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Analisis Senyawa Bioaktif Ekstrak N-Heksana Jintan Hitam (Nigella sativa) Menggunakan Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis of Bioactive Compounds in the n-Hexane Extract of Black Cumin (Nigella sativa) Using Gas Chromatography–Mass Spectrometry (GC–MS)

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Abstrak

Tumbuhan telah memainkan peran penting dalam pengobatan tradisional dan modern di seluruh dunia, dengan bukti historis yang mendukung aplikasi terapeutiknya. Jintan hitam (Nigella sativa) merupakan salah satu tanaman obat tradisional yang paling banyak digunakan. Ekstrak n-heksana jintan hitam memiliki kandungan senyawa metabolit potensial yang dapat berperan sebagai antioksidan. Tujuan penelitian ini untuk mengetahui senyawa bioaktif yang terkandung dalam ekstrak n-heksana jintan hitam. Metode penelitian ini biji jintan hitam (3 kg) dihaluskan dan diekstraksi menggunakan metode maserasi dengan pelarut n-heksana (1:6), setiap 1x24 jam disaring selama 72 jam hasil filtrat dipekatkan menggunakan evaporator hingga diperoleh ekstrak kental. Ekstrak n-heksana jintan hitam dilakukan identifikasi senyawa bioaktif yang terkandung dalam ekstrak n-heksana jintan hitam dengan analisis Chromatography-Mass Spectrometry (GC-MS). Hasil penelitian analisis GC-MS ekstrak n-heksana jintan hitam mengidentifikasi 13 senyawa bioaktif. Terdapat tiga senyawa dominan yaitu asam 9,12-Octadecadienoic (ZZ) (65.96 %), asam n-Hexadecanoic (14.59%) dan Octadecanoic acid (9.10 %). Kesimpulan bahwa ekstrak n-heksana jintan hitam mengandung berbagai senyawa kimia yang bermanfaat srbagai antioksidan, hipokolesteromik, antiandrogenik, dan hemolitik.

Kata Kunci:
Jintan hitam; Ekstrak n-heksana; Senyawa bioaktif; GC-MS; Antioksidan
Keywords:
Black cumin; n-hexane extract; Bioactive compounds; GC–MS; Antioxidant

Abstract

Plants have played an important role in traditional and modern medicine worldwide, with historical evidence supporting their therapeutic applications. Black cumin (Nigella sativa) is one of the most widely used traditional medicinal plants. The n-hexane extract of black cumin contains potential metabolite compounds that may act as antioxidants. The aim of this study was to identify the bioactive compounds contained in the n-hexane extract of black cumin. In this study, black cumin seeds (3 kg) were ground and extracted using the maceration method with n-hexane as the solvent (1:6). The mixture was filtered every 24 hours over a period of 72 hours, and the resulting filtrate was concentrated using an evaporator to obtain a viscous extract. The n-hexane extract of black cumin was then analyzed to identify its bioactive compounds using Gas Chromatography–Mass Spectrometry (GC–MS). The GC–MS analysis of the n-hexane extract of black cumin identified 13 bioactive compounds. Three dominant compounds were 9,12-Octadecadienoic acid (Z,Z) (65.96%), n-hexadecanoic acid (14.59%), and octadecanoic acid (9.10%). In conclusion, the n-hexane extract of black cumin contains various chemical compounds that are beneficial as antioxidants, hypocholesterolemic agents, antiandrogenic agents, and hemolytic agents.

INTRODUCTION

Black cumin (Nigella sativa), commonly known as black seed, belongs to the Ranunculaceae family and grows in southern Europe, North Africa, the Middle East, and Western Asia (Mahmoudvand et al., 2014). Nigella sativa, known as black cumin, is one of the traditional medicinal plants widely used to maintain health and to treat various diseases (Darakhshan et al., 2015). Black cumin contains amino acids, saturated and unsaturated fatty acids (31–35.5%), tannins, minerals (phosphate, calcium, and zinc), alkaloids, carbohydrates (33–34%), and vitamins (folic acid, thiamine, ascorbic acid, and niacin) (Forouzanfar F., 2014), which exhibit

anticancer, antiparasitic, antibacterial, and antioxidant effects (Ping et al., 2014). The antioxidant compounds present in black cumin are believed to have nephroprotective potential.

Bioactive compounds in black cumin can be obtained through an extraction process. The yield of extraction depends on the type of solvent used based on its polarity. Hexane is a nonpolar solvent capable of extracting nonpolar compounds and has several advantages, including volatility, stability, and selectivity (Constanty & Tukiran, 2021). A study by Tiji et al. (2021) reported that the hexane extract of black cumin analyzed using GC–MS contained fatty acid compounds such as linoleic acid, palmitic acid, hexadecanoic acid, and oleic acid, and showed an IC_{50} value of 23.25 mg/mL.

The analysis of bioactive compound content was conducted using the Gas Chromatography–Mass Spectrometry (GC–MS) method. GC–MS is a sample separation technique performed through gas chromatography followed by analysis using mass spectrometry. Gas chromatography is used to detect compounds that are volatile or easily evaporated under high vacuum and low pressure when heated. Meanwhile, mass spectrometry is used to determine molecular formulas, molecular weights, and to generate charged molecules (Hotmian et al., 2021).

The advantages of compound analysis using GC–MS include higher sensitivity toward volatile compounds compared to other methods, ease of operation, simpler maintenance, and lower cost when compared to LC–MS (Revanthi et al., 2015).

RESEARCH METHODS

Preparation of Black Cumin Extract

Three kilograms of black cumin seeds were ground using a blender until a fine powder was obtained and then sieved through a 60-mesh sieve. The sieved black cumin powder was macerated with n-hexane using a ratio of 1:6 (one part black cumin powder to six parts n-hexane) and soaked for 3 × 24 hours with occasional stirring. The solvent was replaced every 24 hours, after which the filtrate was filtered and collected in a beaker glass. All macerates were then concentrated by solvent evaporation using a rotary evaporator to obtain a viscous extract.

GC–MS Analysis

Identification of bioactive compounds was carried out at the laboratory of the National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia. The analysis was performed using Gas Chromatography–Mass Spectrometry (GC–MS).

A total of 1 mg of the n-hexane extract of black cumin was dissolved in 1 mL of chloroform. The GC–MS analysis employed a capillary column of Agilent 19091S-433 HP-5MS type with a phenyl methyl siloxane stationary phase, a maximum column temperature of 325°C, a length of 30 m, an internal diameter of 250 µm, and a film thickness of 0.25 µm. Helium was used as the carrier gas at a flow rate of 15 mL/min, with an injection volume of 1 µL. The column oven temperature was programmed and gradually increased at a rate of 40°C per minute up to 300°C.

The number of compounds present in the extract was indicated by the number of peaks in the chromatogram, while the identity of each compound was interpreted based on the mass spectral data of each peak using similarity matching with the GC–MS library database (Novitasari et al., 2017).

RESULTS AND DISCUSSION

RESULTS

The n-hexane extract of black cumin (Nigella sativa) seeds exhibited a characteristic yellowish-brown color. In terms of texture, the extract was viscous and had an oil-like appearance. These properties are consistent with the use of n-hexane as a nonpolar solvent, which selectively extracts nonpolar components, particularly fatty oils that constitute the major chemical components of black cumin seeds.

The GC–MS profile of the n-hexane extract of black cumin revealed the presence of several peaks (Figure 1). The chromatographic peaks were interpreted using a known component spectral database from the GC–MS library, and Table 2 lists the identified compounds along with their peak areas, retention times, molecular formulas, and molecular weights.

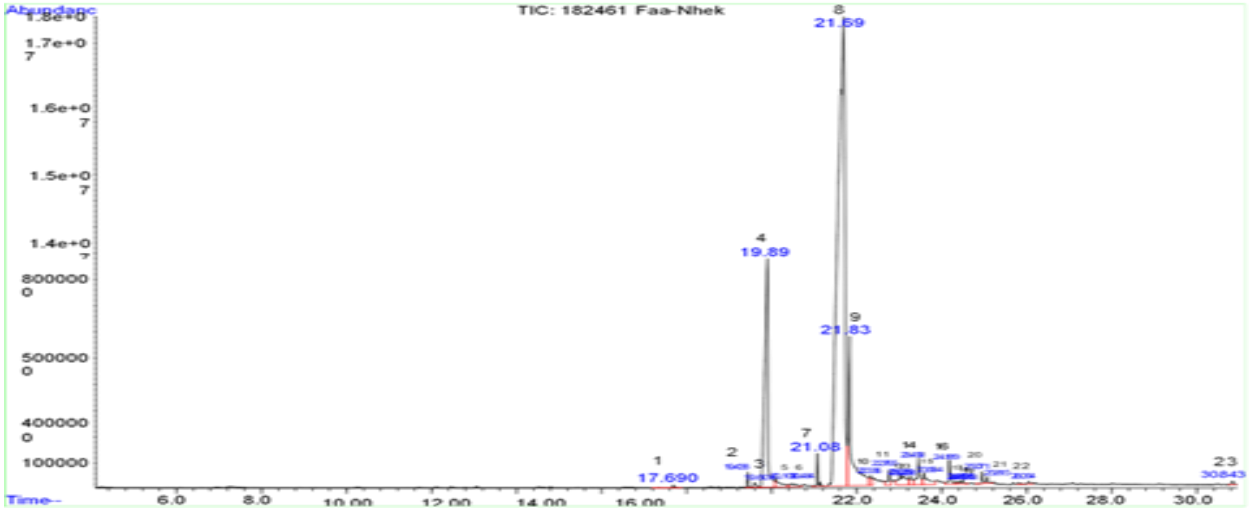

Figure 1. GC–MS chromatogram of the n-hexane extract of black cumin

The GC–MS chromatogram of the n-hexane extract of black cumin showed the presence of 23 peaks. The highest peak was peak number 8, followed by peak number 4 and peak number 9. Furthermore, each peak appearing in the GC–MS chromatogram was fragmented using mass spectrometry (MS), and the resulting fragmentation spectra were compared with the NIST20.L database. Based on a literature review, compounds with potential biological activity are presented in Table 2.

Table 2. Bioactive Components Identified in the n-Hexane Extract of Black Cumin

No.	Retention Time (min)	Compound	Molecular Formula	Compound Class	Molecular Weight (g/mol)	Peak Area (%)
1	17.686	Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid	228	0.09
2	19.438	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Methyl palmitate	270	0.33
3	19.601	Palmitoleic acid	$C_{16}H_{30}O_2$	Palmitoleic acid	254	0.26
4	19.891	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	256	14.59
5	20.105	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Palmitic acid ester	284.4	0.85
6	21.076	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	Linoleic acid ester	294	0.77
7	21.693	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid	280	65.96
8	21.832	Octadecanoic acid	$C_{18}H_{36}O_2$	Stearic acid	284.3	9.10
9	22.326	Linoelaidic acid	$C_{18}H_{32}O_2$	Linoleic acid	280.4	0.51
10	23.256	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	Unsaturated aldehyde	264.4	0.51
11	23.458	Eicosanoic acid	$C_{20}H_{40}O_2$	Arachidic acid	312	1.31
12	25.071	Docosanoic acid	$C_{22}H_{44}O_2$	Behenic acid	340	0.22
13	30.843	Campesterol	$C_{28}H_{48}O$	Phytosterol	400	0.15

Based on the GC–MS analysis results presented in Table 2, a total of 13 bioactive compounds were identified in the n-hexane extract of black cumin. These compounds consisted predominantly of unsaturated fatty acids, with linoleic acid (65.96%) as the major component, followed by stearic acid (9.10%), while the main saturated fatty acid was palmitic acid (14.59%). In addition, phytosterols, myristic acid, methyl palmitate, palmitoleic acid, palmitic acid ester, behenic acid, and unsaturated aldehydes were also detected. The levels of 9,12-octadecadienoic acid and n-hexadecanoic acid in black cumin observed in this study were higher than those reported by Hagr et al. (2018), who found 48.72% of 9,12-octadecadienoic acid (Z,Z) and 7.97% of n-hexadecanoic acid in black cumin extract.

n-Hexane is a nonpolar solvent; therefore, nonpolar compounds present in black cumin, such as steroids and fatty acids, can be efficiently extracted. The n-hexane extract of black cumin contains linoleic acid, which may serve as a precursor for antioxidant activity. A study by Li et al. (2022) reported that linoleic acid exhibited an IC_{50} value of 23.1 µg/mL. According to Liu et al. (2020), samples with high fatty acid content also tend to show strong antioxidant activity.

9,12-Octadecadienoic acid (Z,Z) was the major bioactive compound identified in the n-hexane extract of black cumin. This compound, also known as α-linoleic acid, possesses antioxidant properties and inhibitory effects on cancer cell proliferation (Yuan et al., 2021).

Another bioactive compound detected in the n-hexane extract of black cumin was n-hexadecanoic acid, which has been reported to exhibit antioxidant, hypocholesterolemic, antiandrogenic, hemolytic, and 5-alpha-reductase inhibitory activities (Oni et al., 2020). n-Hexadecanoic acid, also known as palmitic acid (PA), has been reported to have anti-inflammatory effects (Syed et al., 2022). Octadecanoic acid, or stearic acid, acts as an anti-inflammatory lipid. Stearic acid is the third most abundant fatty acid in human hepatocytes and is associated with several liver functions, including cholesterol metabolism and lipoprotein biogenesis (Goradel et al., 2016).

Minor compounds identified in the n-hexane extract of black cumin included 9,12-octadecenoic acid, methyl ester, which has considerable potential as an antioxidant, anticancer, and anti-inflammatory agent (Hagr et al., 2018). Tetradecanoic acid, also known as myristic acid, can contribute to fat accumulation in the body but may also have positive effects on cardiovascular health (Verruck et al., 2019).

Hexadecanoic acid (methyl ester) has been reported to reduce blood cholesterol levels, exhibit anti-inflammatory properties, and possess antioxidant activity (Nugraha et al., 2020). Palmitoleic acid functions as a lipokine capable of regulating various metabolic processes, including improving insulin sensitivity in muscle tissue, promoting β-cell proliferation, preventing endoplasmic reticulum stress, and enhancing lipogenic activity in adipocytes (Fauziah et al., 2022).

Hexadecanoic acid, ethyl ester, a derivative of palmitic acid ester, exhibits antioxidant, hemolytic, hypocholesterolemic, and antiandrogenic activities, and also functions as an antifungal and antibacterial agent (Sukarti et al., 2023). Linoelaidic acid shows effective anticancer activity against MCF-7 cells. Furthermore, linoelaidic acid significantly decreases reduced glutathione levels and increases oxidized glutathione levels in MCF-7 cells, indicating the induction of oxidative stress within the cells (Dutta et al., 2023).

9,17-Octadecadienal (Z) has demonstrated antimicrobial activity and acts as a selective inhibitor of human monoamine oxidase (hMAO) (Das et al., 2022; Krishnamoorthy & Subramaniam, 2014). Arachidic acid exhibits neuroprotective effects (Sandhir et al., 2021). Behenic acid has been reported to reduce basal blood glucose levels and improve insulin tolerance (Moreira

et al., 2017). Campesterol is a phytosterol compound that exhibits antidiabetic, cardiovascular protective, anticancer, and antimicrobial activities (Uttu et al., 2023).

A study by Modak et al. (2021) on compounds isolated from Drynaria quercifolia rhizome docked with COX-2 and TNF-α proteins showed that n-hexadecanoic acid and 9,12-octadecadienoic acid (Z,Z)-, methyl ester exhibited efficient binding affinity to inflammatory target markers, which may lead to downregulation of inflammatory responses. Recent studies on plant extracts and herbal products have revealed that plant-based herbal medicines can directly act on the transcription of specific genes responsible for the production of cytokines, cyclooxygenase, and other inflammatory biomarkers (Saleem et al., 2020).

Furthermore, a study by Phan et al. (2023) demonstrated that n-hexadecanoic acid and 9,12-octadecadienoic acid (Z,Z) from Eclipta prostrata L. extract complied with all five Lipinski's rules. These findings indicate that these compounds possess favorable drug-like properties and high potential for development as therapeutic agents.

CONCLUSION

Based on the results of this study, it can be concluded that the n-hexane extract of black cumin identified 13 bioactive compounds. Three dominant compounds were 9,12-octadecadienoic acid (Z,Z) (65.96%), n-hexadecanoic acid (14.59%), and octadecanoic acid (9.10%). These findings indicate that the n-hexane extract of black cumin exhibits antioxidant potential and may be utilized in pharmaceutical products to improve health standards. The presence of these bioactive compounds also validates the traditional use of black cumin seeds by practitioners for the treatment of various diseases.

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