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The Effect of Different Solvents on the Content of Black Cumin Seed Extract (*Nigella sativa*)

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ABSTRACT

Seeds of black cumin (*Nigella sativa L.*), also called "black cumin," are an herbal plant from the Ranunculaceae family. The objective of this study was to determine the yield of black cumin seed extract using 96% ethanol, N-hexane, and ethyl acetate as solvents, as well as to determine the results of phytochemical screening tests and the active ingredient content using GCMS. The stages of the research included the extraction process, phytochemical screening test, and GCMS. The study's findings on the yield of black cumin seed extract using the three solvents showed that the solvent N-Hexane produced the highest yield, at 28.09 percent. Based on phytochemical screening tests, black cumin seeds were positive for containing secondary metabolites such as alkaloids, saponins, phenolic flavonoids, triterpenoids, steroids, and glycoxides. Other potential compounds vizhexadecenoic acid, ethyl ester, Methyl (8e,11e)-8,11-octadecadienoate, 12-octadecadienoic acid (z,z)-, Grape seed oil.

***Keywords*:** Phytochemical screening; GCMS; Black cumin seeds

INTRODUCTION

Herbal medicine-based traditional medical systems continue to play a significant role in the current healthcare system. Because medicinal plants are thought to be natural products with fewer side effects and greater effectiveness than their synthetic counterparts, more people have come to accept them in recent decades. About 80% of people worldwide today receive their primary care from traditional medicine. The pharmacological composition of many herbal plants includes, fungicidal, virucidal, and bactericidal properties. They are used in food preservation and embalming, and they also have anti-inflammatory, antibacterial, spasmolytic, sedative, analgesic, and local anesthetic qualities. Plant phytoconstituents include tannins, alkaloids, glycosides, saponins, flavonoids, steroids, and terpenes, among others, have been shown to

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possess pharmacological qualities in a variety of plant species. At the moment.[1]

For more than a millennium, Nigella sativa, also referred to as black cumin or black caraway seeds, has been utilized as a spice and alternative medicine. Empirical and medicinal evidence for this plant has been gathered by researchers from the United States, Europe, Africa, and the Middle East. Numerous studies on black cumin oil and extract have advanced significantly throughout the past 20 years, both in vivo and in vitro. Numerous pharmacological effects, including as immune-stimulating, antihistamine, antidiabetic, anti-hypertensive, anti-inflammatory, and antibacterial properties, are known to be present in black cumin seed extract. [2] Several active chemicals are found in black cumin seeds (Nigella sativa), including p-cymene (7–15%), carvacrol (6–12%), thymoquinone (30–48%), thymohydroquinone, dithymo-quinone, 4-terpineol (2–7%), and t-anethole (1%–4%).[3]

The use of solvent is a component that influences the success of the extraction process. When choosing a solvent to use, there are two main factors to consider. This means that the solubility must be high and the solvent must not contain harmful or toxic substances. Solvents are an important part of the compound extraction process. The way the target compound is extracted from the starting material is strongly influenced by the polar nature of the solvent. Traditional methods for isolating plant extracts require large amounts of solvent, long periods of time, and low recovery rates. By choosing the right solvent, compound extraction can be optimized. [4,5]

The goal of this research was to identify the phytocomponents of extracts with 96% N-Hexane and Ethyl Acetate ethanol solvents from black cumin seeds (Nigella sativa) by applying GCMS and phytochemical screening techniques.

METHODS

This research is experimental laboratory. Plant identification is the first step in this research process. Next are the steps of extract yield, black cumin seed extract preparation, phytochemical screening, and GCMS testing.

Tools and materials

The equipments used in this research were laboratory glassware (Pyrex®), oven (Memmert), analytical scales (Mettler Toledo), Rotary evaporator (Buchi), and GCMSD (Agilent Technologies 7890).

The ingredients include: 15% hydrochloric acid (Merck), glacial acetic acid (Merck), N-Hexane (Merck 104367, ≥ 99.0%), ethyl acetate (Merck 109623, ≥ 99.5%), black cumin seeds (Tanibuni), 96% ethanol solvent (Merck 100971, 95.1 - 96.9%), Mayer's, Dregendorff's, and Bouchardat's reagents, 10% aluminum chloride (Merck), 5% sodium nitrite (Merck), 1% ferric (III) chloride (Merck), 1% natrium hydroxide (Merck), 1% hydrochloric acid (Merck), 1% acetic acid anhydride (Merck), and 1% sulfuric acid P (Merck).

Determination of black cumin seeds (*Nigella sativa)*

Determination of black cumin seeds (*Nigella sativa)* carried out at the Bogoriense Herbarium Laboratory, Directorate of Scientific Collection Management, BRIN Cibinong with

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letter number B-1626/IV/DI.05.07/6/2022. The results of the determination showed that the plants used were black cumin seeds (*Nigella sativa*). The purpose of plant determination is to ensure the correct identity of the plants used

Preparation of black cumin seed extract

The maceration process is used to extract the seeds of black cumin. The Spice and Medicinal Plants Research Center (BALITRO) produced the extract using ethyl acetate, 96% ethanol, and n-hexane as the solvent. A 1:5 ratio is used to soak black cumin seed powder in solvent (1000 grams of black cumin seed plants (*Nigella sativa*) powder: 5000 liters of solvent). Then, for two to three hours, a mixer is used to combine the solvent and black cumin seed powder. We let this mixture sit for a full day. To get a pure filtrate, this combination is then filtered using a filter. Next, the filtrate was heated to 50^{o}C and evaporated using a rotary evaporator. The solvent is drawn in and distilled until it evaporates in a rotary evaporator. After the solvent has completely evaporated.[6,7]

$$\% \text{ Yield} = \frac{\text{Extract Weight (final)}}{\text{Simplicia Weight (initial)}} \text{ x } 100\%$$

Phytochemical analysis

Qualitative analysis of phytochemical components was carried out using conventional analysis methods to determine whether there were secondary metabolites in the extract. The extract is mixed with standard qualitative analysis reagents for alkaloid, flavonoid, saponin, triterpenoid and phenol tests. This reagent functions to show secondary metabolite compounds in the extract by observing the test color results.[8,9]

Alkaloid test

The Bouchardat, Mayer, and Dragendorff reagents were employed in precipitation procedures to identify alkaloids. One milliliter of each reagent (Mayer, Dragendorff, and Bouchardat) was combined with three milliliters of AETT, and the mixture was allowed to sit for ten minutes. The test is deemed positive when the following precipitates appear: an orange one with Dragendorff's reagent, a yellowish-white one with Mayer's reagent, and a brown one with Bouchardat's reagent.[10]

Saponin test

To determine whether saponins are present, perform the foam test. In order to conduct this test, 5 mg of extract was diluted in 5 mL of distilled water, added to the test tube, and violently shaken for around 15 seconds. Plenty of saponins are present when stable foam (more than 1 cm high) forms and lasts for 15 minutes.[9]

Tannin test

After mixing 1 g of the ingredients with 100 ml of hot water, the mixture is filtered. Take 5 mL and add a few drops of 1% iron (III) chloride solution if the filtrate test is positive for tannin. The outcome is a shade of green or black.[6,11]

Phenol test

A total of 500 mg of sample was added with 2 mL of heated ethanol and filtered. The filtrate was evaporated and 2 mL of chloroform and 1 mL of water were added. If it doesn't foam, add 2 mL of 0.1 N HCl and 1 – 2 drops of FeCl3. If it is red then it is positive for phenol.[7]

Flavonoid test

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After mixing with distilled water, the sample was filtered as much as 2 g. Up to 5 ml of filtrate is used, add 1 ml of 10% aluminum chloride, 1 ml of 5% sodium nitrite, and 2 ml of 1 N sodium hydroxide through the tube wall. If a red or yellow-orange hue appears, then there are flavonoids.[12]

Glycoside test

Glycosides are present when 1 mL of the extract is added to concentrated sulfuric acid, glacial acetic acid, and FeCl3. A purple ring forms when this happens.[8]

Terpeneoid/Steroid test

A total of 500 mg of sample was added with 2 mL of heated ethanol and filtered. The filtrate was evaporated and 2 mL of chloroform and 1 mL of water were added. The chloroform layer was added with Lieberman Bouchardat's reagent, 10 drops of anhydrous acetic acid, and 2 drops of concentrated sulfuric acid. If a green-blue color is formed, it is positive for terpenes, if it is red, it is positive for steroids.[11,12]

GCMS analysis

The content of black cumin seed extract was examined using the Agilent Technologies 7890 Gas Chromatograph with Auto Sampler, 5975 Mass Selective Detector, and Chemstation data system. After 900 µL of methanol, ethyl acetate, and ethanol pro analysis were added to 100 µL of liquid black cumin seed extract, the mixture was vortexed and injected into the GCMSD apparatus. For GC–MS detection, an electron ionization device with an ionization energy of 70 eV was employed. The carrier gas was helium at a flow rate of 1.2 mL/minute. Temperatures of 250 and 300 0C were established for the injector and MS transfer line, respectively. The temperature of the column was kept between 80 and 280 °C, increasing by 20 °C per minute. The injection of a 5 ml sample.[9,13,14]

RESULTS AND DISCUSSION

Yield Analysis

There are variations in the temperature, kind of solvent, and extraction time while using the maceration process. To calculate the yield, the extract that is left over after the extraction procedure is weighed. Table 1 illustrates the variations in the yield data for black cumin seeds.

Table 1. Yield of black cumin seed extract with 96% Ethanol, N-hexane and Ethyl acetate solvents

Black Cumin Seed Extract	Yield (%)
Ethanol Solvent 96%	27.57
N-Hexane Solvent	28.09
Ethyl Acetate Solvent	25.81

Three distinct types of solvents yielded varying yields based on the extraction results. The bioactive components' solubility determines the yield amount. Table 1 shows that 96% ethanol solvent (27.57%), n-hexane solvent (28.09%), and ethyl acetate solvent (25.81%) were the yield values that the black cumin seed extract produced. Given that Table 1 indicates that the N-Hexane solvent produced the best yield, it is probable that the bioactive chemicals present in Nigella sativa (black cumin) seeds are more non-polar. This is because N-Hexane solvent is a non-polar solvent.

The use of N-Hexane solvent using the maceration method resulted in a higher average yield compared to 96% ethanol solvent and ethyl acetate solvent. The N-Hexane solvent has the

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ability to bind polar and non-polar compounds, which is thought to be the cause of the difference in yield results. The extract yield is said to be good if the value is more than 10%. [15] So it can be concluded that the yield of black cumin seed extract in this study is in accordance with the requirements, namely >10%. The yield results are also related to the active compounds of a sample, if the yield value is high then the active compound components contained in it are also high. [16]

Phytochemical Screening Test

Plant secondary metabolites with specific uses for humans are called phytochemical substances (Figure 1 and Table 2).

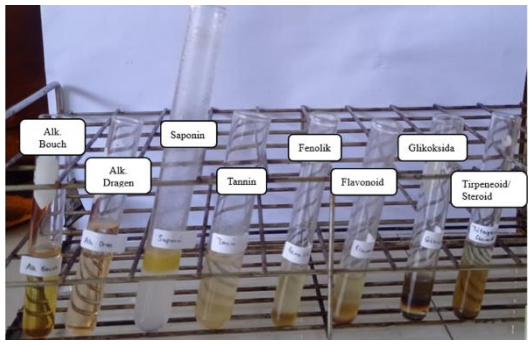
Table 2. Phytochemical screening test results for black cumin seed extract using 96% Ethanol, N-hexane and Ethyl acetate

Type of Testing/Inspection	Test/Inspection Results Black Cumin Seed Extract		
	Ethanol 96%	N-Hexane	Ethyl Acetate
Alkaloid	+	+	+
Saponin	+	+	+
Tannin	-	-	-
phenolic	+	+	+
Flavonoid	+	+	+
Triterpenoids	+	+	+
Steroid	-	-	+
Glycosides	+	+	+

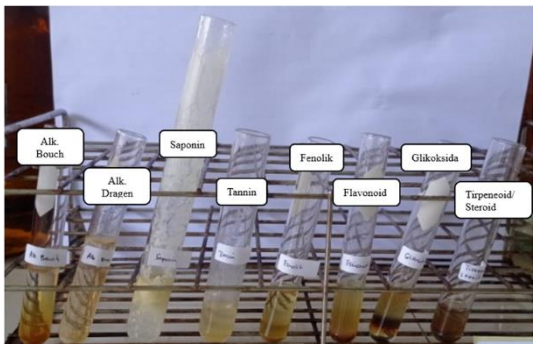
Note:
+: Positive contains secondary metabolites
-: Negative contains secondary metabolites

We identified eight different types of phytochemical compounds in this study that are hypothesized to be present in black cumin seed extract in order to identify these phytochemical compounds. Alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides are some of these phytochemical substances. (Figure 1).

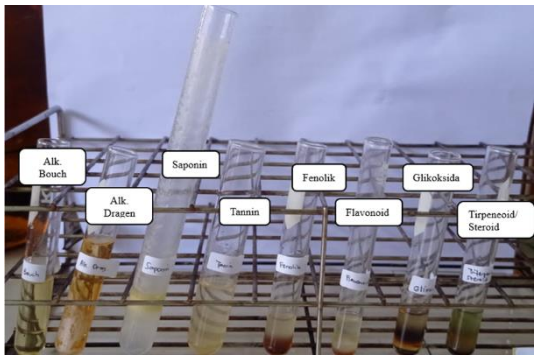
Table 2 shows that, for the 96% ethanol and N-Hexane solvent maceration methods, the phytochemical screening test results are identical and positive for containing alkaloids, saponins, phenolic flavonoids, triterpenoids, and glycoxides; on the other hand, the ethyl acetate solvent is positive for containing alkaloids, saponins, phenolic flavonoids, triterpenoids, steroids, and glycoxides.



(a)



(b)



(c)

Figure 1. Phytochemical screening test for black cumin seeds with 96% ethanol, n-hexane and ethyl acetate

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Phytochemicals are compounds originating from plants, this group also includes secondary metabolic chemicals. Tannins bind to proteins and carbohydrates, making feed unavailable for digestion.[17]

Alkaloids are natural chemical compounds that contain nitrogenous organic molecules. Because they contain nitrogen atoms, alkaloid compounds are classified as basic compounds. Because alkaloids are basic, which requires a solution containing an acid, the addition of a solution of hydrochloric acid and water is done to test the alkaloid compound. By adding Mayer, Bouchardat, and Dragendorf reagents, identification of alkaloid compounds produces yellow, brown, and brick red precipitates. This happens because nitrogen compounds bind to K+ ions in each reagent. The color change of this precipitate is caused by the change of metal ligands in the Mayer, Bouchardat, and Dragendorf reagents. Although alkaloids can function as antidiarrheal, antidiabetic, antimicrobial and antimalarial, some compounds in this group can be dangerous.[10,18]

Because saponin compounds are easily hydrolyzed in water and have physical features that enable foam to form when shaken, it is possible to identify them by looking for their hydrophilic and hydrophobic groups. The principle of the saponin test is the hydrolysis reaction of saponin compounds into aglycones, which are non-sugar compounds, and glycones, which are sugar compounds. The results of this test indicate that a stable foam is formed as a result of this reaction. By reducing surface tension, saponin can help stop fungal growth.[14,18]

In testing flavonoid compounds, the yellow layer of amyl alcohol was caused by the reduction of hydrogen gas after adding concentrated hydrochloric acid and magnesium powder to become the aglycone. The compound that undergoes reduction will then form a complex compound with magnesium which is yellow in color. Due to the hydroxyl groups contained in the structure of flavonoid compounds, they have the ability to function as antioxidants.[19] Phenol derivative compounds known as flavonoids have benefits for reducing cholesterol and lipids because they are antibacterial.[10] Phenolic compounds are known to be the most diverse class among bioactive secondary metabolites synthesized by medicinal plants. They are often used to fight pathogenic microbes.[18]

Terpenes can be categorized as essential or non-essential compounds depending on how important these metabolites are to plant survival. Essential compounds perform important functions in the basic and vital metabolic activities of plants, while non-essential compounds help plants in stressful conditions. Under normal conditions, these terpenes are not harmful to plants and do not show any phenotype if their biosynthetic pathways are disrupted. Monoterpenes are one of the many antimicrobial substances included in nonessential terpenes.[11] Triterpenoid chemicals aid in the body's organic synthesis and cell restoration processes, whereas steroid molecules have antibacterial, antifungal, anticancer, neurotoxic, and anti-inflammatory qualities. Through isolation and identification of their activities, these two bioactive compounds have antibacterial and

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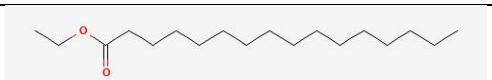
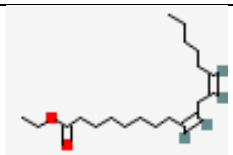
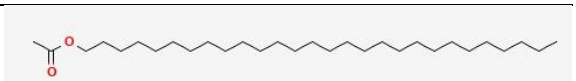
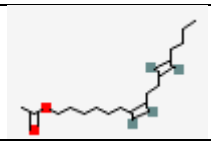
antioxidant properties. Triterpenoid compounds found in plants protect plants from insects and bacteria. Steroid compounds can interact with cell phospholipid membranes, which become impermeable to lipophilic compounds. This reduces the integrity of the membrane, changes its morphology, and can ultimately cause lysis and brittleness.[20]

Analisis GCMS

GCMS is a gas chromatography method based on separation in which heat-stable and volatile solutes move through a column at a certain speed. Analysis of this chemical content can be done using tools *Gas Chromatography-Mass Spectrometry* (GCMS). This analysis is carried out by comparing the mass of the GC separation results for each peak that appears in the chromatogram with the mass of the compounds in the data *Library Wiley*.[13] While gas chromatography (GC) will break down molecules into their constituent components and can be used to determine the purity of a compound, mass spectrometry (MS) will measure the molecular mass of each component that has been separated from the gas chromatography system. So you will get the molecular formula of the chemical components that make up essential oils.[21,22]

When it comes to determining the components of volatile chemicals, long chains, branched chain hydrocarbons, alcohols, acids, esters, etc. GCMS is one of the best methods available. Table 3, Table 4 and Table 5 display the phytochemicals found by GCMS analysis, which are associated with numerous biological activities that are pertinent to this work and are listed on Pubchem(https://pubchem.ncbi.nlm.nih.gov).

Table 3. Content of black cumin seed extract with 96% ethanol solvent

No	Compound name	Content (%)	Rt	Quality	Molecular Formula
1.	Hexadecenoic acid, ethyl ester	26.14	31.330	98	$C_{18}H_{36}O_2$
	Structure of chemical compounds				
2.	Ethyl (9Z, 12Z)-9,12-octadecadienoate	16.8	31.633	99	$C_{20}H_{36}O_2$
	Structure of chemical compounds				
3.	Octacosyl acetate	11.58	32.861	95	$C_{30}H_{60}O_2$
	Structure of chemical compounds				
4.	Z,E-7,11-hexadecadien-1-yl acetate	9.35	33.275	93	$C_{18}H_{32}O_2$
	Structure of chemical compounds				
5.	Ethanamine,2'2'-oxybis [n,n-dimethyl-	5.72	33.764	59	$C_8H_{20}N_2O$

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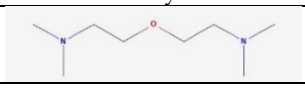
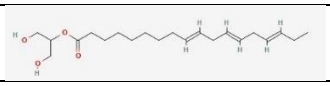
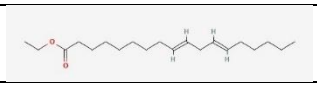
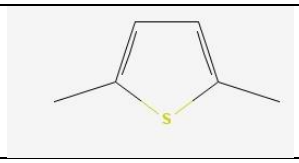
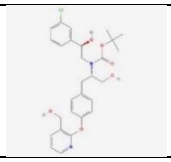
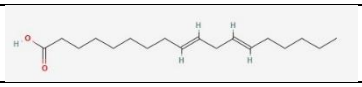
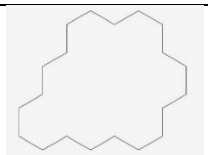
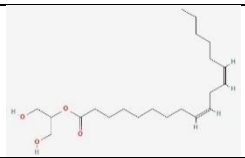
No	Compound name	Content (%)	Rt	Qualit y	Molecular Formula
	Structure of chemical compounds				
6.	2-hydroxy-1-(hydroxymethyl) ethyl (9e,12e)-9,12-octadecadienoate	8.70	36.040	91	$C_{21}H_{36}O_4$
	Structure of chemical compounds				
7.	9,12-octadecadienoic acid, ethyl ester	2.21	32.606	99	$C_{20}H_{36}O_2$
	Structure of chemical compounds				
8.	2,5-dimethyl-thiophene-3-sulfonic acid (3-methoxy-phenyl)-amide	2.70	33.585	91	C_6H_8S
	Structure of chemical compounds				
9.	Hexadecenoic acid, 2-hydroxy-l- (hydroxymethyl) ethyl ester	3.43	34.199	91	$C_{28}H_{33}CLN_2O_6$
	Structure of chemical compounds				
10.	(9e,12e)-9,12-octadecadienoic acid	1.36	34.398	99	$C_{18}H_{32}O_2$
	Structure of chemical compounds				
11.	Cycloicosane	2.34	34.529	56	$C_{20}H_{40}$
	Structure of chemical compounds				
12.	Ethyl (9z, 12z)-9-12-octadecadienoate	1.44	32.433	99	$C_{21}H_{38}O_4$
	Structure of chemical compounds				
13.	14-.beta.-h-pregna	1.98	33.137	99	
14.	Grape seed oil	1.32	35.074	95	

Based on the data in table 3, it can be seen that the main compounds in black cumin seed extract with 96% ethanol solvent are Hexadecenoic acid, ethyl ester (26.14%), Ethyl (9Z, 12Z)-9,12-octadecadienoate (16.8%), Octacosyl acetate (11.58%), Z,E-7,11-hexadecadien-1-yl acetate (9.35%) And

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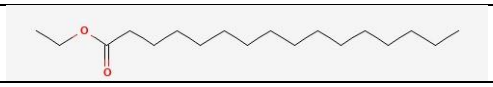
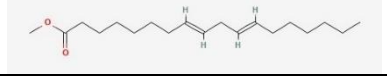
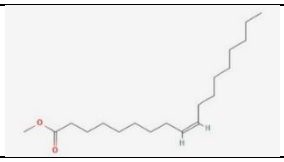
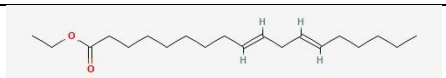
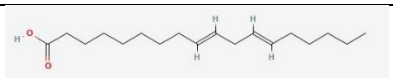
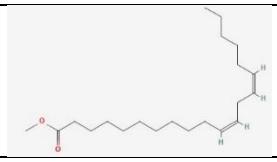
2-Hydroxy-1- (Hydroxymethyl) ethyl (9e,12e)-9,12-octadecadienoate (8.70%)

There have been reports of antispasmodic,anti-inflammatory, antiviral, and anticancer properties for hexadecenoic acid, ethyl ester. Moreover, it demonstrates antibacterial, antioxidant, and anticancer qualities..[23] Ethyl (9Z, 12Z)-9,12-octadecadienoate, also known as ethyl linoleate, has several functions, namely: As a building block for fats and oils, as a precursor to other compounds. Some research suggests that linoleic acid, the parent compound of ethyl linoleate, might provide certain health advantages, such lowering the risk of heart disease and inflammation.[24] Octacosyl acetate has several functions, namely: anti-inflammatory, antirheumatic, anthelmintic, laxative and tonic, used for leprosy, vertigo, skin diseases, bronchitis, dysentery, asthma, leucoderma, hemorrhoids, tremors. muscles, and diarrhea. [25,26] (Z,E7,11-Hexadecadien-1-yl acetate, also known as (Z,E)-7,11-hexadecadienyl acetate or cis-7,trans-11-hexadecadienyl acetate, is a chemical compound with several properties and has been identified as new compounds in today's learning. [27] (Z,E)-7,11-hexadecadienyl acetate has antitumor, anticancer, antistatic, and antioxidant properties.[28]

Table 4. Content of n-hexane solvent black cumin seed extract

No	Compound name	Content (%)	Rt	Quality	Molecular Formula
1.	Hexadecenoic acid, ethyl ester	7.99	30.151	99	$C_{18}H_{36}O_2$
	Structure of chemical compounds				
2.	Methyl (8e,11e)-8,11-octadecadienoate	27.50	31.330	99	$C_9H_{34}O_2$
	Structure of chemical compounds				
3.	9-octadecenoic acid (z)-, methyl ester	9.64	31.385	99	$C_{19}H_{36}O_2$
	Structure of chemical compounds				
4.	9,12-octadecadienoic acid, ethyl ester	11.01	31.654	99	$C_{20}H_{36}O_2$
	Structure of chemical compounds				
5.	(9e,12e)-9,12-octadecadienoic acid	12.69	31.978	95	$C_{18}H_{32}O_2$
	Structure of chemical compounds				
6.	Cis-11,14-eicosadienoic acid, methyl ester	5.08	32.378	99	$C_{21}H_{38}O_2$
	Structure of chemical compounds				

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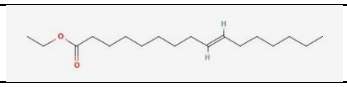
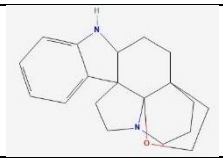
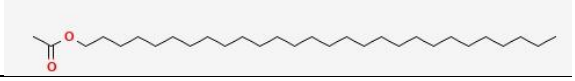
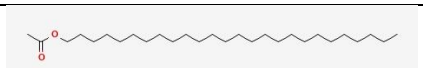
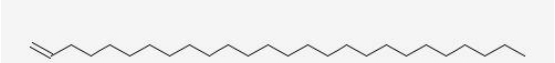
No	Compound name	Content (%)	Rt	Quality	Molecular Formula
7.	Hexadecenoic acid, ethyl ester	2.13	30.565	99	$C_{18}H_{34}O_2$
8.	Structure of chemical compounds				
9.	13a,3a-(epopxyethano)-1h-indolizino [8,1-cd] carbazol-7-ol, 6-acethyl-2,3,4,5,5a,6,11,12-octahydro-8,9-dimethoxy-	1.37	30.840	92	$C_{19}H_{24}N_2O$
10.	Structure of chemical compounds				
11.	Octacosyl acetate	1.91	32.516	95	$C_{30}H_{60}O_2$
	Structure of chemical compounds				
12.	Hexacosyl acetate	3.84	32.744	96	$C_{28}H_{56}O_2$
	Structure of chemical compounds				
13.	1-hexacosene	1.10	33.116	95	$C_{26}H_{52}$
	Structure of chemical compounds				

Based on the data in table 4, it can be seen that the main compounds in black cumin seed extract with N-Hexane solvent are Methyl (8E,11E)-8,11- octadecadienoate (27.50%), 9-octadecenoic acid (z)-, methyl ester (9.64%), 9,12-octadecadienoic acid, ethyl ester (11.01%), and (9e,12e)-9,12-octadecadienoic acid (12.69%).

Methyl (8E,11E)-8,11-octadecadienoate it has been reported to have Antibacterial activity and has potential use in research on lipid metabolism and as a precursor to other chemicals.[29] 9-octadecenoic acid (z)-, methyl ester has the biological activity of increasing VLDL and reducing HDL cholesterol.[30] 9,12-octadecadienoic acid, ethyl ester has antioxidant and anti-inflammatory biological activity. (9e,12e)-9,12-octadecadienoic acid has anti-inflammatory biological activity , low blood cholesterol, preventative measures for cancer hepatoprotective Insecticidal, nematode, hypoallergenic, acne-preventive, 5. inhibitor of alpha reductase, Anti-arthritic, Anti-androgenic, and Anticoronary.[30]

Based on the data in table 5, it can be seen that the main compound in black cumin seed extract with Ethyl acetate solvent is 9,12-octadecadienoic acid (z,z)- (20.41%), 9,12-octadecadienoic acid, methyl ester (7 .89%), 13-tetradece-11-yn-1-ol (5.25%), 9,17-octadecadienal, 9,12-octadecadienoic acid (z,z)- has biological activity as anti-inflammatory, hypocholesterolemia, cancer preventive, hepatoprotective, nematicide, insecticide, antihistamine, antiischemic, anti-acne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritis and anticoronary.

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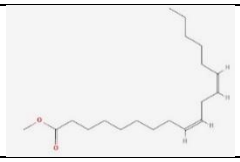
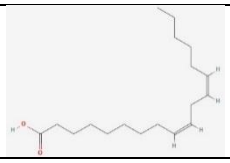
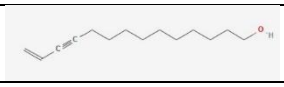
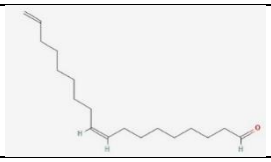
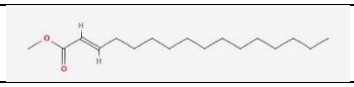
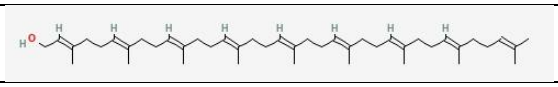
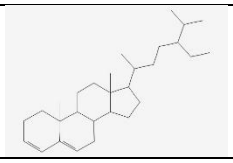
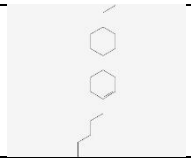
Table 5. Content of black cumin seed extract, ethyl acetate solvent

No	Compound name	Content (%)	Rt	Quality	Molecular Formula
1.	9,12-octadecadienoic acid, methyl ester	7.89	31.185	99	$C_{19}H_{34}O_2$
	Structure of chemical compounds				
2.	9,12-octadecadienoic acid (z,z)-	20.41	31.896	99	$C_{18}H_{32}O_2$
	Structure of chemical compounds				
3.	13-tetradece-11-in-1-ol	5.25	32.820	95	$C_{14}H_{24}O$
	Structure of chemical compounds				
4.	9.17-octadecadienal, (z)- (9z)	5.70	33.420	95	$C_{18}H_{32}O$
	Structure of chemical compounds				
5.	Hexadecenoic acid, methyl ester	1.95	30.089	98	$C_{17}H_{32}O_2$
	Structure of chemical compounds				
6.	Solanesol	1.06	33.564	90	$C_{45}H_{74}O$
	Structure of chemical compounds				
7.	Stigmastan-3,5-diene	1.62	41.411	50	$C_{29}H_{48}$
	Structure of chemical compounds				
8.	Cyclohexene,4-(4-ethylcyclohexyl)-1-pentyl-	1.34	52.485	96	$C_{19}H_{34}$
					
9.	Grape seed oil	15.05	32.020	99	

9,12-octadecadienoic acid, methyl ester Anti-inflammatory, Nematicide, Insecticide, Anti-acne, Hypocholesterolemia, Cancer prevention, Hepatoprotective, Antihistamine, Anti-arthritis, Anti-eczema. [30] 13-tetradece-11-yn-1-ol has antifungal activity.[31] 9,17-

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octadecadienal, (z)- (9z)used as a surfactant and emulsifying agent.[32]

CONCLUSION

The maceration process yielded the maximum yield of 28.09% for N-hexane solvent, 27.57% for 96% ethanol solvent, and 25.81% for ethyl acetate solvent. The same 96% ethanol and N-Hexane solvents tested positive for alkaloids, saponins, phenolic flavonoids, triterpenoids, and glycoxides, while the ethyl acetate solvent tested positive for alkaloids, saponins, phenolic flavonoids, triterpenoids, steroids, and glycosides. These results were based on the results of the phytochemical screening test conducted using the maceration method. The primary chemicals identified by GCMS analysis of black cumin seed extract in 96% thanol solvent were hexadecenoic acid and ethyl ester (26.14%). Using N-Hexane solvent to extract black cumin seeds revealed the

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them. All authors contributed equally to this work.

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