International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 06 Issue 08 August 2026

Page No : 1199-1209

DOI: https://doi.org/10.47191/ijpbms/v6-i8-06, Impact Factor: 8.428

FTIR Method for Determining Oil Quality and the Effects of Sonication and Soxhlet Extraction Methods on the Phenolic Content and Antioxidant Activity of Black Cumin (Nigella Sativa) Oil

Youstiana Dwi Rusita[1*], Rini Tri Hastuti[2]

[1,2]Department of Pharmaceutical and Food Analysis, Health Polytechnic of Health Ministry Surakarta, Indonesia

ABSTRACT

Black cumin *(Nigella sativa L.)* is a plant that contains various bioactive compounds, particularly phenolic compounds, which have the potential to exhibit antioxidant activity. This study aimed to determine the characteristics of black cumin seeds, compare the yield and phenolic content of extracts obtained via maceration and sonication using 96% ethanol, and identify the functional groups of compounds in the extracts using Fourier Transform Infrared (FTIR) spectroscopy. Black cumin seeds were characterized macroscopically and microscopically, then extracted using the maceration method for 3 × 24 hours and sonication at a frequency of 60 kHz and a temperature of 40°C for 60 minutes. The extracts were subsequently analyzed using FTIR, and total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. The results showed that the yield of the maceration extract was 10.55% ± 0.001, while the yield of the sonication extract was 7.80% ± 0.001. FTIR identification results indicated the presence of functional groups associated with unsaturated C–H bonds, CH_2, carbonyl (C=O), CH_2/CH_3, and C–O. A qualitative test using $FeCl_3$ yielded positive results for phenolic compounds in both extraction methods. The total phenolic content of the maceration extract was 42.678 mg GAE/g ± 4.0934, while that of the sonication extract was 32.007 mg GAE/g ± 0.077. Based on these results, the maceration method under the study conditions yielded a higher yield and phenolic content compared to the sonication method. These findings serve as a basis for optimizing the extraction method and testing antioxidant activity in subsequent research phases.

KEYWORDS: black cumin, FTIR, maceration, sonication, total phenolics.

ARTICLE DETAILS

Published On:
25 August 2026

Available on:
https://ijpbms.com/

INTRODUCTION

Black cumin (Nigella sativa L.) is a plant widely used as a food ingredient, traditional medicine, and a source of natural compounds with pharmacological potential. Black cumin seeds contain fixed oil and volatile fractions rich in various bioactive compounds. Components that have been widely reported to contribute to biological activity include thymoquinone, thymohydroquinone, thymol, carvacrol, as well as various terpenoids and phenolic compounds. The composition of these compounds may vary depending on the variety, raw material conditions, growing location, and the processing and extraction methods used (1)(2)

Black cumin oil is of significant value because it contains unsaturated fatty acids and bioactive components associated with antioxidant activity. Thymoquinone is one of the most extensively studied marker compounds in black cumin, but the oil's biological activity is not determined solely by thymoquinone. Volatile fractions and other minor components may also contribute to the oil's characteristics and biological activity. Therefore, the quality of black cumin oil must be comprehensively evaluated based on its chemical characteristics and the presence of functional groups associated with its constituent components (1).

The quality of vegetable oils can change due to extraction processes, heating, oxidation, storage, or environmental conditions. Parameters such as free fatty acids, peroxide value, iodine value, fatty acid composition, and the presence of bioactive compounds can be used to characterize the oil's properties and stability. In black cumin oil, differences in extraction methods have been reported to result in variations in physicochemical characteristics, the composition of bioactive components, and antioxidant activity. Soxhlet solvent extraction, for example, can yield different extraction results compared to pressing or other technology-assisted methods (3)

The quality control process for oils requires analytical methods capable of providing information on chemical characteristics quickly, simply, and with minimal sample preparation. Fourier Transform Infrared Spectroscopy (FTIR) is one spectroscopic technique with potential for this purpose. FTIR provides spectra based on the vibrations of functional groups within a compound, making it suitable for obtaining the chemical fingerprint of an oil. In vegetable oils, the FTIR band at approximately 3008 cm^{-1} corresponds to the =C–H vibration of unsaturated fatty acids, while the bands at approximately 2923 and 2853 cm^{-1} correspond to the C–H stretching of the CH_2 groups, while the band around 1740 cm^{-1} is associated with the carbonyl (C=O) vibration of esters in triglycerides. Thus, FTIR spectral patterns can provide information regarding the structural characteristics and composition of oils (4).

The application of FTIR to black cumin oil has also demonstrated potential for authentication and quality evaluation. Rohman and Ariani (2013) used FTIR combined with partial least squares (PLS) to authenticate black cumin seed oil in mixtures with corn oil and soybean oil. The developed models yielded very high coefficients of determination, with one reaching $R^2 = 0.9984$.

These results indicate that FTIR spectra contain sufficient chemical information to distinguish and identify the characteristics of black cumin oil. In addition to authentication, FTIR can be utilized as a method for oil quality screening, as changes in fatty acid composition and oxidation products can affect the spectral patterns. Research on black cumin oil also shows that FTIR characteristics can reflect the predominance of unsaturated fatty acids as well as changes in quality during storage. Physicochemical parameters such as free fatty acids and the peroxide value also undergo changes during storage, so the use of FTIR has the potential to be developed as a rapid analytical approach to support quality control of black cumin oil (4).

In addition to oil quality aspects, the extraction method is a critical factor determining the quantity and composition of the bioactive compounds obtained. Extraction efficiency is influenced by the type of solvent, polarity, temperature, extraction time, particle size of the material, material-to-solvent ratio, and extraction technique. Differences in these conditions can lead to variations in the amounts of phenolic compounds and antioxidant components successfully extracted. In black cumin, changes in solvent polarity and boiling point have been shown to affect the yield and composition of oil components, including the presence of thymoquinone (3)

The Soxhlet method is a conventional extraction method that uses repeated heating and circulation of the solvent to enhance contact between the material and the solvent. This method offers the advantage of a relatively thorough extraction process; however, the use of high temperatures over a relatively long period can increase the risk of changes or degradation of heat-sensitive compounds. In contrast, ultrasound-assisted extraction (UAE) or sonication utilizes the phenomenon of acoustic cavitation, which can enhance solvent penetration into the material matrix and accelerate mass transfer. Therefore, sonication has the potential to enhance the release of bioactive compounds with shorter extraction times and lower heat exposure compared to conventional extraction (5)

Recent research on black cumin indicates that the sonication method shows great potential for increasing the yield of bioactive compounds. Dar et al. (2024) reported that ultrasound-assisted extraction yielded higher antioxidant activity compared to Soxhlet extraction under the conditions studied. The ultrasonic method yielded an IC_{50} value of 203.56 µg/mL, whereas conventional extraction resulted in a higher IC_{50} value. The researchers attributed this increase to the phenomenon of acoustic cavitation, which enhances mass transfer and facilitates the release of phytochemicals from the seed matrix.

Other research findings also indicate that the extraction method can influence the phenolic content and antioxidant activity of black cumin. Ultrasound-assisted phenolic extraction from defatted black cumin seeds resulted in an approximately 70% increase in total phenolics and a roughly 38% increase in DPPH radical scavenging activity compared to conventional extraction under optimal conditions. The optimal conditions obtained included an ethanol concentration of 59.1%, a temperature of 44.6 °C, and an extraction time of 32.5 minutes. These findings indicate that sonication has the potential to enhance the efficiency of phenolic compound extraction from the black cumin matrix (6)

Although various studies have evaluated black cumin extraction methods, there remains a need to integrate the characterization of oil quality using FTIR with an evaluation of the effect of extraction methods on phenolic content and antioxidant activity within a single research approach. Previous studies have largely focused on a single aspect, such as oil component characterization, extraction optimization, or biological activity testing. In fact, changes in extraction methods can affect not only the oil yield but also the composition of functional groups, the content of bioactive compounds, and the antioxidant capacity of the resulting oil.

Therefore, the study titled "FTIR Method for Determining Oil Quality and the Effects of Sonication and Soxhlet Extraction Methods on Phenolic Content and

Antioxidant Activity of Black Cumin Oil" is important to conduct. This study aims to determine the FTIR spectral characteristics of black cumin oil as a basis for oil quality assessment, as well as to compare sonication and Soxhlet extraction methods in producing oil with optimal phenolic content and antioxidant activity. FTIR parameters, total phenolic content, and antioxidant activity can then be used in an integrated manner to evaluate the relationship between extraction methods and the bioactive quality of black cumin oil.

The research results are expected to provide a scientific basis for selecting more effective and efficient extraction methods, while also supporting the development of FTIR as a rapid analytical method for the characterization and quality control of black cumin oil. Furthermore, the research results have the potential to support the utilization of black cumin oil as a raw material in the development of functional food products, nutraceuticals, and natural-based pharmaceutical preparations.

IMPLEMENTATION STAGES

a) Determinattion

Black cumin crude drug was obtained from "Toko Jamu Rumah Rempah Lestari" in Klaten. Subsequently, the samples were identified in the laboratory at Universitas Setia Budi Surakarta.

b) Raw Material Preparation

The black cumin seeds were dry-sorted and then ground into a powder using a herb grinder

c) Macroscopic and Microscopic Examination of Black Cumin Seeds

Examination was conducted with the naked eye or with the aid of a magnifying glass on various organs of living organisms. Macroscopic identification is based on shape, size, color, and surface characteristic (1). Microscopic testing was performed on the crude drug powder by placing the powder on a slide, adding a drop of chloroform, covering it with a cover slip, and observing general identifying features under a microscope (2).

d) Preparation of Black Cumin Extract

The sonication method was performed by weighing 400 grams of black cumin powder, then adding 4.000 mL of 96% ethanol solvent (1:10) w/v. The mixture was placed in an ultrasonic device at 60 kHz and 40 °C for 60 minutes. The filtrate was filtered through filter paper, then evaporated in a water bath at 50 °C.

The Soxhlet extraction method was performed by weighing 400 grams of black cumin powder, which was then soaked in 4.000 mL of 96% ethanol. The liquid extract was collected, filtered using a vacuum pump, and the solvent was evaporated using a rotary evaporator; to obtain a concentrated extract, it was further evaporated in a water bath at 50°C(3) . For each extraction, the extract yield was calculated using the formula:

$$\text{Yield=} \quad \frac{\text{Initial Weight}}{\text{Extract Weight}} \quad \text{x} \quad 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

e) FTIR (*Fourier Transform Infrared*) Analysis

Oil samples were measured using a Nicolet 6700 FTIR spectrometer (Thermo Nicolet, Madison, WI). FTIR spectra were collected in the mid-infrared region of 4,000–650 $cm^{-1}$with 32 scans and a resolution of 8 cm^{-1}. All spectra were compared to a background air spectrum. Afterward, a new reference air background spectrum was acquired for each scan. The spectra were recorded as absorbance values at each data point in triplicate (5).

f) Identification of Black Cumin Oil

1) Phenolic Test

Three drops of 1% $FeCl_3$ were added to the extract. A positive result was indicated by the appearance of green, red, purple, blue, or deep black color(6).

2) Determination of Phenolic Content

The phenolic content is determined based on the absorbance curve of a gallic acid standard solution. A 1000 ppm standard solution was prepared by weighing 10.0 mg of gallic acid and dissolving it in p.a. ethanol to a final volume of 10.0 mL. From this stock solution, 2.5 mL was pipetted and diluted with p.a. ethanol to a volume of 25.0 mL, resulting in a concentration of 100 ppm. From this solution, a series of concentrations of 10, 20, 30, 40, and 50 ppm was prepared. For each concentration of gallic acid solution, 5.0 mL was taken and 0.4 mL of Folin-Ciocalteu reagent was added; the mixture was then shaken and allowed to stand for approximately 4–8 minutes. Next, 3 mL of a 7% Na_2CO_3 solution was added, and the mixture was shaken until homogeneous and made up to 10.0 mL with distilled water. The mixture was allowed to stand for 2 hours at room temperature, after which its absorbance was measured at a maximum wavelength of 744.8 nm using a UV-Vis spectrophotometer(8).

3) Antioxidant Activity Test of Cumin Oil

Pipette 2 mL of each solution in the concentration series, then add 2 mL of a 50 ppm DPPH solution, and mix the solution thoroughly for 2 minutes. Cover the solutions with aluminum foil and let them stand for 30 minutes in a dark place away from sunlight. Measure the absorbance using a UV-Vis spectrophotometer at the predetermined maximum wavelength.(9)

RESEARCH RESULT

a) Macroscopic and Microscopic Characteristics of Cumin Seeds

Macroscopic:

These seeds are small, oblong, and angular, measuring 2–3.5 mm in length and 1–2 mm in width. The surface of the cumin seeds is smooth with a slightly aromatic odor, but the interior of the seeds is oily and has a very bitter taste.

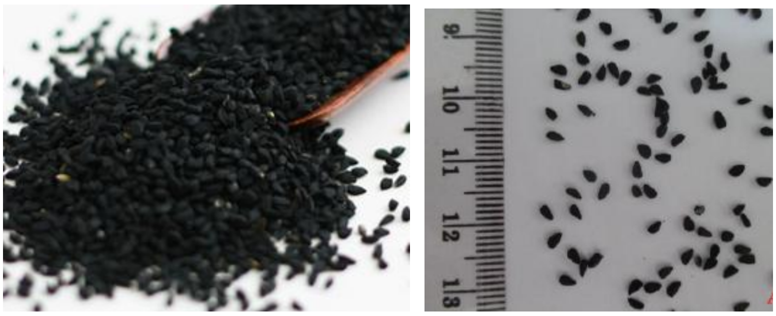
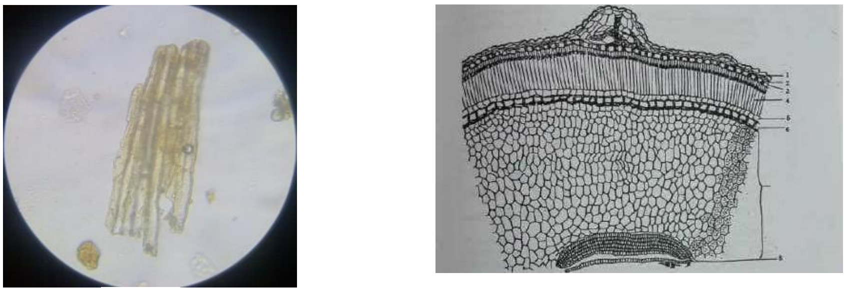


Figure 1. Black Cumin Seeds

Microscopic: Based on observations of black cumin seed powder under a microscope at 400x magnification, the following image is visible:



A B

Figure 2: Black cumin pollen cells viewed under a microscope at 400x magnification (A) Black cumin powder cells, based on reference (B)

Keterangan :

1. Outer epidermis
2. Compressed parenchyma cell layer
3. A layer of cells containing prismatic crystals
4. Palisade cell layer
5. A layer of parenchyma cells
6. Endodermis visible tangentially
7. Endosperm
8. Embry

b) Extraction Result of Black Cumin *(Nigella sativa L.)*

Table 1. Yield (%) of 96% Ethanol Extracts by Soxhlet Extraction and 96% Ethanol Extracts by Sonication

Extraction Method	Replication	Initial Weight	Extract Weight	Yield	Average	SD
Soxhlet extraction (96% ethanol)	1	400,0540	42.2018	10.55%	10.55%	0.001
	2	401,0042	42.5003	10.60%		
	3	400,8404	42.0450	10.49%		
Sonication (96% ethanol)	1	400,9840	31.3780	7.83%	7.80%	0.001
	2	401,0044	31.5800	7.88%		
	3	400,7720	30.8560	7.70%		

c) FTIR (*Fourier Transform Infrared*) Analysis Results

Based on the FTIR analysis results at wavelengths of 4000–650 nm, the following wavelength spectrum was obtained.

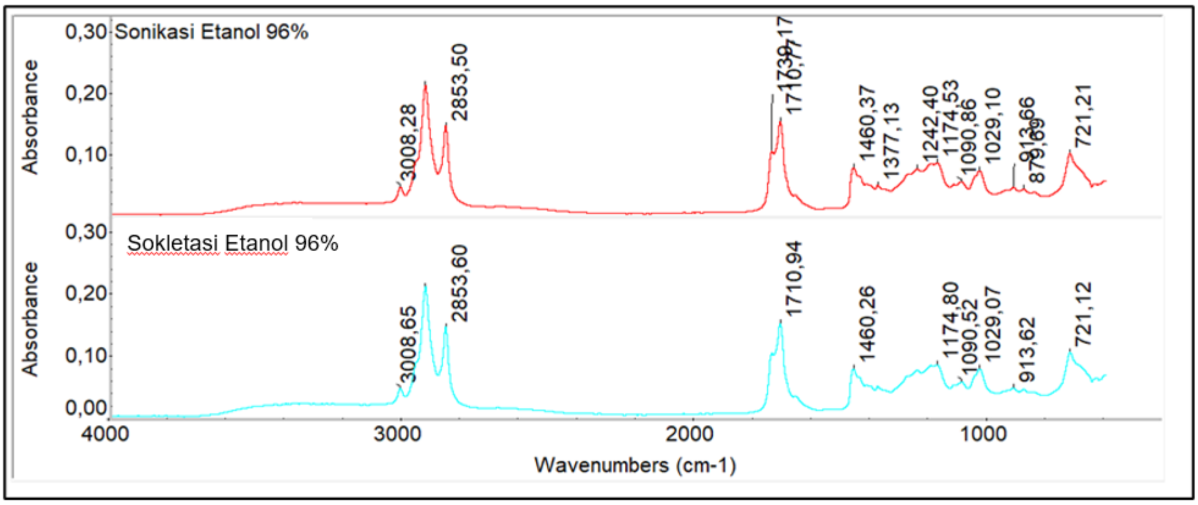


Figure 3. FTIR spectrum of a black cumin oil sample in 96% ethanol, at wavelengths of 4000–650 nm.

Table 2. Interpretation of the 400–3600 nm wavelength spectrum for black cumin oil

Wavenumber (cm^{-1})	**Literatur Wavenumber (cm^{-1})**	**Functional group /vibration**	**Interpretation**	**Reference**
3008.28	3006–3010	=C–H stretching	C–H stretching in a cis double bond indicates the possible presence of an unsaturated structure. In lipids/oils, this band is associated with unsaturated fatty acids.	(10)
2922.96	2920–2924	C–H stretching asimetris, CH_2	Asymmetric stretching of the methylene group. Commonly found in hydrocarbon/aliphatic chains and fatty acid chains.	(11)
2853.50	2850–2854	C–H stretching simetris, CH_2	Symmetric CH_2 stretching in aliphatic chains. Together with the band at 2923 cm^{-1}, this indicates the presence of long hydrocarbon chains.	(12)
1739.17	1740–1750	C=O stretching	A strong carbonyl band. If the sample contains lipids or oils, this region is highly characteristic of triglyceride ester carbonyls..	(13)
1710.17	±1700–1720	C=O stretching	With regard to carbonyl groups, including free fatty acids and ketones, the results depend on the sample matrix. A band around 1719–1700 cm^{-1} has been reported in oil as a carbonyl group associated with ketones and free fatty acids..	(12)
1460.37	1458–1466	CH_2/CH_3 bending	Scissoring vibrations in the aliphatic CH_2 and/or CH_3 groups.	(12)
1377.13	1376–1380	CH_3 bending simetris	Symmetric deformation/bending of the methyl group. This band is found in	(14)

Wavenumber (cm^{-1})	Literatur Wavenumber (cm^{-1})	Functional group /vibration	Interpretation	Reference
			both aliphatic structures and the ethanol spectrum.	
1242.40	1236–1244	C–O stretching / CH_2 bending	The lipid matrix may be associated with C–O ester vibrations and CH_2 bending.	(12)
1174.53	±1160–1180	C–O stretching	The fingerprint region may be associated with C–O vibrations, particularly in esters/alcohols, depending on the sample composition..	(13)
1090.86	1088–1098	C–O stretching	Closely related to C–O vibrations. Ethanol is reported to have a band around 1088–1091 cm^{-1}.	(15)
1029.10	±1000–1050	C–O stretching	Characteristic C–O stretching band of alcohols. Ethanol has a strong C–O band around 1045–1051 cm^{-1}	(15)
983.66	±965–990	=C–H bending, out-of-plane	Deformation region outside the plane of the olefinic bond; interpretation requires caution because it lies in the fingerprint region.	(12)
879.98	±870–890	C–C/C–O atau C–H vibration	The band around 880–882 cm^{-1} is one of the characteristic bands of ethanol and is associated with C–O/C–C vibrations.	(12)
721.21	720–723	$(CH_2)n$ rocking/cis =CH	The rocking vibration of CH_2 in long chains that can overlap with out-of-plane deformations in the cis olefinic structure.	(12)

d) Result of Phenolic Compound Identification

Table 3. Result of Compound Identification in Cumin Oil

Extraction Methode	Replication	Result	Standard	Interpretation
Soxhlet extraction (96% ethanol)	1	Purple to Bluish	Green, Red, Purple, Blue, Black (8)	Positive
	2	Purple to Bluish		Positive
	3	Purple to Bluish		Positive
Sonication (96% ethanol)	1	Purple to Bluish		Positive
	2	Purple to Bluish		Positive
	3	Purple to Bluish		Positive

e) Result of Phenol Concentration Determination Jinten oil was subjected to quantitative testing to determine its phenol content.

- Determination of the Maximum Wavelength
  The maximum wavelength was determined in the range of 725–770 nm. The measured maximum wavelength for gallic acid was 735 nm with an absorbance of 0.322.
- Determination of the Gallic Acid Standard Curve
  A gallic acid standard curve was prepared at several concentrations: 10, 20, 30, 40, and 50 ppm. The resulting linear regression equation for gallic acid was $y = 0.013x + 0.0179$ with an R^2 value of 0.996.

Tabel 4. Hasil penetapan kadar Fenol Pada Minyak Jinten*(Nigella sativa L.)*

Extraction Method	Replication	Phenolic Content (mg GAE/g)	Mean and SD
Soxhlet (96% ethanol)	1	42.531	42.678 ± 4.0934
	2	38.660	
	3	46.843	
Sonication (96% ethanol)	1	32.007	32.007 ± 0.077
	2	31.930	
	3	32.084	

f) Results of the DPPH Antioxidant Activity Test

The antioxidant activity test was conducted to determine the antioxidant potency of black cumin oil (Nigella sativa L.). Antioxidant activity analysis was performed using the DPPH method. Measurements of maximum wavelength absorption were taken across a range of 450–550 nm. The results showed a maximum wavelength at 518 nm with an absorbance of 0.632. Data on the determination of antioxidant activity in black cumin oil (*Nigella sativa L.*).

Table 5. Results of the antioxidant activity test of black cumin oil using the Soxhlet and sonication methods

Extraction Methode	Replication	IC_{50} (ppm)	Mean and SD
Soxhlet (96% ethanol)	1	42.531	42.678 ± 4.0934
	2	38.660	
	3	46.843	
Sonication (96% ethanol)	1	32.007	32.007 ± 0.077
	2	31.930	
	3	32.084	

DISCUSSION

a. Macroscopic and Microscopic Characterization of Black Cumin Seeds

Preliminary characterization of materials is a crucial step in medicinal plant research because it is used to confirm the identity and quality of the materials to be extracted. Based on macroscopic observations, the black cumin seeds (Nigella sativa L.) used were approximately 2–3.5 mm long and 1–2 mm wide, oblong and angular in shape, black in color, with a relatively smooth surface, an aromatic scent, an oily interior, and a bitter taste. These characteristics are consistent with the general characteristics of N. sativa seeds.

Microscopic observation at 400× magnification revealed anatomical structures including an outer epidermis, a compact parenchymatous layer, a layer containing prismatic crystals, a palisade layer, parenchyma, endodermis, endosperm, and an embryo. The presence of these identifying features confirms the identity of the crude drug used in this study. Microscopic characterization is important because it helps ensure the authenticity of the material, especially when the material has undergone a grinding process, making its macroscopic characteristics more difficult to observe. Phytochemically, N. sativa is known to contain various groups of secondary metabolites, including flavonoids, phenolic compounds, terpenoids, saponins, and major bioactive components such as thymoquinone. These compounds are associated with various biological activities, particularly antioxidant activity. Recent studies indicate that the antioxidant activity of N. sativa is related to a combination of various bioactive components, not just a single compound (Aslani dkk., 2024; Dar dkk., 2024). Thus, the results of the macroscopic and microscopic characterization in this study provide a sufficient basis for proceeding with the extraction and phytochemical analysis processes.

b. Extract Yield Based on Soxhlet and Sonication Methods

The results show that the 96% ethanol extract obtained via the Soxhlet method yielded an average extraction yield of 10.55%, whereas the sonication method yielded an average extraction yield of 7.80%. Thus, under the experimental conditions used, the Soxhlet method produced a higher extraction yield compared to sonication. The extraction yields for each method were also relatively consistent across replicates. The extraction yields for Soxhlet extraction ranged from 10.49% to 10.60%, while those for sonication ranged from 7.70% to 7.88%.

Yield is a parameter that describes the total amount of extract successfully obtained from a given amount of starting material, but it does not directly indicate the high content of specific bioactive compounds. Yield can be influenced by the type of solvent, solvent polarity, solid-to-solvent ratio, particle size, extraction time, temperature, and plant matrix characteristics.

In this study, the difference in yield is likely related to the difference in contact time between the material and the solvent. Soxhlet extraction was performed for 3 × 24 hours, whereas sonication was performed for 60 minutes at a frequency of 60 kHz and a temperature of 40°C. The longer contact time during Soxhlet extraction allows more time for the diffusion of compounds from the seed matrix into the solvent. However, theoretically, sonication can accelerate the extraction process through the phenomenon of acoustic cavitation.

Ultrasonic waves cause the formation of microbubbles that form and collapse, thereby increasing the penetration of the solvent into plant tissue and accelerating mass transfer. Therefore, sonication does not always result in lower yields. Its effectiveness depends heavily on a combination of time, temperature, solvent concentration, solid-to-solvent ratio, and ultrasonic frequency and intensity (Gueffai dkk., 2022; Dar dkk., 2024). Based on the study(6), for example, it was reported that the extraction of phenolic compounds from N. sativa using ultrasound-assisted extraction (UAE) yielded optimal results at an ethanol concentration of approximately 59.1%, a temperature of 44.6°C, and a time of 32.5 minutes. These conditions resulted in an increase in total phenolic content and DPPH radical scavenging activity compared to conventional extraction.

The differences between the results of this study and that study may be due to differences in the extraction targets, ethanol concentration, materials used, solid-to-solvent ratio, and ultrasonic parameters. This study used 96% ethanol, whereas the optimal conditions reported by Gueffai et al. (2022) utilized approximately 59% ethanol (6). Hal ini menunjukkan bahwa penggunaan etanol dengan konsentrasi lebih tinggi tidak his suggests that using ethanol with a higher concentration does not always result in better extraction of polar compounds. Dar et al. (2024) also demonstrated that ultrasonic extraction parameters significantly influence the yield and antioxidant activity of N. sativa oil. The optimal conditions obtained yielded a recovery of approximately 34.53%(5). The study showed that temperature, time, and the amount of solvent are important factors in determining the success of UAE.

Thus, the results of this study indicate that Soxhlet extraction is more effective at producing extract yield under the applied conditions; however, it cannot yet be concluded that Soxhlet extraction is generally a better method than sonication. To determine the best method, yield must be considered alongside phenolic content, tocopherol content, and antioxidant activity.

c. FTIR Spectral Characterization

FTIR analysis was used to obtain the chemical fingerprint of the extracts and to identify functional groups based on absorption wavenumbers. In this study, spectra were obtained using a Nicolet 6700 FTIR in the 4,000–650 cm⁻¹ range with 32 scans and a resolution of 8 cm⁻¹.

The spectra of the Soxhlet and sonicated extracts showed relatively similar absorption patterns. Several major bands identified were 3008.28; 2922.96; 2853.50; 1739.17; 1710.17; 1460.37; 1377.13; 1242.40; 1174.53; 1090.86; 1029.10; 983.66; 879.98; and 721.21 cm^{-1}. The band at 3008.28 cm^{-1} is associated with the =C–H stretching vibration in the cis double bond. These bands indicate the possible presence of unsaturated structures, particularly those associated with unsaturated fatty acids. Furthermore, the bands at 2922.96 and 2853.50 cm^{-1} correspond to the asymmetric and symmetric stretching of the CH_2 group, respectively. Both bands are characteristic of aliphatic hydrocarbon chains, which are abundant in lipid components.

These results are consistent with the spectral characteristics of N. sativa oil reported in FTIR studies, namely bands around 2923 and 2850 cm^{-1} associated with C–H stretching, a band around 1744 cm^{-1} associated with C=O stretching, and a band around 721 cm^{-1} associated with the rocking of the methylene group(17). A strong band at 1739.17 cm^{-1} indicates C=O carbonyl stretching vibrations and can be attributed to ester groups in triglycerides or lipid components. Meanwhile, the band at 1710.17 cm^{-1} may be associated with carbonyl groups in free fatty acids or other carbonyl compounds. These bands must be interpreted with caution because their positions and intensities can be influenced by the matrix composition. The bands at 1460.37 and 1377.13 cm^{-1} are associated with CH_2/CH_3 bending vibrations, while the bands in the 1242–1029 cm^{-1} region are associated with C–O vibrations and fall within the fingerprint region. The band at 721.21 cm^{-1} indicates the CH_2 rocking vibration in long aliphatic chains and may be associated with the cis structure in fatty acids. The similarity in FTIR profiles between the Soxhlet and sonication extracts indicates that both methods produce extracts with relatively similar major functional groups. Differences in extraction methods are more likely to affect the relative amounts of compounds successfully extracted rather than causing significant changes to the chemical structure of the major compound groups. FTIR can therefore be used as a fingerprinting method to quickly compare the characteristics of extracts.

d. Qualitative Identification of Phenolic Compounds

Test results using 1% $FeCl_3$ showed a bluish-purple color change in all replicates of both the Soxhlet and sonication extracts. Based on the testing criteria used, both extracts were determined to be positive for phenolic compounds. The reaction of $FeCl_3$ with phenolic compounds occurs due to the formation of a complex between Fe^{3+} ions and phenolic hydroxyl groups, resulting in a color change. The positive results indicate that phenolic components are present in both extracts; however, this test is qualitative and cannot specifically determine the types or concentrations of the phenolic compounds. The presence of phenolic compounds in N. sativa has been reported in recent studies. Based on the study by Saleh *et al.* (2023) N. sativa seeds were found to contain total phenolics, flavonoids, and tannins, as well as the ability to scavenge DPPH free radicals. The study also showed that phytochemical content can vary depending on the source of the material and extraction conditions.

Therefore, the positive $FeCl_3$ results in this study can serve as supporting evidence for the quantitative results of total phenolic content but are insufficient to identify individual phenolic compounds.

e. Total Phenolic Content

According to the latest report, the total phenolic content of the Soxhlet extract was 32.007 ± 0.077 mg GAE/g, while that of the sonicated extract was 33.732 ± 0.171 mg GAE/g. Thus, the phenolic content of the sonicated extract was approximately 5.4% higher than that of the Soxhlet extract. These results indicate that although Soxhlet extraction yields a higher total yield, sonication produces a slightly higher phenolic content. This finding is significant because it shows that yield is not always directly proportional to the concentration of bioactive compounds. Yield indicates the total amount of extract obtained, whereas phenolic content indicates the proportion of phenolic compounds in the extract. Thus, the Soxhlet extraction may produce more extract material, but the sonicated extract may have a slightly higher phenolic concentration.

The advantage of sonication over conventional methods for extracting phenolic compounds can be explained by the phenomenon of cavitation. Ultrasonic waves can increase cell wall disruption or enhance cell membrane permeability, allowing the solvent to more easily reach intracellular components. Gueffai *et al*. (2022) reported that optimizing UAE of N. sativa seeds increased total phenolic content by approximately 70% compared to conventional extraction methods(6). hese results reinforce the notion that sonication has the potential to enhance the release of phenolic compounds when extraction parameters are at optimal conditions.

However, the phenolic content obtained via sonication in this study was only slightly higher than that from Soxhlet extraction. This is likely due to the use of 96% ethanol and sonication conditions of 60 kHz for 60 minutes at 40°C, which may not necessarily represent the optimal conditions for phenolic extraction. A study by Gueffai et al. (2022) dentified optimal conditions using an ethanol concentration of approximately 59.1%, a temperature of 44.6°C, and a time of 32.5 minutes(6). This difference indicates that ethanol concentration is a critical factor in the extraction process.

Furthermore, the study by Muzolf-Panek dan Gliszczyńska-Świgło (2022) showed that solvent concentration, time, and temperature affect the content of antioxidant compounds in N. sativa seeds. This supports the interpretation that the differences in phenolic content between Soxhlet extraction and sonication in this study are likely the result of the interaction of various extraction parameters, rather than the influence of a single extraction method alone(18).

The gallic acid standard curve in this study was prepared at concentrations of 10–50 ppm and yielded the regression equation $y = 0.013x + 0.0179$ with $R^2 = 0.996$. An R^2 value close to 1 indicates an excellent linear relationship between gallic acid concentration and absorbance within the concentration range used.

Using gallic acid as the standard yields results in units of mg GAE/g, allowing the results to be compared with other studies that use the Folin–Ciocalteu method. However, the Folin–Ciocalteu method essentially measures the total reducing capacity of the sample and is not entirely specific to all phenolic compounds. Therefore, the results obtained are more accurately referred to as total phenolic content (TPC) based on gallic acid equivalents.

f. Antioxidant Activity Using the DPPH Method

This study also planned to test antioxidant activity using the DPPH method. Measurement of the maximum wavelength yielded 518 nm with an absorbance of 0.632. The DPPH method was used to evaluate the extract's ability to scavenge free radicals through changes in the absorbance of the DPPH solution after reacting with antioxidant compounds.

Secara teoritis, semakin besar kemampuan ekstrak mereduksi radikal DPPH, semakin Theoretically, the greater the extract's ability to reduce DPPH radicals, the greater the decrease in absorbance. A commonly used parameter is IC_{50}, which is the concentration of the extract required to produce 50% inhibition of the radicals. The lower the IC_{50} value, the stronger the antioxidant activity. N. sativa is known to possess antioxidant activity associated with various bioactive compounds, including thymoquinone, phenolic compounds, and other components. A comprehensive review(16) indicates that N. sativa extracts and oils exhibit antioxidant activity in various experimental models and that thymoquinone is one of the key components contributing to this activity.

However, the data in the DPPH table in the latest report need to be reexamined before the IC_{50} values can be discussed quantitatively. In that section, the table is titled "DPPH Activity" and includes an "IC_{50}" column, but the listed numbers are identical to the phenolic content data in the previous section, namely 42.531; 38.660; 46.843 for Soxhlet extraction and 32.007; 31.930; 32.084 for sonication. Therefore, it is premature to conclude that these values represent DPPH IC_{50}. If these figures are indeed DPPH results, verification is needed regarding the absorbance data, inhibition percentages, the regression equation relating concentration to % inhibition, and the IC_{50} calculations. This is important to ensure that the discussion of antioxidant activity does not lead to erroneous interpretations.

Research on N. sativa seeds indicates a relationship between polyphenol content and antioxidant capacity; however, variations in raw material sources can lead to significant differences in phenolic content and antioxidant activity (6)

CONCLUSION

Overall, the study shows that the N. sativa seeds used possess macroscopic and microscopic characteristics that support the identity of the crude drug. Soxhlet extraction yielded a higher recovery rate compared to sonication, at 10.55% and 7.80%, respectively. Conversely, sonication yielded a slightly higher total phenolic content compared to Soxhlet extraction, namely 33.732 ± 0.171 mg GAE/g versus 32.007 ± 0.077 mg GAE/g.

The FTIR spectra of both extracts showed relatively similar functional group patterns, particularly aliphatic C–H, C=O, CH_2/CH_3, and C–O groups. The $FeCl_3$ test results also indicated the presence of phenolic compounds in both extracts. These results suggest that the sonication method has the potential to enhance the extraction of phenolic compounds, although it did not increase the total extract yield under the experimental conditions used.

Thus, it cannot yet be concluded that one method is the best overall, as the success parameters yielded different results. Soxhlet extraction outperformed sonication in terms of yield, while sonication outperformed Soxhlet extraction in terms of phenolic content. Determining the optimal method requires consideration of the tocopherol content and DPPH antioxidant activity after these data have been verified.

REFERENCES

I. Silva AFC, Haris PI, Serralheiro ML, Pacheco R. Mechanism of action and the biological activities of Nigella sativa oil components. Food Biosci. 2020;38(October).

II. Handayani F, Apriliana A, Natalia H. Karakterisasi Dan Skrining Fitokimia Simplisia Daun Selutui Puka (Tabernaemontana macracarpa Jack). J Ilm Ibnu Sina Ilmu Farm dan Kesehat. 2019;4(1):49–58.

III. Al-Naqeep G, Ismail M, Yazan LS. Effects of thymoquinone rich fraction and thymoquinone on plasma lipoprotein levels and hepatic low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase genes expression. J Funct Foods [Internet]. 2009;1(3):298–303. Available from: http://dx.doi.org/10.1016/j.jff.2009.06.003

IV. Rohman A, Ariani R. Authentication of nigella sativa seed oil in binary and ternary mixtures with corn oil and soybean oil using FTIR spectroscopy coupled with partial least square. Sci World J. 2013;2013.

V. Dar IH, Junaid PM, Ahmad S, Shams R, Dash KK, Shaikh AM, et al. Optimization of ultrasound-assisted extraction of Nigella sativa seed oil for enhancement of yield and antioxidant activity. Discov Appl Sci [Internet]. 2024;6(3). Available from: https://doi.org/10.1007/s42452-024-05714-7

VI. Gueffai A, Gonzalez-Serrano DJ, Christodoulou MC, Orellana-Palacios JC, Ortega MLS, Ouldmoumna A, et al. Phenolics from Defatted Black Cumin Seeds (Nigella sativa L.): Ultrasound-Assisted Extraction Optimization, Comparison, and Antioxidant Activity. Biomolecules. 2022;12(9).

VII. Triyanti SB, Lestari FP, Fitriana PAN, Rostiana HR, Silalahi DD, Syalsabina TD, et al. Pengaruh Metode Ekstraksi Maserasi, Sonikasi, dan Sokletasi Terhadap Nilai Rendemen Sampel Kulit Buah Naga (Hylocereus polyrhizus). J Sains dan Edukasi Sains. 2025;8(1):71–8.

VIII. Sugiarna R, Farhan N, Rusdi M, Arsul MI. Total Phenolic and Flavonoid Content of Grapevine (Vitis vinifera L) Leaves Ethanol Extract. ad-Dawaa'JPharmSci. 2019;2(2):97–8.

IX. Angelia A, Putri GR, Shabrina A, Ekawati N. Formulasi Sediaan Spray Gel Ekstrak Kulit Jeruk Manis (Citrus Sinensis L.) sebagai Anti-Aging. Generics J Res Pharm. 2022;2(1):44–53.

X. Ferreira MCM, Macedo dos Santos P, Rampazzo V, Bona E, Sanchez JL, Maciel GM, et al. Common Components and Specific Weights Analysis for The Discrimination and Evaluation of Vegetable Oil Quality. Int J Food Sci Technol. 2017;52(9):1995–2005.

XI. Tsai YH, Chiang D, Li YT, Perng TP, Lee S. Thermal Degradation of Vegetable Oils. Foods. 2023;12(9):1–14.

XII. Szmatoła M, Chrobak J, Grabowski R, Iłowska J, Woch J, Szwach I, et al. Spectroscopic Methods in The Evaluation of Modified Vegetable Base Oils From Crambe Abyssinica. Molecules. 2018;23(12).

XIII. Barbosa N, Santos L, Seguchi L, Silva B, Carvalho T, Wanderley E, et al. Avaliação Rápida Da Qualidade Do Óleo Vegetal Isolante De Transformador Por Espectroscopia Ftir E Quimiometria. Quim Nova. 2022;45(8):994–1003.

XIV. Golub P, Doroshenko I, Pogorelov V, Sablinskas V, Balevicius V, Ceponkus J. Temperature Evolution of Cluster Structures in Ethanol. Dataset Pap Sci. 2013;2013(1):10–3.

XV. Zhou Y, Gu Y, Guo R, Jiao L, Wang K, Zhu Q, et al. In Situ Detection of Fruit Spoilage Based on Volatile Compounds Using The Mid-Infrared Fiber-Optic Evanescent Wave spectroscopy. Front Plant Sci. 2022;13(October):1–12.

XVI. Aslani MR, Saadat S, Boskabady MH. Comprehensive and updated review on anti-oxidant effects of Nigella sativa and its constituent, thymoquinone, in various disorders. Iran J Basic Med Sci. 2024;27(8):923–51.

XVII. Elmosalami TA, Kamel MM, Tomashchuk I, Alzaid M, Mostafa M. Characterization and Modeling

Quality Analysis of Edible Oils Using Electrochemical Impedance Spectroscopy. Int J Food Sci. 2022;2022.

XVIII. Muzolf-Panek M, Gliszczyńska-Świgło A. Extraction optimization for the antioxidants from Nigella sativa seeds using response surface methodology. J Food Meas Charact. 2022;16(6):4741–53.