



Research Article

Qualitative and Quantitative Phytochemical Screening and GC-MS Analysis of Aqueous Extract of *Nigella sativa* (Black Seed)

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ABSTRACT

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Nigella sativa, commonly known as black seeds, is a medicinal plant native to Europe, Northern Africa, and Western Asia. Traditionally, it has been used to treat a variety of ailments, including respiratory and digestive issues, and is recognized for its anti-inflammatory and immune-boosting properties. This study aims to conduct qualitative and quantitative phytochemical screening and GC-MS analysis of the aqueous extract of *Nigella sativa* to identify its bioactive compounds and evaluate their potential medicinal properties. The *Nigella sativa* seeds were collected, processed, and subjected to qualitative and quantitative phytochemical tests. Gas Chromatography-Mass Spectrometry (GC-MS) was utilized to identify bioactive compounds. The qualitative analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, and steroids. Quantitative analysis indicated the abundance of flavonoids (2.946 mg/g), polyphenols (1.519 mg/g), and tannins (1.02 mg/g). The GC-MS analysis identified 24 bioactive compounds, with Linoelaidic acid (69.034%) being the most abundant. The findings highlight a diverse presence of alkaloids, flavonoids, tannins, polyphenols, and saponins, all of which contribute to the plant's antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. The most abundant compound, Linoelaidic acid, highlighted strong anticancer activity, while flavonoids and polyphenols emphasized potent antioxidant effects. Future research should focus on elucidating the mechanisms of action of these bioactive compounds and conducting clinical trials to validate their efficacy and safety for therapeutic applications.

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INTRODUCTION

Nigella sativa, commonly known as black seeds, belongs to the Ranunculaceae family and is native

to Europe, Northern Africa, and Western Asia, particularly Iran and Turkey (Ostadpoor & Gholami-Ahangaran, 2021). It's an annual plant,

which grows up to 30-60 cm tall (Thakur *et al.*, 2021). The stem of the *Nigella sativa* is thin and finely divided into its branches. The leaves are finely divided and long and the lower ones are opposite, while the upper ones are alternating. The Flowers are blue and white and grow in clusters on the ends of long flowering stems. The seeds are black, and arranged in follicles that are long and slender, unlike the seeds of other plants in the same family that have round seeds similar to poppy seeds (Thakur *et al.*, 2021). Traditionally, several parts of the *Nigella sativa* plant, specifically the seeds, were used in traditional medicine to cure a variety of diseases. It has been used to ease respiratory ailments, digestive troubles, and inflammatory conditions, as well as to enhance the immune system. It has also been utilised as a culinary spice and flavouring component in numerous cuisines (Sadeghi *et al.*, 2023). Black seed is a medicinal plant with anti-bacterial, antifungal, anti-oxidant, anti-viral, anti-parasite, analgesic, antipyretic, anti-tussive, and anti-inflammatory activities (Sadeghi *et al.*, 2023). Thymoquinone, the main bioactive compound in black seeds, has been shown to promote the function of various vital organs and exhibit diverse pharmacological activities, including neuroprotective, nephronprotective, Cardio-protective, gastroprotective, hepatoprotective, anticancer, and immune system support, as well as general well-being (Hannan *et al.*, 2021; Sadeghi *et al.*, 2023). It is important to emphasize the current limitations in the understanding of *Nigella sativa*'s phytochemical composition, particularly with respect to its aqueous extract. Despite the widespread use of *Nigella sativa* in traditional medicine for its therapeutic benefits, such as antioxidant, antimicrobial and anti-inflammatory effects, previous studies ((Adebayo-Tayo *et al.*, 2021; Canayakin *et al.*, 2016; Dinagaran *et al.*, 2016; Gültekin *et al.*, 2024; Hemadri Reddy *et al.*, 2018; Tiji *et al.*, 2021; Zouirech *et al.*, 2022) predominantly focused on its oil extracts, with limited exploration into the bioactive compounds present in its aqueous extract.

Moreover, while the pharmacological potential of *Nigella sativa*'s key bioactive compound, thymoquinone has been well-documented (Ahmed *et al.*, 2023; Anwar *et al.*, 2020; Karimi *et al.*, 2019; Mashayekhi-sardoo *et al.*, 2020; Thakur *et al.*, 2021), there is a noticeable lack of

comprehensive phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) analysis specifically targeting the aqueous extract. This creates a gap in understanding the full spectrum of phytochemicals present in water-based extracts and how they contribute to the plant's therapeutic properties, particularly in environments where water extraction is preferred for its safety and accessibility.

MATERIALS AND METHODS

Plant Collection and Identification

The fresh *Nigella sativa* seeds were purchased from Bakin dogo Central Market, Kaduna, and authenticated at the herbarium unit of the Department of Biological Sciences, Nigerian Defence Academy, Kaduna (Voucher No. NDA/BioH/2023/55).

Plant Preparation and Extraction

The fresh *Nigella sativa* seeds was thoroughly rinsed with clean water to remove any dirt or impurities, dried under the shade and ground into powder using an electric blender. A total of 324.51g of powdered seeds was soaked in 1.8 liters of distilled water, stirred and left for 48 hours. The mixture was filtered using Whatman filter paper and the filtrate was evaporated using a water bath at 40°C, yielding 34.3g of dried extract and the dried extracts were kept at room temperature.

Qualitative phytochemical tests

The qualitative phytochemical tests was carried out using the standard procedure for the identification of phytochemical constituents as described by Alamgir, (2017); Shaikh & Patil, (2020); Abdelfatah *et al.* (2021); Pant *et al.* (2017) and Mamta *et al.* (2023). The extract solution was prepared by dissolving 5g of extract in distilled water to make up a 100ml solution. The extract solution was then used for phytochemical screening. Tests were done for confirmation of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, phenols and steroids

Quantitative Phytochemical Analysis

The quantitative phytochemical constituents of the aqueous seeds extracts of *Nigella sativa* (ASENS) was determined using the standard procedures. The estimation was done in triplicates and the results expressed as mean and standard deviation.

Estimation of Alkaloids

A 5 mg of the extract were weighed into clean test tubes, and 2 ml of 2 N HCl were added. The solution was shaken until fully dissolved. Then, 1

ml was transferred to a separating funnel, followed by the addition of 5 ml chloroform. The mixture was shaken to wash the solution, after which 2.5 ml bromocresol green and 2.5 ml phosphate buffer (pH 7) were added. The solution was shaken again, and the lower layer was removed. The upper layer was dispensed into a graduated cylinder, and absorbance was measured for both standard and test solutions were obtained using a reagent blank at 470 nm via UV/Visible spectrophotometry. A series of reference standard atropine solutions (20, 40, 60, 80, and 100 µg/mL) was prepared under identical conditions. Absorbance readings for the standards and test samples were again taken at 470 nm, and the alkaloid content was quantified as milligrams of atropine equivalents (AE) per gram of plant extract (Alkali *et al.*, 2024; Chandan & Krishna, 2024).

Estimation of Flavonoids

A 20 µl of samples and standard were pipetted into each well of a microtiter plate, followed by the addition of 15 µl of 5% sodium nitrite. After incubating for 6 minutes, 15 µl of 10% aluminum chloride were added and left to stand for 6 minutes. Finally, 80 µl of 4% sodium hydroxide were added, and the plate was incubated for 15 minutes. The absorbance of both test and standard solutions was measured against a reagent blank at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was quantified and expressed as milligrams of quercetin equivalents (QE) per gram of extract (Alkali *et al.*, 2024; Chandan & Krishna, 2024).

Estimation of Polyphenols

Twenty µl of sample and standard were pipetted into the wells of a microtiter plate, and 150 µl of Folin-Ciocalteu reagent were added. After standing for 6 minutes, 80 µl of 7.5% sodium carbonate were added, and the mixture was left to stand for 1 hour before the absorbance was measured at 765 nm using a UV-Visible spectrophotometer, with a reagent blank serving as the reference. The total phenolic content was calculated using a standard calibration curve of gallic acid (ranging from 10 to 100 µg), applying the correlation equation $y = 0.923x$. The phenolic content was expressed as gallic acid equivalents (GAE) (Alkali *et al.*, 2024; Chandan & Krishna, 2024).

Estimation of Tanins

The tannin content was quantified using the Folin-Denis reagent method. In this procedure, a

standard calibration curve was generated by plotting absorbance (A) against the concentration of tannins at a specific wavelength. 1.5 ml of distilled water were pipetted into clean test tubes, followed by 20 µl of the sample and standard (methanol). Next, 100 µl of Folin-Ciocalteu reagent and 200 µl of 35% sodium carbonate were added. The volume was adjusted to 2 ml by adding 180 µl of distilled water. Absorbance was measured at 725 nm. The total phenolic content was calculated as tannic acid equivalents (TAE) based on the standard calibration curve (Alkali *et al.*, 2024; Chandan & Krishna, 2024).

Estimation of Saponins

A concentration of 5 mg/ml of samples was prepared. Then, 20 µl of each sample and standard were pipetted into clean test tubes. Subsequently, 250 µl of vanillin reagent and 2.5 ml of 72% sulfuric acid were added to each test tube. The tubes were incubated in a water bath at 60°C for 10 minutes. Afterward, they were cooled by transferring them to an ice-filled beaker. The absorbance was measured at 544 nm using a UV/Visible spectrophotometer, with a reagent blank as the reference. Diosgenin was utilized as the standard, and the assay results were expressed in diosgenin equivalents (DE) for comparison (Alkali *et al.*, 2024; Chandan & Krishna, 2024).

GC-MS Phytochemical Analysis

The gas chromatography and mass spectrometry (GC-MS) analysis was conducted at Ahmadu Bello University's Multi-User Science Research Laboratory in Zaria, Nigeria. GC-MS is a powerful method for identifying and quantifying different bioactive compounds in plant extracts (Olivia *et al.*, 2021).

Gas chromatography-mass spectrometry (GC-MS) procedure

The GC-MS analysis of the aqueous extract of *Nigella sativa* was done using the following conditions: The Elite-1 fused silica capillary column (30 m × 0.25 mm ID × 1µm df, composed of 100% dimethyl polysiloxane) was used in electron impact mode at 70 eV, with helium gas (99.999%) as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2µl (split ratio of 10:1). The injector temperature is set to 250 °C, while the ion source temperature is 280 °C. The oven temperature was designed to rise from 110 °C (isothermal for 2 minutes) to 200 °C/min, then 5 °C/min to 280 °C/min, and finally 9

minutes at 280°C. Mass spectra were collected at 70 eV with a scan interval of 0.5 seconds with fragments ranging from 45 to 450 Da. The total GC running duration was 36 minutes. Each component's relative percentage quantity was computed by dividing its average peak area by the overall area. The mass spectrum of GC-MS was interpreted using the database of the National Institute of Standards and Technology (NIST) (Ondeko *et al.*, 2020).

Complex organic and biological combinations are analysed using gas chromatography (GC) and mass spectrometry. The gas chromatography component separates distinct compounds in the sample into pulses of pure chemicals according on their volatility by passing an inert gas (mobile phase) through a stationary phase placed in the column (Arora & Kumar, 2018). The mass spectrometer collects compound spectra as they exit a chromatographic column, identifying and

RESULTS

Qualitative Phytochemical Analysis of Aqueous Extract of *Nigella sativa* Seeds

The qualitative phytochemical analysis of the aqueous extract of *Nigella sativa* seeds, demonstrating the presence of various bioactive compounds. The analysis reveals that alkaloids, flavonoids, saponins, tannins, cardiac glycosides, phenols, and steroids are all present in the extract. This comprehensive detection underscores the diverse phytochemical composition of *Nigella sativa*, with no compound category indicated as absent. This suggests that the aqueous extract of the seeds contains a wide array of phytochemicals commonly associated with therapeutic properties.

Quantitative Phytochemical Analysis of *Nigella sativa* Seeds

The quantitative phytochemical analysis of *Nigella sativa* (black seed) provides crucial insights into its bioactive compounds, as represented in Table 2 of the study. The analysis focuses on several major classes of phytochemicals: alkaloids, flavonoids, saponins, tannins, and polyphenols, detailing their relative abundance in milligrams per gram (mg/g). Alkaloids, with a concentration of 1.567 ± 0.002 mg/g, are noted for their potent biological activities, including analgesic, anti-inflammatory, and antimicrobial properties. Alkaloids in *Nigella sativa* are linked to its traditional use in treating

quantifying molecules based on their mass-to-charge ratio (m/z). These spectra can be saved and analysed on the computer. A GC-MS chromatogram is a plot that shows the separation of compounds in a sample as they elute from the gas chromatography column over time. The x-axis of the chromatogram shows the retention time, the time it takes for each compound to move through the column and reach the mass spectrometer detector. The y-axis typically shows the intensity or concentration of the compounds, which is proportional to the number of counts recorded by the mass spectrometer detector at each retention time (Arora & Kumar, 2018).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 24. All results were expressed as Mean \pm Standard Deviation (SD).

pain, infections, and inflammation-related ailments.

Flavonoids, which represent the most abundant group at 2.946 ± 0.015 mg/g, are renowned for their strong antioxidant properties. Flavonoids are known to neutralize free radicals, preventing oxidative stress, and thereby contributing to anti-inflammatory and anticancer activities. The high flavonoid content suggests that *Nigella sativa* has substantial potential for preventing diseases related to oxidative stress, such as cardiovascular diseases and cancer. Saponins are found in relatively low amounts (0.036 ± 0.001 mg/g), yet they play a significant role in enhancing immune response and lowering cholesterol levels. Although their concentration is minimal, saponins contribute to *Nigella sativa*'s reputation as a medicinal plant, especially for its cholesterol-lowering and immune-modulating properties. Tannins, present at 1.02 ± 0.022 mg/g, possess strong antioxidant and astringent properties. These compounds are known for their role in wound healing, reducing inflammation, and protecting against microbial infections. The moderate presence of tannins in *Nigella sativa* indicates its use in treating inflammatory conditions and microbial infections. Polyphenols, which are recorded at 1.519 ± 0.008 mg/g, are another key component with notable antioxidant capabilities. Polyphenols are vital for

scavenging harmful free radicals and are linked to anticancer and anti-inflammatory effects. This concentration of polyphenols supports the use of *Nigella sativa* in promoting general health, especially in preventing degenerative diseases.

GC-MS Bioactive Compounds Identified in *Nigella sativa* Seed Extract

GC-MS analysis identified 24 bioactive compounds in *Nigella sativa* seed extract, each with specific bioactivities. The analysis revealed a diverse range of compounds, each contributing to the pharmacological potential of the plant. The most abundant compound identified was Linoelaidic acid, accounting for 69.034% of the extract. This compound is notable for its anti-cancer properties, suggesting its potential in cancer prevention or treatment. The significant concentration of Linoelaidic acid may be a key factor in the traditional use of *Nigella sativa* as a therapeutic agent for various ailments, particularly those involving abnormal cell growth. 2-Octen-4-ol, comprising 14.515% of the extract, exhibits antimicrobial and anti-inflammatory activities. This compound plays a crucial role in defending the body against infections and reducing inflammation, which is consistent with *Nigella sativa*'s reputation for treating inflammatory diseases and microbial infections. The presence of this compound also enhances the plant's therapeutic range, making it relevant in both external and internal treatments. Another significant compound, n-Hexadecanoic acid (2.1349%), is associated with multiple

bioactivities, including antioxidant, anti-androgenic, and hypocholesterolemic effects. These activities suggest its potential in promoting cardiovascular health by reducing oxidative stress and cholesterol levels. Additionally, its antipsychotic properties indicate potential applications in mental health treatments, while its pesticidal and lubricant uses showcase its versatility beyond human health.

12-Benzenedicarboxylic acid butyl 2-ethylhexyl ester (1.6152%) is primarily industrial in use, being involved in the production of polyesters, but it plays a less significant role in medicinal contexts. However, 9-Octadecenoic acid (Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester (1.5895%) demonstrates notable antibacterial activity, supporting its role in managing infections, particularly bacterial-related diseases.

Other compounds, such as 917-Octadecadienal (Z)- (3.0205%) and 15-Hydroxypentadecanoic acid (0.2528%), further enhance the anti-inflammatory and antimicrobial profile of the extract. These compounds are also associated with anticancer, digestive aid, and relaxation/stress reduction properties, underscoring the extract's broad therapeutic potential.

The presence of Tetradecanoic acid (0.078%) with its antioxidant and hypocholesterolemic properties suggests potential benefits for heart health, while Dodecanoic acid 123-propanetriyl ester (2.2217%) contributes anti-inflammatory and moisturizing effects, which could be useful in dermatological applications

Table 1: Qualitative phytochemical analysis of aqueous extract of *Nigella sativa* seeds

Phytochemicals	Aqueous Extract of <i>Nigella sativa</i>
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Cardiac Glycosides	+
Phenols	+
Steroids	+

Key: + = Present, - = Absent

Table 2: Quantitative phytochemical analysis of *Nigella sativa*

Phytochemicals	Relative abundance (mg/g)
Alkaloids	1.567±0.002
Flavonoid	2.946±0.015
Saponins	0.036±0.001
Tannins	1.02±0.022
Polyphenols	1.519±0.008

Values are expressed in mean ± SD., n= 3.

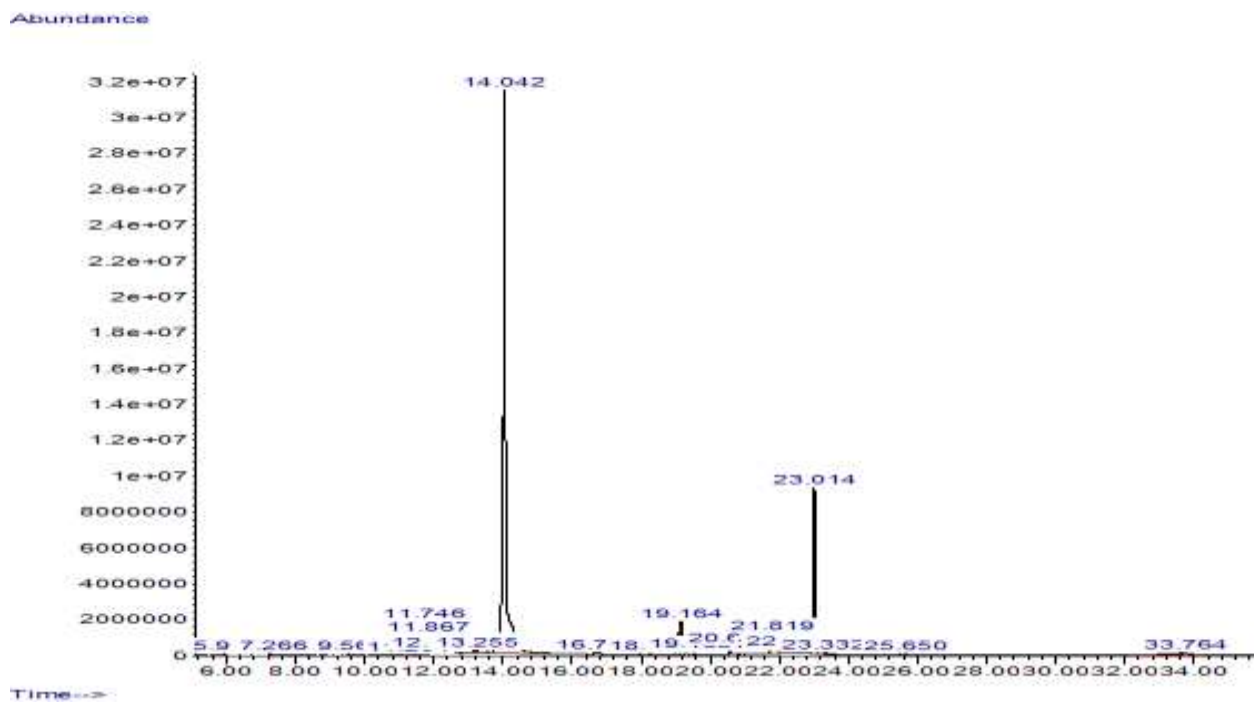
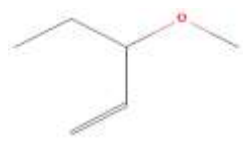
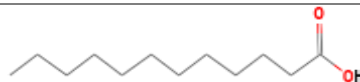
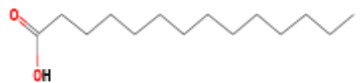

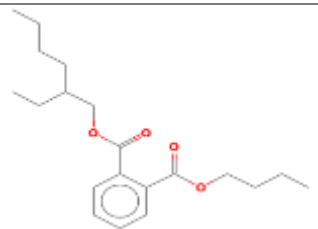

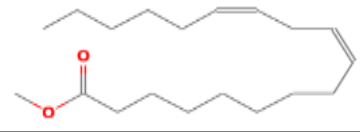

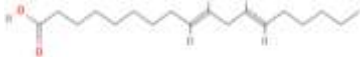

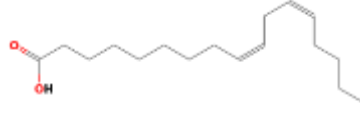

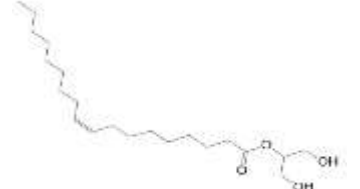


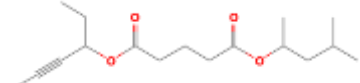


Figure 1: GC-MS Chromotogram of Aqueous Extract of *Nigella Sativa* (Black Seed)

Table 3: Activity of bioactive compounds identified in aqueous extract of *Nigella sativa* (Black seed) seed

S/N	RT (min)	Peak Area	Compounds	Qualitative (%)	Molecular Formular	Molecular Structure	Bioactivity
1	5.9512	0.083	3-Methoxy-1-pentene	64	C ₆ H ₁₂ O		It's used as a flavouring agent, fragrance ingredient, or as an intermediate in organic synthesis.
2	7.2657	0.0668	Dodecanoic acid	90	C ₁₂ H ₂₄ O ₂		Antimicrobial
3	9.5669	0.078	Tetradecanoic acid	98	C ₁₄ H ₂₈ O ₂		Antioxidant, Anticancer, Hypocholesterolemic
4	11.2635	0.0554	Hexadecanoic acid, methyl ester	97	C ₁₇ H ₃₄ O ₂		Antioxidant, Insecticide, Nematicide, Lubricant, Hemolytic, Antiandrogenic, Hypo-cholesterolemic
5	11.7459	1.6152	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	78	C ₂₀ H ₃₀ O ₄		It's used for the production of polyesters with aliphatic diols as the comonomer
6	11.8672	2.1349	n-Hexadecanoic acid	99	C ₁₆ H ₃₂ O ₂		Antioxidant, Anti-androgenic, Hypocholesterolemic, Nematicide, antipsychotic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor.
7	13.1899	0.2115	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	99	C ₁₉ H ₃₄ O ₂		Hepatoprotective, Antieczemic, Anti-histaminic, Hypocholesterolemic
8	13.2555	0.1292	trans-13-Octadecenoic acid, methyl ester	96	C ₁₉ H ₃₆ O ₂		Anti-Inflammatory, Cardiovascular health,

						Improve cholesterol levels
9	14.0422	69.034	Linoelaidic acid	99	C ₁₈ H ₃₂ O ₂	 Anti-cancer
10	16.6674	0.2528	15-Hydroxypentadecanoic acid	59	C ₁₅ H ₃₀ O ₃	 Anti-inflammatory, Anti-oxidant and Antimicrobial
11	16.7685	0.0502	9,12-Octadecadienoic acid (Z,Z)-	97	C ₁₈ H ₃₂ O ₂	 Anti-Inflammatory, Hepatoprotective, Antiarthritic, Antibacterial, Anticoronary, Anti-histaminic,
13	19.1245	3.0205	9,17-Octadecadienal, (Z)-	97	C ₁₈ H ₃₂ O	 Anti-inflammatory, Antimicrobial, Digestive Aid, Anticancer, Relaxation and Stress Reduction,
14	19.1638	1.5895	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	90	C ₂₁ H ₄₀ O ₄	 Antibacterial activity
15	19.4627	0.3228	15-Hydroxypentadecanoic acid	38	C ₁₅ H ₃₀ O ₃	 Anti-inflammatory, Antimicrobial, Neuroprotective effects and Cardiovascular
16	20.6013	0.6117	Adipic acid, di(but-2-en-1-yl) ester	30	C ₁₄ H ₂₂ O ₄	 It's plasticizer in pharmaceutical formulations. It's used in topical creams, lotions, and ointments to improve the flexibility, spreadability, and overall texture of the product
17	21.8193	2.8034	Glutaric acid, hex-4-yn-3-yl pent-4-en-2-yl ester	43	C ₁₇ H ₂₈ O ₄	 Neuroprotective effects



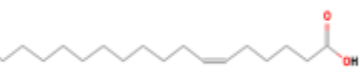
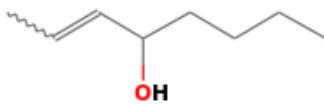


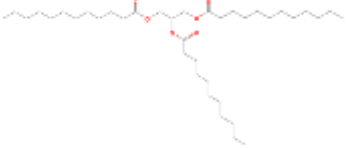
18	22.0281	0.2178	9,17-Octadecadienal, (Z)-	86	$C_{18}H_{32}O$		Relaxation and Stress Reduction, Anti-inflammatory, Antimicrobial, Digestive Aid, Anticancer
19	22.1805	0.1375	9-Octadecenal, (Z)-	81	$C_{18}H_{34}O$		Antioxidant, Anti-inflammatory, Skin health, Immune system support, Cardiovascular health, Neuroprotective, Weight management
20	22.2826	0.325	6-Octadecenoic acid, (Z)-	47	$C_{18}H_{34}O_2$		Anti-inflammatory and Antioxidant
21	23.0138	14.515	2-Octen-4-ol	32	$C_8H_{16}O$		Antimicrobial, Anti-inflammatory and flavouring agent
22	23.3324	0.1403	2-Heptenoic acid, tetradecyl ester	35	$C_{21}H_{40}O_2$		Antimicrobial activity, Anti-inflammatory properties, Cell signalling, Enzyme inhibition
23	25.6502	0.0718	5-Undecyne	25	$C_{11}H_{20}$		Antifungal, Antimicrobial, Insecticidal, Odor control, Skin conditioning
24	33.7641	2.2217	Dodecanoic acid, 1,2,3-propanetriyl ester	62	$C_{39}H_{74}O_6$		Anti-inflammatory, Antimicrobial, Moisturizing properties, Emollient properties, Non comedogenic

Table 4: Major peaks in GC-MS Chromatogram with their corresponding compounds and bioactivities

S/N	RT (min)	Peak Area	Compound Names	Conc. (%)	Bioactivity
1	14.0422	69.034	Linoelaidic acid	99	Anti-cancer
2	23.0138	14.515	2-Octen-4-ol	32	Antimicrobial, Anti-inflammatory and flavouring agent
3	19.1245	3.0205	9,17-Octadecadienal, (Z)-	97	Anti-inflammatory, Antimicrobial, Digestive Aid, Anticancer, Relaxation and Stress Reduction,
4	21.8193	2.8034	Glutaric acid, hex-4-yn-3-yl pent-4-en-2-yl ester	43	Neuroprotective effects
5	33.7641	2.2217	Dodecanoic acid, 1,2,3-propanetriyl ester	62	Anti-inflammatory, Antimicrobial, Moisturizing properties, Emollient properties, Non comedogenic
6	11.8672	2.1349	n-Hexadecanoic acid	99	Antioxidant, Anti-androgenic, Hypocholesterolemic, Nematicide, antipsychotic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor.

DISCUSSION

The study on *Nigella sativa* (black seed) provides a detailed analysis of its phytochemical composition and bioactive potential, as demonstrated through qualitative and quantitative assessments, alongside Gas Chromatography-Mass Spectrometry (GC-MS) results. Qualitative phytochemical analysis confirmed the presence of key bioactive compounds, including alkaloids, flavonoids, saponins, tannins, cardiac glycosides, phenols, and steroids. These compounds are well-known for their medicinal properties, offering analgesic, antioxidant, antimicrobial, and anti-inflammatory effects. Similar findings have been reported by Adebayo-Tayo *et al.*, (2021); Hemadri Reddy *et al.*, (2018) and Nivetha & Prasanna, (2016). The crude methanol extract of *Nigella sativa* seeds was found to contain flavonoids, saponins, alkaloids, tannins, anthraquinones, and terpenoids but lacked cardiac glycosides. The ethanol extract revealed anthraquinones, alkaloids, amino acids, saponins, tannins, flavonoids, steroids, proteins, and terpenoids, with the absence of carbohydrates, phlobatannins, polyphenols, phytosterols, and cardiac glycosides. These compounds align with the traditional medicinal

uses of *Nigella sativa*, reaffirming its therapeutic value.

Quantitative analysis provided further insights into the relative abundance of these phytochemicals, with flavonoids being the most prevalent (2.946 ± 0.015 mg/g). Flavonoids, known for their strong antioxidant properties, play a vital role in neutralizing free radicals and reducing oxidative stress, thereby aiding in the prevention of chronic diseases like cancer and cardiovascular disorders. Polyphenols (1.519 ± 0.008 mg/g), the second most abundant group, share similar antioxidant and anti-inflammatory effects, supporting the traditional use of *Nigella sativa* in promoting health and preventing disease. The high concentration of flavonoids and polyphenols which exhibit strong antioxidant properties. In contrast, oil extracts of *Nigella sativa* generally report lower concentrations of these compounds (Badr *et al.*, 2020; Dinakaran *et al.*, 2016; Gültekin *et al.*, 2024; Pachaiappan *et al.*, 2023; Thomas *et al.*, 2022), as many phenolic compounds and flavonoids are more soluble in water than in oil. This elevated presence in the aqueous extract suggests that water-based extractions may be particularly beneficial for antioxidant-related applications, potentially enhancing the extract's

anticancer and anti-inflammatory effects via mechanisms tied to oxidative stress reduction. Alkaloids (1.567 ± 0.002 mg/g), present in significant amounts, are recognized for their antimicrobial, anti-inflammatory, and analgesic properties. Tannins (1.02 ± 0.022 mg/g) are recognized for their astringent, anti-inflammatory, and wound-healing properties, which may enhance the therapeutic scope of the aqueous extract in treating inflammation and infections. Although saponins were detected in lower concentrations (0.036 ± 0.001 mg/g), contribute to immune modulation and cholesterol regulation, further differentiating the potential applications of the aqueous extract from oil-based options. These findings align with studies by Adebayo-Tayo *et al.*, (2021); Shafodino *et al.*, (2022), who reported abundant flavonoids and alkaloids, along with moderate levels of saponins, tannins, and phenols in methanolic extracts of *Nigella sativa*.

GC-MS analysis identified twenty-four (24) compounds, with six being the most abundant. Linoelaidic acid, a primary component in the aqueous extract, makes up 69.034% of the total compounds identified via GC-MS, was the most predominant. Previous oil-based studies of *Nigella sativa* have reported thymoquinone as the major bioactive compound, known for its anticancer and anti-inflammatory properties (Hannan *et al.*, 2021; Sadeghi *et al.*, 2023). The high concentration of Linoelaidic acid in the aqueous extract introduces a novel compound of interest, particularly in anticancer research. The absence or lower presence of thymoquinone in the aqueous extract emphasizes how solvent choice affects compound extraction, pointing to distinct therapeutic applications based on extract type. Linoelaidic acid found in *Nigella sativa* (black seed), a hypothesis regarding its mechanism of action could involve its interaction with molecular pathways associated with cancer cell proliferation and survival. Linoelaidic acid, which constitutes 69.034% of the *Nigella sativa* aqueous extract, is noted for its potential anticancer properties due to its ability to induce apoptosis and inhibit cell growth. This might be explained by its interaction with pathways like the PI3K/Akt and MAPK pathways, which play significant roles in cell survival and proliferation. A research by

Choo *et al.*, (2016) and Morgos *et al.*, (2024) has shown that the inhibition of these pathways can lead to reduced cancer cell viability, as they are critical in many cancers for maintaining uncontrolled cell growth. Additionally, the anti-inflammatory effects of Linoelaidic acid may contribute to its anticancer effects by reducing chronic inflammation, a known risk factor for cancer development, through the suppression of inflammatory mediators like TNF- α and IL-6 (Bahman *et al.*, 2024; Florescu *et al.*, 2023; Paramita Pal *et al.*, 2023). These actions could potentially disrupt the microenvironment that supports cancer progression. Other major compounds included 2-Octen-4-ol (14.515%) and 9,17-Octadecadienal (Z)- (3.0205%), which exhibits anti-inflammatory, antimicrobial, anticancer, stress-relief effects and flavoring properties. Both aqueous and oil extracts contain antimicrobial compounds, yet there are notable differences in composition and abundance. The aqueous extract identified several unique compounds with antimicrobial activity, such as 2-Octen-4-ol (14.515%), which is less frequently noted in oil-based extracts. This compound's presence may contribute to *Nigella sativa*'s effectiveness against a broader range of pathogens in its aqueous form, indicating its potential as a more versatile antimicrobial agent when compared to oil-based extracts that rely heavily on thymoquinone for microbial inhibition. Additionally, Glutaric acid, hex-4-yn-3-yl pent-4-en-2-yl ester (2.803%), recognized for its neuroprotective properties open potential for applications in neurodegenerative disorders like Alzheimer's disease and Parkinson's disease. It may help mitigate oxidative stress in neurons, which is linked to neurodegeneration, making it a candidate for further research in cognitive and mental health treatments. Other notable compounds such as Dodecanoic acid, 1,2,3-propanetriyl ester (2.222%), is promising in dermatological treatments, especially for inflammatory skin conditions, such as eczema or psoriasis, and as a moisturizing agent in treating dry, cracked skin. Its non-comedogenic properties make it suitable for sensitive or acne-prone skin formulations, and n-Hexadecanoic acid (2.135%), known for its antioxidant, hypocholesterolemic, and antipsychotic activities, were also identified. n-Hexadecanoic

acid and other long-chain fatty acids, common in oil extracts, were either present in significantly lower concentrations in the aqueous extract. This difference likely results from the inability of water to dissolve non-polar lipid-based compounds, underscoring the oil extract's superior capacity for harnessing lipid-soluble constituents associated with anti-inflammatory and hypocholesterolemic activities. Consequently, the oil extract may retain advantages in cardiovascular and cholesterol-related applications, whereas the aqueous extract offers stronger antioxidant and antimicrobial potential. These results align with the studies of Nivetha & Prasanna, (2016), who detected twenty-two (22) compounds using ethanol extract of *Nigella sativa* seeds, and Krishnaveni & Saranya, (2016), who identified nineteen (19) compounds using ethanol extract of *Nigella sativa* seeds. Other minor compounds contribute to the pharmacological activities of *Nigella sativa*, reinforcing its therapeutic potential.

CONCLUSIONS

AND

RECOMMENDATION

The research on *Nigella sativa* (black seed) concludes that the aqueous extract of the seeds contains a wide array of bioactive phytochemicals, including flavonoids, alkaloids, saponins, tannins, polyphenols, and steroids. These compounds, as identified through qualitative, quantitative, and GC-MS analyses, exhibit a broad range of medicinal properties such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. The high concentration of flavonoids and polyphenols suggests strong antioxidant potential, while the presence of linoelaidic acid highlights the plant's anticancer capabilities. The study supports the traditional use of *Nigella sativa* for treating various ailments, particularly those involving inflammation, infections, and oxidative stress. Additionally, the identification of 24 bioactive compounds through GC-MS analysis reinforces its potential for pharmaceutical applications. The findings encourage further research into the mechanisms of these compounds and the development of *Nigella sativa* based therapeutic agents.

CONFLICT OF INTEREST

The authors confirm no conflict of interest.

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