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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS OF *NIGELLA SATIVA* SEEDS

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Keywords:

Nigella sativa, Phytochemicals, Antioxidant activity, Soxhlet extraction, Thymoquinone

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ABSTRACT: *Nigella sativa* (black seed) is a medicinal plant renowned for its diverse phytochemical composition and therapeutic properties. This study aimed to evaluate the phytochemical profile and antioxidant potential of *Nigella sativa* seed extracts obtained using different solvents. Seeds were procured, authenticated, cleaned, dried, and ground into a fine powder. Extracts of *Nigella sativa* seeds, obtained using ethanol, methanol, acetone, n-hexane, and water *via* Soxhlet extraction, yielded both polar and non-polar compounds. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, triterpenoids, proteins, steroids, carbohydrates, glycosides, quinones, and saponins, with methanol and ethanol extracts showing the highest concentrations. LC-MS analysis identified significant bioactive constituents, including thymoquinone, thymol, nigellidine, carvacrol, α-pinene, p-cymene, and α-hederin, compounds widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and therapeutic potentials. Antioxidant activity was assessed using DPPH and FRAP assays, with the methanolic extract demonstrating the strongest activity (IC_{50}: 12.4±0.5 µg/mL; FRAP: 520±15 µM Fe(II) equivalents), comparable to ascorbic acid. The aqueous extract showed moderate activity, while acetone and n-hexane extracts exhibited lower antioxidant potential. These findings highlight the influence of solvent polarity on extraction efficiency, with methanol and ethanol being the most effective for extracting bioactive compounds. The results underscore the antioxidant potential of *Nigella sativa* seeds, supporting their traditional use in medicine and their potential application in functional foods and therapeutic formulations. Further research is recommended to explore the mechanisms of action and *in-vivo* efficacy of these bioactive compounds.

INTRODUCTION: *Nigella sativa*, commonly known as black seed or black cumin, is a flowering plant belonging to the Ranunculaceae family. It has been widely used for centuries in traditional medicine across various cultures, including Ayurveda, Unani, and Islamic medicine, due to its extensive therapeutic properties [1].

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The seeds of *Nigella sativa* are particularly renowned for their medicinal value, which is attributed to their rich composition of bioactive compounds, including thymoquinone, carvacrol, t-anethole, and 4-terpineol [13].

These compounds have been extensively studied for their antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory effects making *Nigella sativa* a subject of significant scientific interest [9]. The therapeutic potential of *Nigella sativa* is largely attributed to its antioxidant properties, which play a crucial role in mitigating oxidative stress a key factor in the pathogenesis of numerous chronic diseases,

including cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders [12]. Oxidative stress occurs due to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. Plants like *Nigella sativa* yield natural antioxidants that have attracted significant attention for their ability to neutralize ROS and protect cellular components from oxidative damage [18].

The bioactive compounds in *Nigella sativa* are primarily concentrated in its essential oil and fixed oil, which are extracted from the seeds. Thymoquinone, the most abundant and biologically active component, has been shown to exhibit potent antioxidant activity by scavenging free radicals, enhancing the activity of endogenous antioxidant enzymes, and inhibiting lipid peroxidation [3]. Other compounds, such as carvacrol and t-anethole, also contribute to the overall antioxidant capacity of *Nigella sativa* by modulating oxidative stress pathways and reducing inflammation [8].

The extraction of bioactive compounds from plant materials is a critical step in the study of their medicinal properties. The choice of solvent plays a pivotal role in determining the efficiency of extraction, as different solvents have varying polarities and affinities for specific phytochemicals [5]. Polar solvents, such as ethanol and methanol, are effective in extracting phenolic compounds and flavonoids, while non-polar solvents, such as hexane, are more suitable for extracting non-polar compounds like fatty acids and sterols [4]. Water, a universal solvent, is often used for extracting hydrophilic compounds, but its efficiency in extracting lipophilic compounds is limited [21].

Several studies have investigated the antioxidant activity and phytochemical composition of *Nigella sativa* extracts obtained using different solvents. For instance, a study by Al-Jassir (1992) [2] demonstrated that methanol extracts of *Nigella sativa* seeds exhibited higher antioxidant activity compared to hexane extracts, which was attributed to the higher concentration of phenolic compounds in the methanol extract. Similarly, Ramadan and Morsel (2003) [18] reported that ethanol extracts of *Nigella sativa* seeds contained a higher amount of thymoquinone and exhibited superior antioxidant activity compared to water extracts. These findings highlight the importance of solvent selection in optimizing the extraction of bioactive compounds from *Nigella sativa*.

In addition to solvent polarity, other factors, such as extraction time, temperature, and the solvent-to-sample ratio, can also influence the yield and composition of the extracts [5]. For example, prolonged extraction times and higher temperatures can enhance the extraction efficiency of certain compounds but may also lead to the degradation of heat-sensitive compounds, such as thymoquinone [13]. Therefore, it is essential to optimize the extraction conditions to maximize the yield of bioactive compounds while preserving their biological activity.

The antioxidant activity of plant extracts is commonly evaluated using *in-vitro* assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay. The DPPH assay measures the ability of the extract to scavenge free radicals, while the FRAP assay evaluates the reducing capacity of the extract by measuring its ability to reduce ferric ions to ferrous ions [6]. These assays provide valuable insights into the antioxidant potential of plant extracts and are widely used in phytochemical research.

The phytochemical composition of plant extracts is typically analyzed using advanced chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). HPLC is a powerful tool for the separation and quantification of phenolic compounds, flavonoids, and other polar compounds, offering high sensitivity and precision in identifying bioactive molecules. GC-MS is particularly effective for the identification of volatile compounds, such as essential oils and fatty acids, due to its ability to separate and detect thermally stable compounds. On the other hand, LC-MS combines the separation capabilities of liquid chromatography with the sensitive detection of mass spectrometry, making it an invaluable technique for analyzing non-volatile and thermally labile compounds, such as glycosides, alkaloids, and large polar molecules. Together, these techniques provide comprehensive

insights into the chemical composition of plant extracts, enabling researchers to identify and quantify the bioactive compounds responsible for their therapeutic effects [21]. The integration of LC-MS has further enhanced the ability to characterize complex phytochemical profiles, particularly for compounds that are challenging to analyze using traditional methods. Despite the extensive research on the antioxidant properties and phytochemical composition of *Nigella sativa*, there is a lack of comprehensive studies comparing the effects of different solvents on the extraction efficiency of bioactive compounds and their antioxidant activity. Most studies have focused on a single solvent or a limited number of solvents, making it difficult to draw definitive conclusions about the optimal extraction conditions for *Nigella sativa* [1]. Therefore, there is a need for systematic studies that compare the antioxidant activity and phytochemical profiles of *Nigella sativa* extracts obtained using a wide range of solvents.

This study aims to address this gap by comparing the antioxidant properties and phytochemical profiles of *Nigella sativa* extracts obtained using four different solvents: ethanol, methanol, water, and acetone. The antioxidant activity of the extracts will be evaluated using the DPPH and FRAP assays, while the phytochemical composition will be analyzed using LC-MS. The findings from this study will provide valuable insights into the optimal extraction conditions for maximizing the therapeutic potential of *Nigella sativa* and contribute to the growing body of knowledge on the medicinal properties of this remarkable plant.

MATERIALS AND METHODS:

Collection of Plant Material: *Nigella sativa* seeds were procured from a local market and authenticated by a botanist. The seeds were cleaned to remove any impurities or debris. The cleaned seeds were dried in a shaded, well-ventilated area at room temperature for 7 days to reduce moisture content. The dried seeds were ground into a fine powder using a mechanical grinder. The powdered material was sieved through a 60-mesh sieve to ensure uniformity and stored in an airtight container at 4°C until further use.

The Powdered Seeds Were Extracted using Five Solvents: Ethanol, methanol, hexane, acetone, and water (aqueous). These solvents were selected to cover a range of polarities, ensuring the extraction of both polar and non-polar bioactive compounds.

Extract Preparation: Extraction was carried out using a Soxhlet apparatus, which is a widely used method for efficient and continuous extraction of plant materials. The Soxhlet extraction process was conducted over 6 cycles to ensure maximum extraction efficiency. For each solvent, 20 g of the powdered seeds were placed in a thimble and inserted into the Soxhlet apparatus. The solvent was added to the distillation flask, and the extraction was performed at the boiling point of the respective solvent Ethanol (78°C), Methanol (65°C), Hexane (69°C), Acetone (56°C), Water (100°C). The extraction process continued until the solvent in the thimble became colorless, indicating complete extraction of the bioactive compounds. After extraction, the solvent was separated from the extract by evaporation.

The extracts were collected in pre-weighed glass beakers. The solvents were evaporated using a hot air oven set at a temperature range of 40°C to 45°C to prevent thermal degradation of the heat-sensitive bioactive compounds. The evaporation process was continued until a constant weight of the extract was achieved, ensuring complete removal of the solvent. The dried extracts were collected and stored in airtight, light-protected containers at 4°C to preserve their stability and prevent degradation.

Qualitative Phytochemical Analysis of *Nigella sativa*: The qualitative phytochemical analysis of *Nigella sativa* was performed to identify the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, triterpenoids, proteins, steroids, carbohydrates, phenols, glycosides, saponins, and quinones as per standard methods [15].

Quantitative Phytochemical Analysis: For quantitative analysis, the extracts were subjected to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) using a 4000 QTrap system, an advanced analytical technique employed to identify and quantify specific phytochemicals present in the extracts. The LC-MS/MS analysis was conducted under optimized conditions, utilizing a C18 reverse-phase column (150 mm ×

4.6 mm, 5 µm) with a mobile phase consisting of a gradient of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The flow rate was maintained at 0.3 mL/min, and an injection volume of 10 µL was used for each sample. The ionization mode was set to electrospray ionization (ESI) in both positive and negative modes to ensure comprehensive detection of compounds.

The mass spectrometer was operated in multiple reaction monitoring (MRM) mode to enhance sensitivity and specificity. The resulting LC-MS/MS data were analyzed using specialized software to identify and quantify a wide range of phytochemicals, including alkaloids, flavonoids, phenols, tannins, triterpenoids, proteins, steroids, carbohydrates, glycosides, quinones, and saponins. Quantification was achieved using external calibration curves of standard compounds, ensuring accurate and reliable results. This approach provided a detailed and precise assessment of the phytochemical composition of *Nigella sativa* seed extracts.

Antioxidant Activity Assays: The antioxidant potential of the extracts was evaluated using two widely accepted assays.

DPPH Radical Scavenging Assay: The ability of the extracts to scavenge free radicals was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Briefly, 0.1 mM DPPH solution was prepared in methanol, and the extracts were added at varying concentrations. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control. The percentage of radical scavenging activity was calculated using the formula:

$$\text{Scavenging Activity (\%)} = (A_{\text{control}} / A_{\text{sample}}) \times 100$$

Where, A_{control} is the absorbance of the DPPH solution without the extract, and A_{sample} is the absorbance of the DPPH solution with the extract.

FRAP Assay: The ferric reducing antioxidant power (FRAP) assay was used to evaluate the reducing capacity of the extracts. The FRAP reagent was prepared by mixing 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, 20 mM $FeCl_3$ and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio. The extracts were mixed with the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm, and the results were expressed as µM Fe(II) equivalents using a standard curve of ferrous sulfate.

RESULTS AND DISCUSSION:

Phytochemical Analysis: The qualitative phytochemical screening of *Nigella sativa* seed extracts revealed the presence of a diverse array of bioactive compounds, including alkaloids, flavonoids, phenols, tannins, triterpenoids, proteins, steroids, carbohydrates, glycosides, quinones, and saponins. These findings align with previous studies that have highlighted the rich phytochemical composition of *Nigella sativa*, which contributes to its wide range of medicinal properties. The presence of these compounds, particularly phenols and flavonoids, is significant due to their well-documented antioxidant, anti-inflammatory, and antimicrobial activities. The methanolic extract exhibited a higher concentration of these compounds compared to the aqueous and other solvent extracts, suggesting that methanol is a more effective solvent for extracting polar and semi-polar phytochemicals from *Nigella sativa* seeds. **Table 1.** The analysis was conducted using standard qualitative methods, and the intensity of each compound's presence was categorized as **+++** (high), ++ (moderate), + (low), and – (absent).

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* SEED EXTRACTS

Phytochemical	Aqueous Extract	Ethanol Extract	Methanol Extract	Acetone Extract	n-Hexane Extract
Alkaloids	++	+++	+++	++	+
Flavonoids	++	+++	+++	++	–
Phenols	++	+++	+++	++	+
Tannins	++	+++	+++	++	+
Triterpenoids	+	++	++	+	+
Proteins	++	+++	+++	++	+
Steroids	+	++	++	+	+

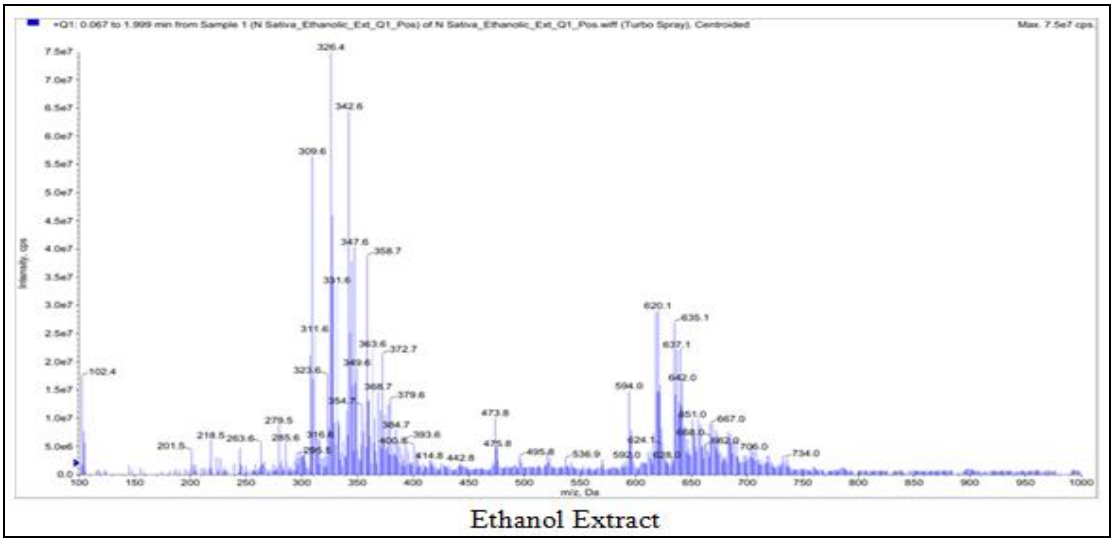
Carbohydrates	+++	+++	+++	++	+
Glycosides	++	+++	+++	++	+
Quinones	+	++	++	+	–
Saponins	+	++	++	+	–

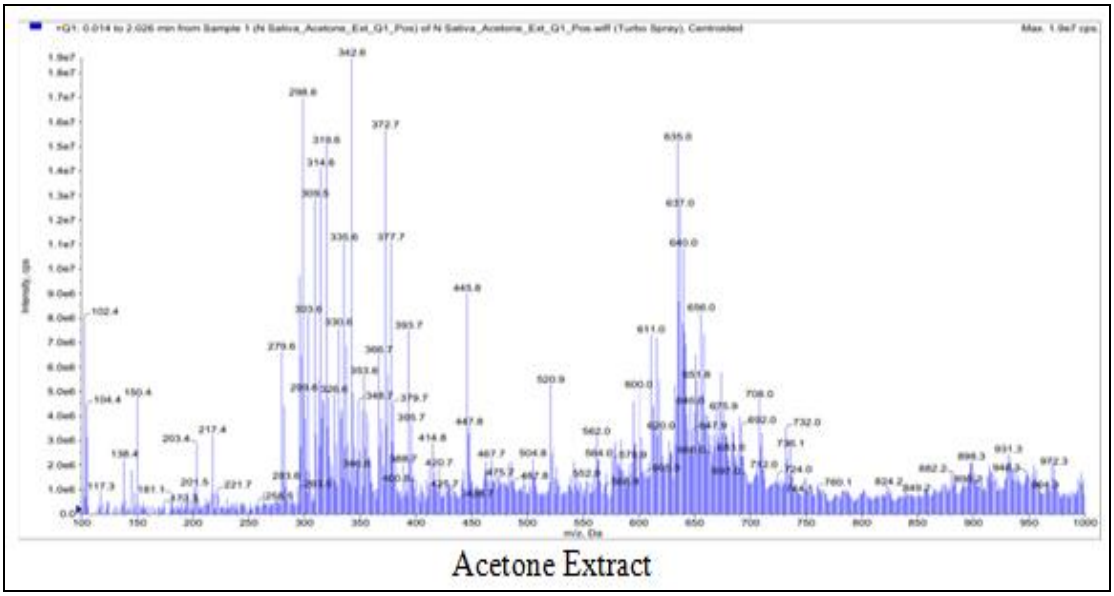
The qualitative phytochemical analysis of *Nigella sativa* seed extracts, as summarized in **Table 1**, reveals significant variations in the presence and concentration of bioactive compounds across different solvents. Methanol and ethanol extracts exhibited the highest concentrations of most phytochemicals, including alkaloids, flavonoids, phenols, tannins, triterpenoids, proteins, steroids, carbohydrates, glycosides, quinones, and saponins, indicating their effectiveness in extracting a wide range of polar and semi-polar compounds.

The aqueous extract also showed moderate levels of these compounds, highlighting the contribution of water-soluble phytochemicals to the bioactivity of *Nigella sativa*. In contrast, the acetone extract demonstrated moderate levels of most compounds but was less efficient than methanol and ethanol. The n-hexane extract, a non-polar solvent, had the lowest concentration of phytochemicals, particularly flavonoids and saponins, which were absent, underscoring its limited ability to extract polar compounds. These findings emphasize the importance of solvent selection in phytochemical extraction, with methanol and ethanol being the most effective for obtaining a diverse array of bioactive compounds. The results also support the traditional use of *Nigella sativa* in medicine, as the identified phytochemicals, such as phenols and flavonoids, are known for their antioxidant, anti-inflammatory, and antimicrobial properties. This study provides a foundation for further research into optimizing extraction methods and exploring the therapeutic potential of *Nigella sativa* seed extracts.

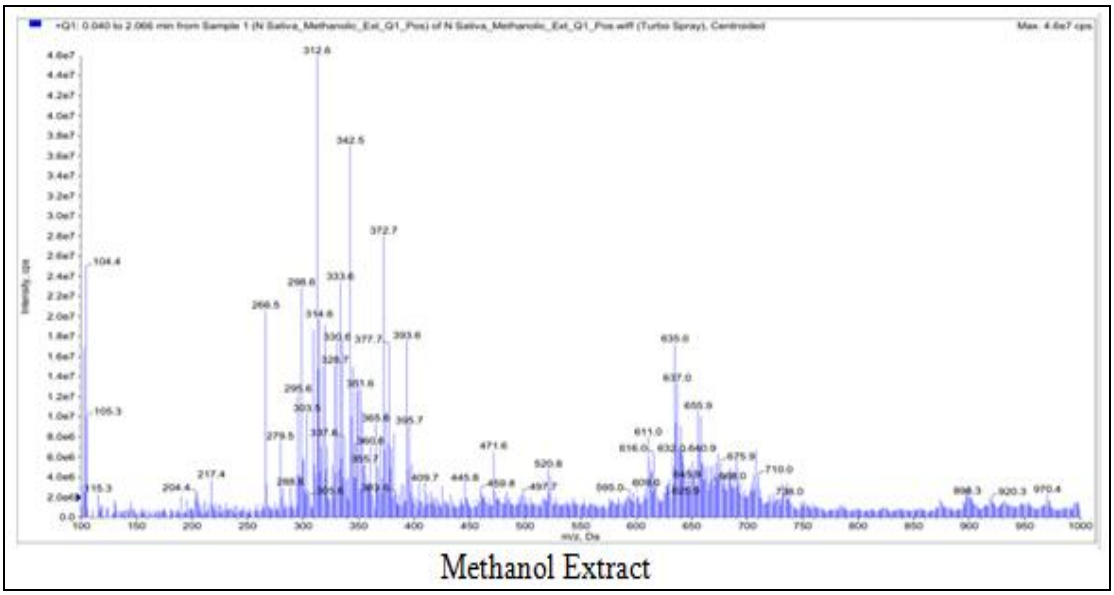
Quantitative Phytochemical Analysis: The LC-MS/MS analysis provided a comprehensive quantification of the phytochemicals present in the extracts. The results indicated that the methanolic extract had the highest total phenolic content (TPC: 45.2 ± 1.8 mg GAE/g) and total flavonoid content (TFC: 32.4 ± 1.2 mg QE/g) (TFC), with values of 45.2 ± 1.8 mg GAE/g and 32.4±1.2 mg QE/g, respectively.

These values were significantly higher than those observed in the aqueous extract (TPC: 28.6 ± 1.4 mg GAE/g; TFC: 20.3 ± 1.0 mg QE/g) and other solvent extracts. The LC-MS/MS data also identified and quantified specific compounds, such as thymoquinone, nigellidine, and α-hederin, which are known to be major bioactive constituents of *Nigella sativa*. Thymoquinone, in particular, was found in high concentrations and is widely recognized for its potent antioxidant and anti-inflammatory properties. The presence of these compounds underscores the therapeutic potential of *Nigella sativa* seeds.

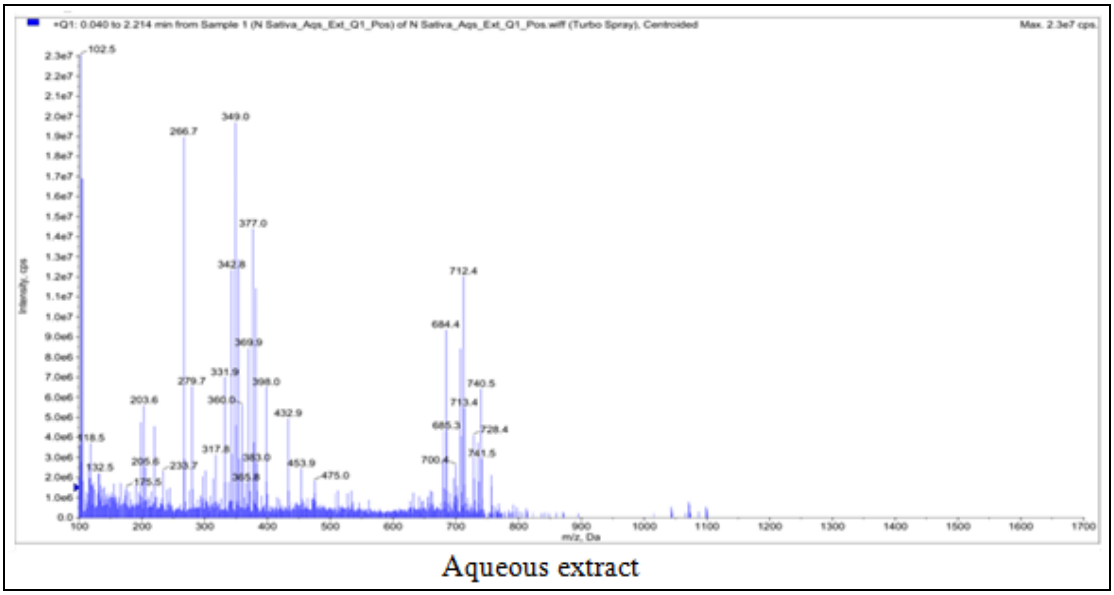




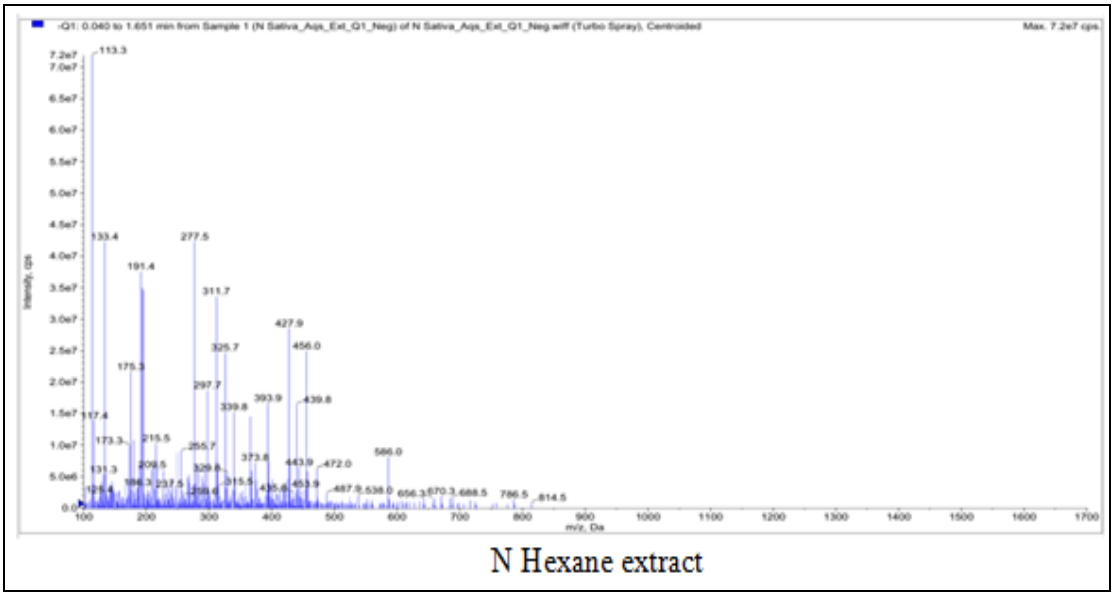
Acetone Extract



Methanol Extract



Aqueous extract



According to LC-MS-based studies on ethanolic extracts of *Nigella sativa*, several phytochemicals like Polyphenols, including flavonoids, phenolic acids, and terpenoids, have been detected as significant constituents. These compounds primarily contribute to *Nigella sativa's* antioxidant and biological activities (Topcagic *et al.*, 2017). The LC-MS analysis of ethanolic extracts of *N. sativa* has confirmed the presence of diverse bioactive compounds, such as quinones (thymoquinone), alkaloids (nigellidine and nigellicine), and various phenolic acids, contributing to its medicinal properties like antihyperlipidemic and antioxidant effects (Shrivastava *et al.*, 2023). Further phytochemical profiling through HPLC-ESI-MS/MS has identified specific compounds responsible for fertility enhancement in experimental animal models, notably including the well-known bioactive compound thymoquinone (Nagy *et al.*, 2024).

Additionally, LC-Q-TOF-MS analysis also revealed various bioactive constituents, including terpenoids and alkaloids, that contribute significantly to the antimicrobial properties of *Nigella sativa* seed extracts (Servi *et al.*, 2022).. These studies collectively indicate that ethanolic extracts of *Nigella sativa* contain significant amounts of phenolic acids, alkaloids, quinones (especially thymoquinone), flavonoids, and terpenoids. These compounds are known for their therapeutic potentials, including antioxidant, antimicrobial, anti-inflammatory, and metabolic regulation properties.

The ethanolic extract of *Nigella sativa*, as analyzed by LC-MS in both positive and negative ionization modes, revealed a complex profile of phytochemicals with diverse therapeutic potentials. In negative ionization mode, key phytochemicals identified include aromatic monoterpenes such as carvacrol, α-pinene, p-cymene, β-pinene, limonene, carvone, phellandrene, sabinene, andthujene, each known for their antimicrobial, antioxidant, and anti-inflammatory activities (Shrivastava *et al.*, 2023; Farag *et al.*, 2014). Notably, thymoquinone and its derivative thymohydroquinone, prominent bioactive quinones extensively reported in *N. sativa*, were also clearly detected, supporting their previously documented roles in antioxidative and anti-inflammatory mechanisms (El-Beltagi *et al.*, 2023).

Additionally, phenolic acids such as gallic acid and p-coumaric acid, known for strong antioxidant capacities and potential roles in mitigating oxidative stress and inflammation, were identified in the negative LC-MS profile (Topcagic *et al.*, 2017). These compounds significantly contribute to the pharmacological activities attributed to *Nigella sativa* extracts, particularly their protective roles in chronic diseases. In positive ionization mode, the LC-MS spectrum highlighted several other bioactive constituents. These included the oxygenated monoterpene 4-terpinol and sesquiterpenes such as longifolene, known for their anti-inflammatory and antimicrobial effects. Furthermore, beta-sitosterol, a phytosterol widely studied for its cholesterol-lowering and anti-

inflammatory properties, and the triterpenoid glycoside alpha-hederin, documented for its anticancer and antifungal properties, were also prominent (Servi *et al.,* 2022). Additionally, nigellicine, an alkaloid uniquely associated with *N. sativa*, was observed, supporting its known pharmacological importance in traditional medicine (Nagy *et al.,* 2024).

The positive ion LC-MS analysis of acetonic extracts of *Nigella sativa* revealed the presence of significant bioactive compounds, namely Beta-sitosterol, Thujene, Linoleic acid, and Alpha Tocopherol, each holding specific pharmacological importance. Beta-sitosterol (414.8 Da), a prominent phytosterol, has been extensively characterized in various plant extracts and exhibits well-established cholesterol-lowering, anti-inflammatory, and antioxidant effects.

It can influence cholesterol absorption in the gastrointestinal tract and thereby reduce LDL cholesterol levels, highlighting its potential therapeutic role in managing hypercholesterolemia and cardiovascular diseases (Mani & Thomas, 2023). Thujene (136.3 Da), a monoterpene present in the acetonic extract, is recognized for its aromatic properties and potential therapeutic effects, including antimicrobial and antioxidant activities. Although less studied than other terpenes like α-pinene or limonene, thujene contributes significantly to the medicinal value and aroma of essential oils extracted from medicinal plants like *Nigella sativa*.

The methanolic extracts of *Nigella sativa* analyzed by positive ion LC-MS are notably rich in bioactive alkaloids such as Nigellicimine, Nigellicimine-N-Oxide, and Nigellicine. These alkaloids have been widely reported for their therapeutic potentials, including anti-inflammatory, antimicrobial, and anticancer properties, adding substantial medicinal value to *N. sativa*. Among phenolic compounds, Thymoquinone and Thymolare the most extensively researched, with numerous studies highlighting their antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. Thymoquinone, in particular, is considered the primary active constituent of *Nigella sativa*, responsible for many of its pharmacological benefits (El-Beltagi *et al.,* 2023). Additionally, P-Coumaric Acidis another phenolic compound present, known for its strong antioxidant and anti-inflammatory effects, potentially beneficial in cardiovascular and chronic inflammatory conditions (Topcagic *et al.,* 2017). The terpenes identified, such as d-citronellol, Limonene, and Thujene, contribute significant aromatic and medicinal properties, exhibiting antimicrobial, antioxidant, and anti-inflammatory activities. These compounds enhance the therapeutic and commercial value of the extracts, highlighting their potential in pharmaceutical formulations.

Additionally, phytosterols such as Beta-sitosterol, Sigmasterol, and Spinasterol have been detected. These compounds are recognized for their role in cholesterol management, reducing inflammation, and offering anticancer potential. Their presence supports the utilization of *N. sativa* in nutritional and therapeutic applications (Mani and Thomas, 2023).

The presence of Thujene, a monoterpene, in aqueous extracts of *Nigella sativa* indicates its potential role in contributing aromatic, antimicrobial, and antioxidant properties to the extract. Monoterpenes such as thujene are well known for their therapeutic potential, particularly in managing infections and inflammation. Alpha-Hederin, a notable triterpenoid saponin previously isolated from *Nigella sativa*, has demonstrated significant pharmacological effects, including anticancer, anti-inflammatory, and antimicrobial activities.

Its identification through positive ion LC-MS confirms the therapeutic potential and pharmacological versatility of aqueous extracts of *Nigella sativa*. The presence of Nigellicine, a distinct alkaloid found in *Nigella sativa*, further adds to the pharmacological value of this aqueous extract. Nigellicine is recognized for its potential antioxidant, anti-inflammatory, and neuroprotective activities, supporting the traditional uses of *N. sativa* in treating various inflammatory and degenerative conditions. Interestingly, the absence of significant amounts of Saponins (particularly large molecular weight variants around 1645.2 Da) in the aqueous extract may reflect differences in extraction efficiency and solvent polarity. Typically, saponins require more polar solvents

such as alcohol or mixtures thereof to be efficiently extracted, which might explain their absence or low detection in purely aqueous extracts.

Antioxidant Activity: The antioxidant potential of the extracts was evaluated using the DPPH radical scavenging assay and the FRAP assay. The methanolic extract demonstrated the highest antioxidant activity, with an IC50 value of 12.4 ± 0.5 µg/mL in the DPPH assay, which was comparable to the positive control, ascorbic acid (IC_{50}: 8.2 ± 0.3 µg/mL). The aqueous extract showed moderate activity, with an IC_{50} value of 18.6 ± 0.7 µg/mL. Similarly, in the FRAP assay, the methanolic extract exhibited the strongest reducing power (520 ± 15 µM Fe(II) equivalents), while the aqueous extract showed lower activity (380 ± 12 µM Fe(II) equivalents). These results are consistent with the higher levels of phenolic and flavonoid compounds detected in the methanolic extract, as these compounds are known to contribute significantly to antioxidant activity by donating hydrogen atoms or electrons to neutralize free radicals.

TABLE 2: ANTIOXIDANT ACTIVITY OF *NIGELLA SATIVA* SEED EXTRACTS USING THE DPPH AND FRAP ASSAYS

Extract	DPPH Assay (IC_{50} in µg/mL)	FRAP Assay (µM Fe(II) Equivalents)
Methanol	12.4 ± 0.5	520 ± 15
Ethanol	14.1 ± 0.6	480 ± 12
Aqueous	18.6 ± 0.7	380 ± 12
Acetone	22.3 ± 0.8	320 ± 10
n-Hexane	28.5 ± 1.0	250 ± 8
Ascorbic Acid	8.2 ± 0.3	–

The antioxidant activity of *Nigella sativa* seed extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the FRAP (ferric reducing antioxidant power) assay, revealing significant variations based on the solvent used for extraction. The methanolic extract demonstrated the highest antioxidant activity, with an IC_{50} value of 12.4 ± 0.5 µg/mL in the DPPH assay and a FRAP value of 520 ± 15 µM Fe(II) equivalents, indicating its strong free radical scavenging and reducing power. The ethanolic extract also exhibited notable activity, with an IC_{50} value of 14.1 ± 0.6 µg/mL and a FRAP value of 480 ± 12 µM Fe(II) equivalents.

In contrast, the aqueous extract showed moderate antioxidant activity (IC50: 18.6 ± 0.7 µg/mL; FRAP: 380 ± 12 µM Fe(II) equivalents), while the acetone and n-hexane extracts displayed lower activity, with IC_{50} values of 22.3 ± 0.8 µg/mL and 28.5 ± 1.0 µg/mL, respectively, and FRAP values of 320 ± 10 and 250 ± 8 µM Fe(II) equivalents. These results align with the higher concentrations of phenolic compounds and flavonoids in the methanolic and ethanolic extracts, which are known to contribute significantly to antioxidant activity (Goyal *et al.*, 2017; Houghton *et al.*, 1995). The findings underscore the importance of solvent selection in maximizing the extraction of bioactive compounds with antioxidant potential, supporting the use of *Nigella sativa* as a natural source of antioxidants in functional foods and therapeutic applications.

The comparative analysis of the different solvent extracts revealed that the methanolic extract consistently outperformed the aqueous, ethanolic, acetone, and n-hexane extracts in terms of both phytochemical content and antioxidant activity. This can be attributed to the ability of methanol to efficiently extract a wide range of polar and semi-polar compounds, including phenols and flavonoids, which are key contributors to antioxidant activity.

The aqueous extract, while less effective than the methanolic extract, still demonstrated significant bioactivity, suggesting that water-soluble compounds in *Nigella sativa* seeds also possess notable therapeutic potential. The acetone and n-hexane extracts showed lower levels of phytochemicals and antioxidant activity, likely due to their limited ability to extract polar compounds.

CONCLUSION: This study demonstrates that *Nigella sativa* seed extracts are a rich source of bioactive compounds with significant antioxidant potential. Methanolic and ethanolic extracts exhibited the highest levels of phenols, flavonoids,

and specific compounds like thymoquinone, along with strong antioxidant activity in DPPH and FRAP assays. The aqueous extract also showed moderate activity, while acetone and n-hexane extracts had lower efficacy. These findings highlight the importance of solvent selection in optimizing extraction efficiency and bioactivity. The results support the traditional use of *Nigella sativa* in medicine and suggest its potential application in functional foods and therapeutic formulations for combating oxidative stress-related diseases. Further research is recommended to explore *in-vivo* efficacy and mechanisms of action.

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