

Green Extraction and Characterization of *Nigella sativa* L. Seed Oil: Chemical Profiling, Antioxidant Activity, and Thermal Stability Kinetics

Fatimah Abdul Razzak Mageed ^{1*}, Saly Naser Abbas ¹ Shatha Abd Al-Jabbar ¹

¹Department of biology, College of Education for pure Science , University of Kerbala , Karbala, Iraq.

* fatima.a@uokerbala.edu.iq

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ABSTRACT

This study aims to explore the efficiency of sustainable green extraction techniques in obtaining essential fatty acids, particularly linoleic acid (omega-6), from black cumin seeds (*Nigella sativa* L.), by comparing the results with the efficiency of well-known conventional extraction methods that rely on the use of organic solvents. The study also examines in depth the stability of these compounds against oxidation during storage. Reference extraction results using a Soxhlet apparatus and normal hexane solvent showed a total fat content in the dry matter of $60.02 \pm 0.78\%$. This environmentally friendly extraction system achieved high efficiency under ambient conditions while maintaining a high degree of chemical composition and structural integrity. The physicochemical properties of the extracted lipid fraction showed an iodine value of 226.04 using method Wiggs with $\text{FeCl}_3/\text{CCl}_4$ solution, indicating a high degree of unsaturation in its constituent fatty acids. A pH value of 16.04 was also recorded. (FTIR) spectroscopy revealed the presence of functional groups characteristic of triglycerides. Major absorption peaks were observed at 1743.71 cm^{-1} (C=O) ester, and at 3009.05 cm^{-1} (C-H) alkenes. Another peak at 375.88 cm^{-1} (O-H). Analysis of methylated fatty acid esters (FAMES) using (GC-MS) spectrometry revealed that linoleic acid (C18:2, omega-6) was the most abundant polyunsaturated fatty acid, followed by oleic acid monounsaturated fatty acids. stearic acids and palmitic were found in low concentrations. DPPH assay results also showed that the extracted oil possessed high antioxidant activity to inhibit free radicals. A 90-day stability study indicated that 4°C was optimal, followed by 25°C and then 40°C .

Key words: Omega-6 Linoleic Acid, *N.sativa*, Green Extraction, GC-MS, FTIR, Oxidative Stability.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is known to be an annual herbaceous plant belonging to the Ranunculaceae family. It is believed to have originated in the Mediterranean region before its cultivation spread to other areas, such as the Hejaz, parts of Asia, and North Africa [1]. Its fruits (seeds) are triangular in shape and, upon ripening, transform into capsules containing tiny seeds. These seeds turn a dark black color when exposed to air and sunlight, which explains its common name [2]. Black seed has been used in traditional medicine since ancient times to treat many ailments, relying on knowledge and experience passed down through generations. With the development of modern scientific studies, its mechanisms of action at the molecular level have been revealed, making it one of the most researched and studied medicinal plants globally and one of the most beneficial among its peers [3]. Phytochemical and nutritional studies confirm that the health benefits of black seed (*Nigella sativa*) are due to its highly complex chemical composition, which includes more than one hundred bioactive and biocompatible compounds [4]. Approximate nutritional analyses also show that it contains about 21.5% protein, 40.5% carbohydrates, and 4.2% fat, although these percentages may vary depending on the geographical region, climatic conditions, and available cultivation methods. There are also characterized by their rich content of micronutrients and secondary compounds bio active, The seeds contain nutrients and active compounds such as thiamine , zinc, and alkaloids, along with a range of impulsive compounds, most notably nigellone, which has a bronchodilator effect. They are also an important source of amino acids, especially arginine, which plays a key role in the growth and infants growth [5]. The pharmacological and physiological properties of black cumin(*Nigella sativa*) are primarily attributed to its fatty acid content. Chromatographic analysis of the seed oil revealed a distinctive composition and results, with linoleic acid being the most abundant at 64.2%, followed by palmitic acid at 16.6%, and oleic acid at 13.7%. It also contains smaller amounts of myristic acid (2.8%), arachidic acid (1.9%), and stearic acid (0.8%). Linoleic acid is an essential polyunsaturated omega-6 fatty acid that the body cannot synthesize and must obtain from food. [6]. Omega-6 fatty acids play an important role in maintaining the integrity of cell membranes and regulating inflammatory responses. However, their high number of double bonds makes them highly susceptible to auto-oxidation, meaning that exposure to environmental factors can lead to their degradation and the formation of toxic secondary compounds that reduce their biological activity [7]. In addition to its fatty composition, the therapeutic efficacy of black seed (*Nigella sativa*) is enhanced by the presence of volatile quinone compounds, most notably thymoquinone. Recent oncology studies indicate that thymoquinone has the ability to inhibit the growth of treatment-resistant human cancer cells, as well as support tissue immunity against malignant metastasis [8]. Black seed oil is also a potent free radical scavenger, contributing to the activation of antioxidant enzymes in the body such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Thymoquinone, flavonoids, and unsaturated fatty acids work synergistically to reduce oxidative stress and enhance cellular protection [9]. Traditional methods for extracting these sensitive bioactive lipids often rely on volatile organic solvents such as hexane. While efficient, these methods are associated with significant environmental risks and can leave toxic residues, in addition to the potential for heat-sensitive polyunsaturated fatty acids to undergo thermal or partial chemical degradation. Accordingly, modern extraction technologies have evolved towards what is known as sustainable green extraction [10], which are environmentally friendly

alternatives (sustainable green environment) aimed at reducing the environmental impact and improving energy efficiency, while preserving the natural chemical structure and biological activity of the targeted compounds [11]. Based on the aforementioned principles, the current study aims to extract omega-6 fatty acids from black cumin seeds (*Nigella sativa*) using sustainable, green solvents, while preserving their natural bioavailability. The study also employed advanced analytical techniques, such as Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS), to accurately determine the lipid composition and evaluate the antioxidant activity of the extracts. In conclusion, this study seeks to highlight the nutritional and therapeutic value of black cumin seeds (*Nigella sativa*) and promote their role as a sustainable source of bioactive compounds with added value that can be utilized in the food, pharmaceutical, and cosmetic industries[12].



Figure 1. Real image and the chemical structure of omega-6 linoleic acid.

This work aims to focus on the study of the efficiency for the sustainable green extraction techniques depending on the essential fatty acids, particularly linoleic acid (omega-6), from black cumin seeds (*Nigella sativa* L.).

MATERIAL AND METHODS

Materials and Reagents

Dried (*Nigella sativa*) black cumin seeds were obtained after careful cleaning and air drying. All chemicals and solvents used in the study, such as, petroleum ether, and ethanol n-hexane, were used at analytical purity levels and without any further purification prior to use.

Apparatus

Fourier-transform infrared(FTIR)spectra were recorded using a Shimadzu FTIR spectrophotometer(IRAffinity-1S, Shimadzu, Kyoto,Japan) across a scanning range of 4000–400

cm⁻¹ [13]. The Gas chromatography-mass spectrometry (GC-MS) analysis (Shimadzu GCMS-QP2020 NX) was detected the bioactive compound in extraction samples.

Sample Preparation

To increase the efficiency of the extraction process, the air-dried black cumin seeds (*N. sativa*) were ground into a fine powder using a laboratory grinder. The powder was then stored in airtight containers (special storage boxes) until it was used in subsequent experiments [14].

Extraction Procedures

Baseline Quantification via Soxhlet Extraction

To determine the initial total fixed oil content of black cumin (*Nigella sativa*) seeds, a conventional extraction process was performed using a Soxhlet extraction apparatus. Approximately 8 grams of finely ground seed powder were used in this process, and the powder was extracted for 7 continuous hours using 180 mL of n-hexane. Petroleum ether was also used in some preliminary tests to characterize the chemical properties of the extracted oil. After extraction, the total fat content in the dry matter, representing the total oil content of the sample, was calculated to be $60.02 \pm 0.78\%$. This final value was determined based on the average of three independent experiments (replications), in order to ensure accuracy and raise the level of statistical reliability of the results [15].

Green Solvent Extraction

As part of environmentally friendly extraction experiments, 50 grams of seed powder were used and subjected to a green extraction process using a dual-solvent system consisting of ethanol and water (food-grade solvents). The process was carried out at room temperature with continuous magnetic agitation for 3 hours to ensure optimal extraction efficiency. After the process was complete, the resulting mixture was filtered to remove solid impurities and then centrifuged to separate and isolate the fat fraction.. The solvent was then removed under reduced pressure using a rotary evaporator to yield the crude oil [16].

Batch Extraction Configuration

Batch extraction experiments were carried out using 14 g of ground *N. sativa* seeds transferred into a specialized glass reactor equipped with a water-cooled reflux condenser. The operational temperature of the system was precisely controlled using a magnetic stirrer equipped with an integrated heating plate. After a designated reaction period, the mixture was vacuum-filtered, and the solvent was distilled under reduced pressure. The isolated oil was dried until a

constant weight was achieved. The relative oil extraction yield (Y %) was calculated using Equation (1):[17]

$$Y(\%) = \frac{m}{m_{0,oil}} \times 100 \quad \dots(1)$$

Chemical Characterization of Extracted Oil

Determination of Iodine Value[18]

The iodine content was determined using Wijs solution, prepared by dissolving 9 g of ferric chloride (FeCl_3) in 300 mL of carbon tetrachloride (CCl_4), then bringing the volume up to 1000 mL by adding 700 mL of glacial acetic acid. Ten grams of iodine were then added to the solution and dissolved completely by continuous stirring. The solution was then left to stand for a specified time before being filtered to obtain a clear solution [19]. For quantitative analysis, 0.02 g of the extracted oil was accurately weighed and mixed with 10 mL of the freshly prepared Wijs solution in a sealed flask. The flask was then sealed, shaken thoroughly, and kept in the dark for 30 minutes to ensure the reaction was complete. In the final stage of the experiment, 20 mL of potassium iodide solution with a concentration of 100 g/L, along with 150 mL of distilled water, were added to the mixture, and titration was performed using a standard solution of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) at a concentration of 0.05 mol/L, with phenolphthalein used as an indicator to determine the endpoint with high accuracy [20].

Determination of Acid Value (Acidity)

The acidity value of the extracted oil was measured according to standard procedures. A 5-gram sample of oil was transferred to a 250 mL conical flask and then dissolved in 50 mL of a pre-neutralized ethanol-ether mixture at a volumetric ratio (1:1). The titration was then carried out using a standard alkaline solution of sodium hydroxide (NaOH), and the reaction continued until the final equilibrium point was reached, which is the end of the reaction of free fatty acids with the base [21].

Fatty Acid Methyl Ester (FAME) Preparation and GC-MS Analysis

To analyze the available fatty acid components, a conversion esterification process was performed on the samples, in which the fatty acids were converted into volatile methyl esters (FAMEs) using a solution of methanol and potassium hydroxide (KOH). These esters were then analyzed, identified, and quantified using gas chromatography-mass spectrometry (GC-MS-QP2010 Ultra, Shimadzu, Kyoto, Japan). The compounds were separated using a 30-meter HP-

5MS capillary column with an internal diameter of 0.25 mm and a membrane thickness of 0.25 μm . High-purity helium (99.999%) was also used as the carrier gas at a constant flow rate of 1.0 ml/min. The injector temperature was set at 250°C, with a 1:50 split injection system employed. The column furnace program started at 60°C for 2 minutes, then the temperature was gradually increased to 200°C at a rate of 10°C/minute, before being held at 240°C for 5 minutes. The electron ionization (EI) mass spectrometer was also operated at an energy of 70 electron volts, maintaining the ion source temperature at 200°C and a mass scanning range of 40–500 m/z. [22].

Antioxidant Activity via DPPH Radical Scavenging Assay

The activity antioxidant of the extracted oil was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to measure its free radical scavenging capacity. This activity was determined by monitoring the decrease in absorbance at a wavelength of 517 nm using a UV-Vis spectrometer.

Stability and Accelerated Degradation Study

Oxidized lipids were studied and evaluated over time by keeping oil samples under three different environments for 90 days, with periodic sampling every 15 days.

- Room temperature (25°C)
- Refrigerated storage (4°C)
- Accelerated degradation conditions(40°C) [23].

RESULTS

Extraction Yield and Physicochemical Indices

Traditional extraction of black cumin seeds (*Nigella sativa*) using an organic solvent (petroleum ether) resulted in an oil yield of 39.0%, which is generally consistent with the values reported in scientific studies, ranging between 40.0% and 44.0%. The chemical quality and nutritional value of the oil fraction extracted from black cumin seeds were assessed by quantitative measurement of both pH and iodine content.

Acid Value Determination

The acidities value (AV) were determined via volumetric titration with potassium hydroxide (KOH)solvent. The experimental data obtained from the titration process are summarized in Table 1.

Table 1. Titration data for the determination of the acid value of *N. sativa* seed oil.

Read the beginning	Final reading	Consumed volume
27	41.30	14.30

The acid value was calculated using the following standard relation:

$$\begin{aligned} \text{Acidity number} &= \frac{M.wt(KOH) * Consumed volume(Oil) * M(KOH)}{wt.(oil)} \quad \dots(2) \\ &= \frac{56.1 * 14.30 * 0.1}{5} \\ \text{Acidity number} &= 16.04 \end{aligned}$$

Iodine Value Determination

The "iodine (IV) value", which expresses the degree of unsaturation and the oil's susceptibility to drying, was measured using the Wijs method based on "reverse titration" with a sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution. The corresponding titration parameters are detailed in Table 2.

Table 2. Titration data for the determination of the iodine value.

Substance	Read the beginning	Read the beginning	Consumed volume
Oil	0.00	12.50	12.50
Blank	12.50	41.0	28.5

The iodine value was calculated according to the following mathematical expression:

$$\begin{aligned} \text{Iodinenumber} &= \frac{M.wt(iodine) * M(K_2S_2O_3) * V(ConsumedOil) * V(ConsumedBlank)}{wt(oil)} \quad \dots(3) \\ \text{Iodinenumber} &= \frac{M.wt(iodine) * M(K_2S_2O_3) * V(253.8 * 0.05 * 28.5 * 12.50)}{0.02} \\ \text{Iodinenumber} &= 226.04 \end{aligned}$$

Spectroscopic Characterization (FTIR)

The functional groups present in the extracted *N. sativa* oil were characterized using Fourier-transform infrared (FTIR) spectroscopy (Figure 2). The spectrum exhibited a distinct diagnostic peak at 3745.88cm^{-1} , corresponding to the C-OH stretching vibration. Prominent aliphatic stretching bands were observed at 3009.05cm^{-1} (C-H) stretching of cis-alkene, C-H, 2928.04cm^{-1} asymmetric C-H= stretching of CH_3 , and 2858.60cm^{-1} symmetric C-H stretching of CH_2 . Furthermore, a sharp, intense absorption band appeared at 1743.71cm^{-1} , assigned to the ester carbonyl (C=O) stretching vibration of triacylglycerol's. The skeletal stretching of aliphatic C=C

bonds was identified at 1516.10cm^{-1} . The bending vibrations of CH_2 groups were confirmed by the deformation band at 1458.23cm^{-1} , while the strong asymmetric stretching of C--O ester bonds was resolved at 1165.04cm^{-1} [24].

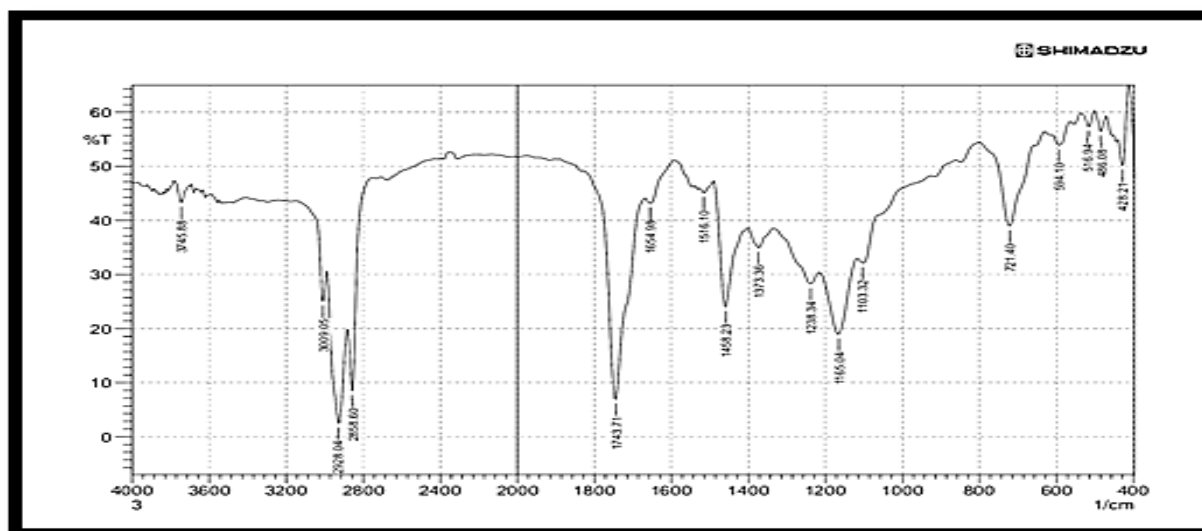


Figure 2. Fatty acid composition profile of *N. sativa* seed oil via FTIR analysis.

Chromatographic Profiling (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to establish the fatty acid profile of the isolated lipids. showing the qualitative distribution and approximate quantitative ratios of the methyl esters of major fatty acids (FAMES) in Table 3.

Table 3. Fatty acid composition profile of *N. sativa* seed oil via GC-MS analysis.

Compounds	Type	Relative Percentage (%)
Linoleic acid (C18:2, Omega-6)	PUFA	High (dominant)
Oleic acid (C18:1)	MUFA	Moderate
Palmitic acid	SFA	Low
Stearic acid	SFA	Low

Antioxidant Capacity and Stability Analysis**DPPH Radical Scavenging Activity**

A(DPPH) activity antioxidant test demonstrated a clear and strong ability to inhibit free radicals. The calculated (IC₅₀)value also indicated that the oil possesses moderate to high antioxidant activity, comparable to standard reference antioxidants.

Storage Stability and Thermal Degradation Kinetics

The stability oxidative of (*Nigella sativa*) black seed oil was evaluated over time under different thermal storage regimes, the resulting changes were analyzed comparatively. The comparative degradation data are presented showing in Table 4.

Table 4. The levels oxidation rates and Oxidative stability under distinct storage temperature.

Storage	Stability Level	Oxidation Rate
4°C	High stability	Low
25°C	Moderate stability	Medium
40°C	Low stability	High

DISCUSSION

The results of the current study demonstrate that extraction using green solvents is a safe, environmentally friendly, and highly efficient option compared to the hazardous conventional organic solvents used in extracting oil from black cumin seeds (*Nigella sativa*). This method also significantly reduces the environmental and health impacts resulting from toxic chemical residues. Mechanistically, the sustainable green solvent system proved highly effective in preserving the chemical stability of oxidation-sensitive bioactive compounds, particularly omega-6 polyunsaturated fatty acids (PUFAs), thereby enhancing the nutritional and therapeutic value of the resulting seed extracts [25]. Furthermore, FTIR spectroscopy analysis confirmed that the chemical structure of these bioactive lipids remained unchanged, with the spectra showing distinct absorption bands reflecting the presence of key functional groups. The unsaturated double bonds (C=C) and ester carbonyl groups (C=O) were also preserved, indicating that the extraction process did not cause any structural degradation or alteration to the spatial (geometric) arrangement of the compounds. These results are consistent with previous

studies that confirm that black seed (*Nigella sativa*) is a rich source of beneficial unsaturated fatty acids and bioactive phenolic compounds.[26]. Analysis Chromatographic using GC-MS confirmed these findings, revealing a high concentration of linoleic acid (C18:2, omega-6) along with a variety of other fatty acids and volatile compounds. From a biophysiological perspective, linoleic acid is an important and essential component in regulating inflammatory responses and maintaining the integrity of cell membranes, which enhances the traditional therapeutic efficacy attributed to black seed (*Nigella sativa*) [27]. The results of the fatty extracts showed a remarkable ability to neutralize free radicals and reduce oxidative stress in the DPPH assay. This potent antioxidant activity is attributed to a synergistic effect between unsaturated fatty acids, phenolic compounds, and thymoquinone. The importance of this effect stems from the pivotal role of oxidative stress in the development of many chronic diseases, such as diabetes, cardiovascular diseases, and some types of cancer, such as breast, colon, prostate, and others. It is noteworthy that despite the high content of oxidizable polyunsaturated fatty acids (PUFAs) in black seed, the green extracts showed high oxidative stability under the conditions studied. This potency and resistance to rancidity are attributed to the presence of a natural system of potent antioxidants that were extracted simultaneously with the fatty compounds. Thus, the combination of the high purity of the bioactive compounds and improved stability oxidative enhances the practical value of these extracts and confirms their applicability in high-value-added sectors such as the food, pharmaceutical, and cosmetic industries [28].

Conclusions

This study confirms the practicability of an joined and supportable approach based on the concept of bio refining, by combining green extraction techniques with advanced characterization methods such as GC-MS and FTIR to assess the biological value of black cumin (*Nigella sativa*) seeds. These results confirm the viability of green extraction techniques for obtaining high-purity black cumin (*Nigella sativa*) oil, with promising potential for use in sustainable pharmaceutical, food, and cosmetic applications and The results experimental indicate these seeds are a high-value fatty acids and environmentally sustainable source of omega-6 and natural antioxidants. This study thus contributes to the global trend toward developing safe and environmentally friendly natural products suitable for large-scale developed production.

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