. Values with the different superscript letters are significantly different from each other ($p<0.05$)

and 120±0.15 mg/100 g, in Marib, Sadah and Taiz samples, respectively (Table 4). There were significant differences ($p<0.05$) in α-tocopherol content between the three different samples. Samples from Marib were showed to contain the highest amount of vitamin E (α-tocopherol) followed by samples from Sadah and samples from Taiz.

DISCUSSION

N. sativa seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world (El-Dakhakhani *et al*., 2000; Al-Gamadi, 2001). The seeds are considered a natural food additive and a condiment. It is typically consumed mixed with honey and in baking products or pastries. As reported by Ramadan and Morsel (2002a), present results showed that the total lipids extracted were higher in the chloroform: methanol miscelles (39.2% of seed fresh weight) than in the n-hexane extract (37.9%). The significant difference in percentage of oil extracted using chloroform: methanol was due to the properties of chloroform: methanol which can extract both polar and nonpolar lipids. Samples from Sadah have been observed to contain the highest percentage of oil compared to samples from Marib and Taiz. This could be attributed to the growth conditions in different locations, where Sadah is located in East of Yemen and characterized by different climates than Taiz and Marib, this was expected since it was reported that the composition and nutritional values vary with the country of origin, stage of maturity, type of seeds and subsequent storage conditions. It was reported that *N. sativa* seeds from Egypt showed to have 34.49 to 38.72% of oil (Atta, 2003), from Saudi showed to have 38.2% (Al-Jassir, 1992) and from Turkey showed to have 40.6%. Our results of the oil content from this study are in well agreement with those reported earlier.

Oils of black cumin contain different proportions of fatty acids according to the variety. Varieties from Egypt contain oleic and linoleic acids at relatively high levels (18.9-20.1 and 47.5-49.0%, respectively) (Atta, 2003), however these were lower than those corresponding to the Tunisian variety (25.0 and 50.3, respectively) and to the Iranian variety (23.7 and 49.2), respectively. The variability in fatty acids composition may be attributed to genetic (plant cultivar, variety grown), seed quality (maturity, harvesting-caused damage and handling/storage conditions), oil processing variables, or accuracy of detection as well as lipid extraction method and quantitative techniques (Ramadan and Morsel, 2002a). In this study, we observed that oleic and linoleic acids contents were higher than the contents from the varieties of Egypt, Tunisia or Iran. The ratio of linoleic acid (57-58%) to oleic acid (20.6-21.5%) was more than 2.5:1 in the three samples. These results were in agreement with those reported in soybean oil ($C_{18:2}$ = 52%, $C_{18:1}$ = 25%) and in corn oil ($C_{18:2}$ = 58.7%, $C_{18:1}$ = 26.6%) (Ramadan and Morsel, 2002a). The ratio of saturated to unsaturated fatty acids (S/U %) was 19.21, 19.86, 21.42 in Marib, Taiz and Sadah samples, respectively. These ratios were lower than that reported by Ramadan *et al*. (2003) for black cumin seed oil (25.7%).

Many investigations have been carried out to isolate the possible active components of black seeds oil (Ghosheh *et al*., 1999). The essential oil of black cumin seeds, *N. sativa* L., was tested for a possible antioxidant activity by Burits and Bucar (2000), they found that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging property. These four constituents and the essential oil possessed variable antioxidant activity when tested in the diphenylpicrylhydracyl assay for non-specific hydrogen atom or electron donating activity. In addition they were demonstrated to be effective hydroxyl radical scavenging agents in the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay.

Total antioxidant activity, of seeds oil extracted using the three different solvents, was estimated using FTC and TBA methods and compared with α-tocopherol. The

results of the three solvents extracts were observed to be higher in samples from Marib, Sadah and Taiz than α-tocopherol. Whereas, there was no significant difference observed between the three different solvents extracts in total antioxidant activity of oil extracted from the three different samples. Oil extracted from Marib's samples was shown to have the highest content of total antioxidant activity compared to samples from Sadah and Taiz. Overall, *N. sativa* oil extracted from the three samples using three different solvents inhibited the linoleic acid oxidation as compared to the control and α-tocopherol. These results were in the same line with the previous findings by Houghton *et al.* (1995), who reported that the fixed oil of *N. sativa* had antioxidant effects greater than thymoquinone, the active constituent of *N. sativa*. Earlier performed clinical and experimental investigations have shown that *N. sativa* has a protective effect against oxidative damage in isolated rat hepatocytes (Daba and Abdel Rahaman, 1998; Kanter *et al.*, 2003). In addition, it was suggested that *N. sativa* treatment increases the antioxidants defense activity in experimentally induced diabetic rabbits (Meral *et al.*, 2001).

Few data has reported α-tocopherol content of *N. sativa* seed oil in the literature except that mentioned by Ramadan *et al.* (2003), who estimated α-tocopherol content to be 0.284 g kg^{-1}, while previously reported by Nergiz and Otles (1993) to be 40 μg/100 g. Present results were found to be different from those results, however the contents of α-tocopherol were observed to be 290±1.5, 170±0.40 and 120±0.15 mg/100 g, in Marib, Sadah and Taiz samples, respectively. The variability in the results of α-tocopherol obtained may be due to the different conditions and methods used for estimation.

CONCLUSION

This study revealed that *N. sativa* grown in Yemen is a rich source of many important nutrients that appear to have protective effects for human health. They constitute a good alternative source of essential fatty acids compared with common vegetable oil. present results showed that *N. sativa* seed oils contain relatively high percentage of linoleic acid and the seeds were exhibited higher total antioxidant activity than α-tocopherol. These findings may prove the traditional use of *N. sativa* seeds in preventive and alternative medicine against many diseases.

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