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MINI REVIEW

Nigella sativa oil: physico-chemical properties, authentication analysis and its antioxidant activity

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Abstract

Nigella sativa oil (NSO) is one of the high value oils in fats and oils industry due to its nutritional applications and its beneficial effects on human health. Several biological activities have been reported, especially antioxidant activities due to its active components, especially phenolics compounds. Some methods have been used for extraction of NSO from seeds to obtain high yield with excellent quality which includes solvent extraction, cold press, Soxhlet, and microwave assisted extraction. NSO commands a high price in the market, as a consequence, NSO is a target to be adulterated with cheaper oils such as corn and soybean oils. Indeed, the authentication analysis of NSO by determining several physico-chemical properties to determine the characteristics of NSO must be performed. This review highlighted some physico-chemical properties of NSO along with authentication of NSO from adulterants. The antioxidant activities of NSO were also highlighted in this review. Based on its activity as antioxidant, NSO is a good source to be used in nutraceutical and pharmaceutical products.

1. Introduction

For many centuries, herbal Medicinal plants have evolved as an interesting issue, to be used for preventing and curing several diseases, especially degenerative diseases such as diabetic and hypertension in different systems of folk medicines as well as indigenous medicine. Herbal medicines are interesting for researchers focusing on types of medicinal plants having medicinal properties, mechanism of action, potential, safety evaluation as well as toxicological studies. Among various medicinal plants, *Nigella sativa* has emerged as a miracle herb on the basis of history and religion (Ahmad *et al*., 2013). *Nigella sativa* (*N. sativa*) belong to Family Ranunculaceae is also known as *Habbatus sauda, Habbet el Baraka', Al-Kammoon Al-Aswad', Shunez'* and black cumin (Nurrulhidayah *et al*., 2011).

This plant is widely used in herbal medicines throughout the world, especially in Islamic countries such as the Middle East, Malaysia, and Indonesia. In the literacy of the Islamic community, *N. sativa* is taken into account as one of the most common plants used for healing or preventing of some diseases. Tibb-e-Nabwi or Prophetic Medicine has recommended using this plant on a regular basis. Prophetic hadith stated that black seed is the remedy for all diseases except death (Ahmad *et al*., 2013). Besides, this plant especially seeds and oils are also popular to be used in various traditional systems of herbal medicine such as Ayurveda and Siddha as well as Unani and Tibb (Sultana *et al*., 2015). The seeds and oils of *N. sativa* are also good food components to be used in the dietary systems as a spice and food preservative. Besides, both seeds and oils have been reported to have several biological activities including antioxidant, antimicrobial, antihypertensive, anticancer, anti-inflammatory, diuretics, anti-diarrheal, appetite stimulant, analgesics, and for treatment of skin disorders, therefore seeds of *N. sativa* can be considered as functional food components. *Nigella sativa* oil (NSO) supplementation along with conservative management is safe in averting the progression of chronic kidney disease in stage 3 and 4 patients (Ansari *et al*., 2016).

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Nigella sativa oil (NSO) has a pleasant taste and a specific odor. In the fats and oils industry, NSO commanded a high price, 10–15 times more expensive than other edible oils such as palm oil, corn oil and soybean oil. This fact has attracted the unethical market players to mix or adulterate NSO with other low priced oils to gain economic profit. Therefore, the identification of NSO from any adulteration must be addressed to assure the authenticity of NSO (Rohman and Ariani, 2013). In this review, some physico-chemical methods have been highlighted including authentication studies of NSO.

2. Methods

During performing this review, we used several databases including Scopus, PubMed, and Google Scholar to identify and to download the abstracts, reports, and research papers related to physico-chemical properties, authentication, and antioxidant activities of *Nigella sativa* oil. The keywords used during searching of information was: (1) (characterization + *Nigella sativa* oil or characterization + black seed cumin oil); (1) (authentication or adulteration + *Nigella sativa* oil); (3) (antioxidant activities + *Nigella sativa* oil + *in vitro* or *in vivo*); (4) (nutritional aspects + *Nigella sativa* oil + *human* health) in the month of April-September 2018.

3. Physico-chemical properties and chemical composition of *Nigella sativa* oil

Nigella sativa oil (NSO) was typically obtained by non-polar solvent extraction and cold-press procedure. The oil contents in most Nigella seeds studied were typically 30-40%, depending on environmental conditions such as water-stress, saline conditions, and temperatures (Khan, 1999; Cheikh-Rouhou *et al*., 2007). Gharby *et al.* (2015) have reported that the oil contents of Nigella seeds obtained from Morocco using hexane extraction and cold press-extraction yielded 37% and 27%, respectively. Some physico-chemical parameters were also affected by techniques used for oil extraction. Khoddami *et al.* (2011) also compared the yields of NSO obtained by three different extracts. The highest yield was observed using Soxhlet (37.33±0.15%), using petroleum ether as a solvent, over Modified Bligh–Dyer with a yield of 33.24±0.59% and hexane extraction with a yield of 31.76±0.64%.

NSO obtained by two extraction methods (hexane extraction and cold press) have been compared in terms of Free fatty acids (as oleic %), Iodine value (g of I_2/100 g), K232, K270, PV ($MeqO_2$/kg), and Refractive index at 20°C. Kiralan *et al.* (2014) have compared some physicochemical properties of NSO extracted by three different methods (Soxhlet extraction using hexane, microwave extraction, and cold press). The values of corresponding these properties were compiled in Table 1.

Table 1. Physico-chemical properties and fatty acid composition of *Nigella sativa* oil.

Physico-chemical properties	Cold-press	Hexane extraction	Microwave extraction
Free fatty acids (as oleic acid%)	0.9 ± 0.2[a] (7.49 ± 0.96)[b]	2.3 ± 0.5 [a] (9.28 ± 0.63)[b]	(9.51 ± 0.36)[b]
Iodine value (g of I_2/100 g)	128 ± 2 [a]	126 ± 4 [a]	-
K_{232}	1.585 ± 0.008 [a] (3.71 ± 0.12)[b]	2.21 ± 0.01 [a] (3.11 ± 0.06)[b]	(0.58 ± 0.05)[b]
K_{270}	2.370 ± 0.003 [a] (0.66 ± 0.05)[b]	2.771 ± 0.009 [a] (0.66 ± 0.06)[b]	(0.58 ± 0.05)[b]
Peroxide value ($MeqO_2$/kg)	3.4 ± 0.5 [a] (31.32 ± 0.74)[b]	11.4 ± 2.5 [a] (25.23 ± 1.56)[b]	(21.45 ± 0.79)[b]
Refractive index at 20°C	1.468 ± 0.003 [a] (1.473 ± 0.00)[b]	1.473 ± 0.002 [a] (1.471 ± 0.00)[b]	1.471 ± 0.00)[b]
Fatty acids[c]	Concentration (%)		
Miristic Acid (C 14:0)	0.13 – 0.16		
Palmitic Acid (C 16:0)	12.90 – 13.25		
Palmitoleic Acid C 16:1)	0 - 0.60		
Margaric Acid (C 17:0)	0.06		
Heptadesenoic Acid (C 17:1)	0.03 – 3.29		
Stearic Acid (C 18:0)	2.56 – 2.80		
Oleic Acid (C 18:1)	22.63 – 24.51		
Linoleic Acid (C 18:2)	58.90 – 61.20		
Linolenic Acid (C 18:3)	0.21 – 0.28		
Arashidic Acid (C 20:0)	0.13 - 0.15		
Eicosenoic Acid (C 20:1)	0.27 – 0.35		
Eicosadienoi c Acid (C 20:2)	1.86 – 9.40		
Behenic Acid (C 22:0)	0.50 - 1.30		
Docosenoic Acid (C 22:1)	0.30 – 1.10		

[a]Data taken from Garby *et al.* (2015); [b]Data taken from Kiralan *et al.* (2014); [c]Data taken from Argon and Gokyer (2006).

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Table 2. Some bioactive lipid components present in *Nigella sativa* oil (NSO) with different extraction methods (Argon and Gokyer, 2006; Kiralan *et al.,* 2014).

Bioactive lipid components	Extraction methods		
	Cold-press	Soxhlet extraction	Microwave extraction
α-tocopherol (mg/kg)	7.30 ± 0.46	4.80 ± 0.36	5.33 ± 0.12
β-tocopherol (mg/kg)	15.47 ± 0.29	8.00 ± 0.36	7.80 ± 0.17
γ-tocopherol (mg/kg)	34.23 ± 0.21	9.57 ± 0.51	8.57 ± 0.21
δ-tocopherol (mg/kg)	8.37 ± 0.12	1.80 ± 0.10	1.63 ± 0.06
Campestrol (mg/kg)	14.88 ± 0.19	13.47 ± 0.43	14.71 ± 0.34
Stigmasterol (mg/kg)	17.48 ± 0.54	17.49 ± 0.43	18.70 ± 0.59
β-sitosterol (mg/kg)	58.05 ± 1.01	57.49 ± 0.82	57.41 ± 0.17
Δ^5-Avenasterol (mg/kg)	7.27 ± 0.74	8.80 ± 0.23	7.34 ± 0.58
Δ^7-Avenasterol (mg/kg)	1.24 ± 0.10	1.14 ± 0.26	0.89 ± 0.09
Δ^7-Avenasterol (mg/kg)	1.62 ± 0.31	1.28 ± 0.25	1.58 ± 0.34
p-hydroxy benzoic acid (μg/g)	1.50 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Benzoic acid (μg/g)	4.15 ± 0.07	2.15 ± 0.07	2.65 ± 0.07
Cinnamic acid (μg/g)	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
Thymoquinone (μg/g)	14.40 ± 0.57	5.65 ± 0.07	6.20 ± 0.28

Linoleic and oleic acids are the main fatty acids composed NSO (Argon and Gokyer, 2016). Table 1 also compiled the fatty acid composition of NGO. NSO was characterized by high contents of PUFA (polyunsaturated fatty acids) and MUFA (monounsaturated fatty acids). MUFA have been reported to lower bad LDL (low-density lipoproteins) cholesterol and retain good HDL (high-density lipoproteins) cholesterol, which makes NSO as a good component to be used in the nutritional and pharmaceutical applications (Ramadan *et al*., 2010). Khoddami *et al*. (2011) reported that main triacylglycerols which composed NOS were LLL (19.90 –20.60%), OLL (16.07–16.97%) and PLL (12.40–18.51%). They also contained small quantities of POO and PPO.

Some active components are believed to be responsible for several biological activities such as tocopherols, carotenoid, and phenolics compounds and thymoquinone. Kiralan *et al.* (2014) have reported some lipid components in NSOs extracted by Soxhlet, microwave and press cold, and the results were compiled in Table 2. Compositional analysis of NSO using gas chromatography-mass spectrometry (GC-MS) contained 17.47% caryophyllene, 11.80% thymoquinone, 7.17% 1,4-cyclohexadiene, 3.5% longifolene, and 1.82% carvacrol (Mohammed *et al*., 2016). Some components 9 compounds) were also isolated and identified using GC-MS from essential oils of NSO namely 2-methyl-5(1-methyl ethyl)-bicyclo-hex-2-ene as a major component and alpha-pinene as a minor compound (Adamu *et al*., 2010). Ahmad *et al.* (2013) also reported that NSO extracted by steam distillation contained carvone, d-limonene and nigellone. However, studies reported that the composition of NSO varies with the geographical regions, where the plant is cultivated (D'Antuono *et al*., 2002; Cheikh-Rouhou *et al*., 2007).

4. Authentication of *Nigella sativa* oil

Using literature review in Scopus (www.scopus.com), only one method has been reported for authentication of NSO, namely Fourier transform infrared spectroscopy. Rohman and Ariani (2013) have used Fourier transform infrared (FTIR) spectroscopy in combination with partial least square regression (PLSR) was used for authentication of NSO mixed in binary systems with corn oil and soybean oil (NSO-CO and NSO-SO) as well as in ternary mixtures of NSO-CO-SO. Based on optimization for selecting the best calibration models in terms of highest coefficient of determination (R^2) and lowest errors in calibration and prediction, the second derivative of FTIR spectra at combined wavenumbers of 2977–3028 cm^{-1} , 1666–1739 cm^{-1} , and 740–1446 cm^{-1} was used for prediction of NSO in binary mixtures with R^2 of 0.9984 and root mean square error of calibration (RMSEC) of 1.34% v/v. In addition, NSO mixed with SO was determined using the first derivative FTIR spectra at combined wavenumbers of 2985–3024 cm^{-1} and 752–1755 cm^{-1} with R^2 and RMSEC values of 0.9970 and 0.47% v/v, respectively. The mode of second derivative FTIR spectra at combined wavenumbers of 2977–3028 cm^{-1}, 1666–1739 cm^{-1}, and 740–1446 cm^{-1} was used for quantification of NSO in ternary systems with CO and SO with R^2 and RMSEC values of 0.9993 and 0.86% v/v, respectively. These results confirmed that FTIR spectrophotometry combined with multivariate calibration offered a reliable method for authentication of NSO.

FTIR spectroscopy in combination with PLSR also successfully used for quantitative analysis of NSO in binary mixture with sunflower oil (SFO) and walnut oil (WO) in the systems of NSO-WO, NSO-WO and NSO-SFO-WO. PLSR using normal spectra at whole wavenumbers of mid-infrared (4000–650 cm^{-1}) was

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selected for quantification of NSO in NSO-WO, NSO-WO and NSO-SFO-WO with R^2 of > 0.99 in calibration and prediction or validation models. Classification of NSO and NSO adulterated with SFO and WO was successfully done using chemometrics of principal component analysis (PCA) using absorbances as wavenumbers region of 3009–721 cm^{-1}. Based on score plot of first principle component (PC1) and second principal components (PC2), pure NSO can be clearly separated from NSO adulterated with SFO and WO (Rohman *et al*., 2015). For authentication purpose, the presence of grapeseed oil (GSO) in NSO as adulterant was also analyzed using FTIR spectroscopy. PLSR at combined wavenumbers of 1114–1074, 1734–1382 and 3005–3030 cm^{-1}could be successfully used for quantification of GSO in NSO with R^2 for the relationship between actual (x-axis) and FTIR predicted (y-axis) values are 0.981. The errors in cross-validation and in prediction are 2.34% (v/v) and 2.37% (v/v), respectively.

FTIR spectroscopy using attenuated total reflectance (ATR-FTIR) has also used for quantitative analysis of NSO from alginate beads. ATR-FTIR method has been developed for quantification of encapsulation efficiency of NSO by applying Beer-Lambert law after selecting the combinations of wavenumbers. The developed method was valid with linearity in the range of 25-300 mg/mL with R^2=0.998, precision as expressed with relative standard deviation (RSD) of 8.4%, limit of detection of 0.28 mg NSO/mL and limit of quantification of 0.87 mg NSO/mL (Khismatrao *et al*., 2018).

5. Antioxidant activities of *Nigella sativa* oil

Many beneficial activities of NSO in human health and mechanisms of action has been reported in relation to NSO which include antioxidant either *in vitro* or *in vivo*, immunomodulatory, anti-inflammatory, antibacterial, antiviral, antifungi, and antiparasitic activities (Abd El-Hack *et al*., 2016). In this review, the antioxidant activities of NSO were highlighted either *in vitro* or *in vivo*.

Free radicals can cause several degenerative diseases due to an imbalance between oxidant (free radicals) and antioxidants in the human body. Reactive oxygen species and reactive nitrogen species (ROS/RNS) are believed to cause and aggravate several degenerative diseases in human such as diabetes mellitus and cancer (Kale *et al*., 2015), therefore human body needs endogenous and exogenous antioxidants. Any substances present in low concentrations coming from natural or synthetic sources with the ability to delay or inhibit free radical reaction can be considered antioxidants (NurAlam *et al*., 2013).

Several tests have been used for evaluation of antioxidant activities *in vitro* and in vivo. *In vitro*, the antioxidant activities of plant samples were assessed by: (1) radical scavenging methods using several radicals namely 2,2'-diphenyl-1-picrylhydrazil (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), hydrogen, nitric oxide, and peroxy-nitrite radicals, (2) reducing power including ferric reducing antioxidant power (FRAP), ferric-thiocyanate, phosphomolybdenum method, cupric ion reducing antioxidant capacities, (3) metal chelating power, (4) lipid peroxide inhibition using beta-carotene bleaching linoleic-ferric-thiocyanate, and thiobarbituric acid (TBA) methods (Antolovich *et al*., 2002; Schaich *et al*., 2015). Evaluation of antioxidant *in vivo* typically was performed using animal models in which the animals were administrated with tested samples at certain doses along with positive controls such as tocopherols and phenolic compounds (Moniruzzaman *et al*., 2015). After the specified time of treatment period, the animals were sacrificed, and the tissue or blood was exploited for *in vivo* antioxidant assay and antioxidant enzymes were measured. Several enzymes used as an antioxidant measure are catalase, glutathione reductase, superoxide dismutase, glutathione peroxidase, gamma-glutamyl transpeptidase activity, and glutathione S-transferase. Besides, glutathione levels and lipid peroxidation were also used as indicative of antioxidant activity *in* vivo (NurAlam *et al*., 2013).

Mohammed *et al.* (2016) have extracted NSO using supercritical fluid extraction (SFE) and cold press (CP) and evaluated its antioxidant activities using DPPH radical scavenging assay, ferric reducing activity power (FRAP) and total phenolics contents. Using DPPH assay, NSO extracted using SFE had higher antioxidant activity that extracted using CP with IC_{50}values of 1.58 mg/mL and 2.30 mg/mL, respectively. FRAP values of NSO extracted from SFE and CP were 538.67 mmol/100 mL and 329.00 mmol/100 mL, respectively. These antioxidant activities were correlated with the phenolics contents in which SFE-extracted NSO have phenolics of 160.51 mg/100 mL gallic acid equivalent (GAE), higher than CP-extracted NSO with phenolics contents of 94.40 mg/100 mL GAE. Kiralan *et al*. (2014) have compared NSOs extracted from three methods (Soxhlet extraction using hexane, microwave extraction, and cold press) for its ability to inhibit DPPH radicals. The highest inhibition rate of DPPH radicals was observed for cold-pressed NSO (78.4%), over Soxhlet extraction (65.58%) and NSO-microwave assisted extraction (61.7%). This inhibition was in line with the total phenolics contents in NSOs, i.e. 36.05%, 15.19% and 21.44% for NSO-cold press, NSO-extracted microwave, and NSO-Soxhlet extraction.

NSOs differed in its oxidative stability as indicated by OSI. The lowest OSI value was 76.73 ± 2.45 hrs, while the highest one was 157.58 ± 0.00. The greater OSI value, the longer shelf life of edible fats and oils. As a comparison, OSI values of corn oil and soybean oil were about 66 and 47 h respectively, determined using the same conditions as NSO (Lutterodt *et al*., 2010). These results indicated press cold extracted-NSO have good oxidative stability. All NSOs exhibited DPPH radical scavenging capacity by quenching of 76.4-83.5 μmol from initial 100 μmol DPPH radicals in the reaction mixtures. This activity was supported by ESR study in which cold-pressed NSOs contained natural antioxidants of phenolics compounds ranging 1.02-1.40 mg GAE/g oil and also contained thymoquinone of 3.48-8.73 mg/g (Parry *et al*., 2005).

Mraihi *et al*. (2013) have compared antioxidant activity of NSO using DPPH radical scavenging method from Tunisia (NSO-T), Libya (NSO-L) and Saudi Arabia (NSO-S) extracted using Soxhlet with hexane as solvent. NSO-S revealed the higher antioxidant activity than NST and NSL with IC_{50} of 0.422, 0.507 and 0.528 μg/μL, respectively. The lower the IC_{50} value, the higher the antioxidant activity of NSO. Haron *et al.*(2014) reported antioxidant activities of 5 different bats NSO from Malaysia, Sudan and Iran using DPPH radical scavenging and β-carotene bleaching. Using DPPH assay, NSO from Iran showed the highest DPPH radical scavenging activity with IC_{50} of 1.49 mg/mL, while NSO from Sudan has IC_{50} of 4.48 mg/mL. Using β-carotene-linoleic acid assay, the highest inhibition activity was observed in NSO from Kelantan Malaysia with inhibition activity of 97.86±0.24%.

NSO has been evaluated for its capacity as an antioxidant *in vivo* using forty-two patients with rheumatoid arthritis (RA). The patients were divided into 2 groups, namely patients treated with 2 capsules containing 500 mg NSO and those treated with placebo. Some inflammatory and antioxidant parameters namely serum interleukin (IL)-10, serum antioxidant total capacity, and antioxidant enzymes (superoxide dismutase and catalase) were measured at baseline and the end of the trial. The results showed that levels of IL-10 in patients treated with NSO were increased, while serum malonaldehyde and nitric oxides were decreased compared to baseline. However, there is no significant difference ($P>0.05$) in the levels of superoxide dismutase, catalase, and total antioxidant serum capacity either between-within or before-after intervention of NSO. This study indicated that NSO could reduce oxidative stress in patients with rheumatoid arthritis, and NSO may be used as adjunct therapy in RA patients (Hadi *et al*., 2016).

The effects of NSO concurrent with a low-calorie diet on lipid peroxidation and oxidative status in obese women has been investigated by Namazi *et al.* (2015) by investigating SOD and other antioxidant parameters in vivo. Participants (50 women) were divided randomly into 25 participants who treated with intervention (3 g/ day NSO) and 25 participants who received placebo (3 g/ day) for 8 weeks. SOD in treated participants with NSO was increased significantly compared to those treated with placebo, however, there were no significant differences in lipid peroxidation, glutathione peroxidase, and total antioxidant capacity concentrations.

NSO was also investigated for its activity in inhibiting lipid peroxidation on rats treated with tartrazine. Tartrazine was known to decrease total protein, antioxidants and high-density lipoproteins. The results showed that NSO con protects male rats via inhibition of lipid peroxidation against the adverse effects resulted from the administration of tartrazine (Al-Seeni *et al.,* 2018). NSO given to guinea-pigs also increased the levels of MDA, SOD activity and GSH (Saleh *et al*., 2012). Ilhan *et al*. (2005) reported that NSO remarkably raised the GSH levels and depressed the MDA level in rats. The thymoquinone present in volatile oil of NSO had a protective effect on acute gastric damage in the rats (Arslan *et al*., 2005). These findings have reported that the whole herbal plants, cold pressed or essential oil, extracts and their active molecules, especially, thymoquinone, possess antioxidants, supporting the common folk perception of black cumin as antioxidant and anti-inflammatory factors (Amin and Hosseinzadeh, 2016).

5. Conclusion

Nigella sativa oil is excellent oil to be used in nutritional and pharmaceutical application due to some active components present such as tocopherol, phenolics compounds, and thymoquinone believed to be responsible for beneficial effects to human health including antioxidant. Some physico-chemical analyses indicated that NSO from different regions has bit difference make a need to standardize NSO. Some instrumental analysis of FTIR spectroscopy could be used for authentication analysis of NSO to assure its quality and authenticity.

Conflict of Interest

We declare that we have no conflict of interest.

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