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Original Article

Black Cumin (*Nigella sativa*) as a Natural Food Preservative: GC-MS Profiling and Antimicrobial Activity against Spoilage Microorganisms

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ABSTRACT

Background: *Nigella sativa*, also referred as black cumin and described as the miraculous herb, exhibits many medicinal and pharmacological properties. **Objectives:** This research focused on the antimicrobial effectiveness of different *N. sativa* Seeds (NSS) extracts against spoilage microbes and phytochemical analysis of seed extract via Gas Chromatography-Mass Spectrometry (GC-MS). **Materials and Methods:** Using agar well diffusion method, acetone, hexane, ethanolic and aqueous extracts of *N. sativa seeds* were examined against spoilage causing bacteria and fungi. Extracts showing highest inhibitory activity was subjected to phytochemical screening via GC-MS analysis. **Results:** Both aqueous and ethanolic extract of NSS exhibited significant inhibitory activity against spoilage bacteria and fungi at a concentration of 10 mg/mL, with MIC value 0.5 mg/mL for spoilage fungi and 2.5 mg/mL for spoilage bacteria. Phytochemical screening using Gas Chromatography-Mass Spectrometry (GC-MS) revealed nineteen compounds in the ethanolic extract and seven compounds in the aqueous extract. The identified compounds contributing to antimicrobial activity include thymoquinone, 2,4-Di-tert-butylphenol, 2-methyl-5-(1-methylethyl) phenol, n-Hexadecanoic acid and p-Cymene-2,5-diol. **Conclusion:** *N. sativa* seeds contain a rich profile of bioactive compounds (*e.g.*, thymoquinone, ethyl oleate, 2,4-Di-tert-butylphenol, *etc.*) and exhibits effective antimicrobial activity against food spoilage microorganisms. These findings indicate its potential application as a natural preservative in food industry.

Keywords: Antimicrobial activity, Gas Chromatography-Mass Spectrometry (GC-MS), Minimum Inhibitory Concentration (MIC), *Nigella sativa*, Seeds extracts.

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INTRODUCTION

Medicinal herbs and spices have long been recognized as a rich source of bioactive compounds with therapeutic and preservative properties. Among these, spices such as clove (*Syzygium aromaticum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and cinnamon (*Cinnamomum verum*) have been extensively used in traditional medicine and food preparations across diverse cultures for thousands of years. Their widespread application as remedies for infectious diseases and various ailments has attracted considerable interest from researchers in the pharmaceutical and food industries (Erdogul *et al.*, 2009). The therapeutic potential of these botanicals is attributed to their rich phytochemical composition, which confers a broad spectrum of biological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer and analgesic properties (Amin and Hosseinzadeh, 2015). These activities are due to presence of secondary metabolites such as phenolics, flavonoids, terpenoids, saponins, glycosides and fatty acids, which are biosynthesized by plants as part of their defense mechanisms against environmental stressors and pathogenic threats (Bourgou *et al.*, 2008).

Nigella sativa L., commonly known as black cumin, is an annual flowering plant belonging to the family Ranunculaceae. The plant typically attains a height of 20-30 cm, bearing linear-lanceolate leaves and producing flowers in a range of colors including white, yellow, pink, pale purple and pale blue. The mature plant yields large, inflated fruit capsules enclosing numerous black seeds (Goreza, 2003). *N. sativa* is native to South Europe, Southwest Asia and North Africa. It is widely cultivated across Israel, India, Pakistan, Bangladesh, Syria, Lebanon, Turkey, the Middle East and the Mediterranean basin. Within India, the plant is cultivated across several states including Punjab, Himachal Pradesh, West Bengal, Assam, Bihar, Maharashtra and the Gangetic plains (Krishnakant *et al.*, 2010).

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N. sativa seeds have been employed in traditional medicinal systems for over 2000 years, with documented use in the treatment of a wide range of conditions including asthma, hypertension, inflammation, diabetes, arthritis, tumours and gastrointestinal and gynaecological disorders. In Islamic medicine, it is revered as a miraculous herb, with its seeds believed to be a remedy for all ailments except death. Beyond their medicinal value, the seeds are also widely used as a spice and condiment in culinary preparations (Ramadan, 2015).

Both the volatile and fixed oils of *N. sativa* seeds are rich in bioactive constituents exhibiting antioxidant, antimicrobial and anti-inflammatory activities. The seeds contain a diverse array of phytochemicals including thymoquinone, nigellone, 2-methyl-4-isopropyl-p-quinone, β-sitosterol, anthraquinones, melanin, saponins, glycosides, tannins, proteins, mucilage, resins and glucose (Javed *et al*., 2012). Among these, thymoquinone is the principal active constituent of *N. sativa* essential oil and has been extensively investigated for its antimicrobial, antioxidant, anticancer and anti-inflammatory properties (Gali-Muhtasib *et al*., 2008; Khan *et al*., 2011).

Thymoquinone and thymohydroquinone, an essential component of *N. sativa* essential oil, have demonstrated significant antibacterial activity against both Gram-positive and Gram-negative pathogens including *Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus*, and *Salmonella typhimurium* (Hanofy and Hatem, 1991; Halawani, 2009). *N. sativa* seeds contain antifungal defensins that inhibit the growth of phytopathogenic fungi such as *Fusarium oxysporum, F. culmorum, F. graminearum, Aspergillus niger* and *Bipolaris sorokiniana*. Antifungal activity has also been reported against dermatophytes including *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Microsporum canis*, both *in vitro* and *in vivo* (Rogozhin *et al*., 2011; Forounzar *et al*., 2014). Furthermore, antioxidant and antimutagenic activities have been documented in Tunisian *N. sativa* roots and shoots, attributed to the presence of vanillic acid, a phenolic compound identified in both plant parts (Bourgou *et al*., 2008).

Despite the well-documented medicinal properties of *N. sativa*, its efficacy in controlling food spoilage microorganisms and the specific phytochemicals responsible for this activity remain underexplored. Food spoilage caused by bacteria and fungi results in significant economic losses and poses serious public health risks through the production of harmful metabolites and mycotoxins. Therefore, the present study aimed to evaluate the antimicrobial potential of different solvent extracts of *N. sativa* seeds against food spoilage bacteria and fungi using the agar well diffusion method, and to identify the bioactive phytochemicals present in the most effective extracts through Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Collection of *Nigella sativa* Seeds

Nigella sativa seeds were procured from local vendors in Bengaluru, Karnataka, India. The seeds were thoroughly cleaned with running tap water, surface-disinfected with 2% sodium hypochlorite solution for 5 min, and subsequently rinsed three times with sterile distilled water to remove residual disinfectant. The cleaned seeds were air-dried, ground into a fine powder using a sterile mechanical grinder, and stored in airtight bottles at 4°C until further use.

Antimicrobial activity of *Nigella sativa* seeds extracts

Preparation of Seed Extracts

10 g of finely powdered *N. sativa* seeds were extracted separately using 100 mL each of acetone, hexane, ethanol and sterile distilled water. The mixtures were agitated on a mechanical shaker at 25°C for 72 hr. The resulting suspensions were filtered through Whatman No. 1 filter paper and the filtrates were concentrated to dryness using a rotary evaporator. The dried extracts were re-dissolved in 10% Dimethyl Sulfoxide (DMSO) to prepare stock solutions at a concentration of 100 mg/mL and stored in airtight containers at 4°C until experimental use (De *et al*., 2019).

Test Microorganisms

Five bacterial and five fungal isolates previously isolated from spoiled fruits and oilseeds were used to assess the antimicrobial efficacy of *N. sativa* seed extracts. The bacterial isolates included *Enterobacter mori* (GenBank accession no. ON413771), *Klebsiella oxytoca* (GenBank accession no. ON413770), *Serratia marcescens* (GenBank accession no. ON413914), *Bacillus inaquosorum* (GenBank accession no. ON413773), and *Bacillus subtilis* (GenBank accession no. ON413772). The fungal isolates comprised *Alternaria alstroemeriae* (GenBank accession no. ON470193), *Penicillium citrinum* (GenBank accession no. ON454381), *Aspergillus welwitschiae* (GenBank accession no. ON479653), *Aspergillus aflatoxiformans* (GenBank accession no. ON470194), and *Aspergillus awamori* (GenBank accession no. ON470195). All cultures were maintained on nutrient agar slants and in glycerol stocks at the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru (Rashmi *et al*., 2024).

Inoculum preparation

Bacterial and fungal cultures were grown in nutrient broth and potato dextrose broth (100 mL each), respectively. These cultures were incubated at 30°C for 24-48 hr on a rotary shaker at 100 rpm until a density of 10^6 cells per mL was attained.

Antimicrobial activity of extracts

The antimicrobial activity of different *N. sativa* seed extracts was evaluated using the agar well diffusion method (Nathan *et al*.,

1978). Wells of 7 mm diameter were bored into the agar using a sterile cork borer, and 50 µL of each extract at a concentration of 10 mg/mL was loaded into the respective wells. Dimethyl sulfoxide (10% DMSO) served as the negative control, while acetic acid (5%) and sodium benzoate (5%) were used as standard reference controls for antibacterial and antifungal assays, respectively. The plates were allowed to stand at room temperature for 1 hr to allow diffusion of the extracts, followed by incubation at 37°C for 24-48 hr for bacterial isolates and at 28°C for 72-96 hr for fungal isolates under aseptic conditions. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition (mm) around each well using a Vernier caliper.

Minimum Inhibitory Concentration (MIC)

The extract showing the highest antimicrobial activity at 10 mg/mL against the tested bacterial and fungal isolates was manipulated for determination of Minimum Inhibitory Concentration (MIC) using the broth microdilution method (CLSI, 2018). The extracts were serially diluted in 10% Dimethyl Sulfoxide (DMSO) to obtain final concentrations spanning 0.1 to 10 mg/mL. Ten microliters of each standardized inoculum were added to the respective tubes, along with growth control (broth with inoculum), sterility control (broth only), solvent control (10% DMSO with inoculum), and reference standards using acetic acid for bacteria and sodium benzoate for fungi. Plates were incubated at 37°C for 24 hr for bacterial isolates and at 28°C for 72-96 hr for fungal isolates under aseptic conditions. Bacterial growth was measured with the help of spectrophotometer at 600 nm, with MIC defined as the lowest concentration achieving ≥90% growth inhibition. For fungi, mycelial biomass was collected on pre-weighed Whatman No. 1 filter paper, dried at 60°C for 24 hr, and percentage mycelial inhibition calculated, with MIC defined as the lowest concentration yielding ≥90% mycelial dry weight reduction

Statistical analysis

All experiments were conducted in triplicate; results are presented as mean±Standard Error of Mean (SEM) and differences were considered statistically significant at $p \leq 0.05$.

Phytochemical Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

Preparation of extract for GC-MS Analysis

N. sativa seeds were washed, dried, and homogenized into a fine powder for extraction using ethanol and sterile distilled water as solvents. 10 g of seed powder were separately extracted in 100 mL of each solvent on a mechanical shaker at 110 rpm for 72 hr at room temperature. The resulting suspensions were filtered through Whatman No. 1 filter paper and the crude filtrates were concentrated to dryness under reduced pressure at 45°C using a rotary evaporator. The dried residues were re-dissolved in HPLC-grade methanol and subjected to GC-MS analysis for identification and characterization of bioactive phytochemicals (Ezhilan and Neelamegam, 2012).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the aqueous and ethanolic extracts of *N. sativa* seeds was performed at the Bioenergy and Quality Assurance Laboratory, University of Agricultural Sciences, Bengaluru, using a Shimadzu QP2020 GC-MS system equipped with an Rtx-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µM film thickness). Helium gas (99.99% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. A sample volume of 1 µL was injected in split mode at a split ratio of 1:10. The injector temperature was maintained at 240°C and the ion source temperature at 220°C. The oven temperature program was set as follows: initial hold at 110°C for 2 min (isothermal), ramped at 10°C /min to 200°C, further increased at 5°C /min to 280°C, and finally held at 280°C for 9 min (isothermal), with a total run time of 40 min and a solvent delay of 0-3 min. Compound quantification was based on the relative peak area percentage of each component calculated from its average peak area relative to the total chromatographic peak area (Ezhilan and Neelamegam, 2012).

Identification of phytocomponents

The phytochemical constituents present in *N. sativa* seed extracts were identified by comparing the obtained mass spectra with reference spectra available in the National Institute of Standards and Technology (NIST) library and the Wiley GC-MS library. Compound identification was confirmed based on matching of molecular ion peaks, fragmentation patterns, retention times, and molecular weight with those of known reference compounds. The nature and biological activities of the identified phytochemicals were further characterized using Dr. Duke's Phytochemical and Ethnobotanical Databases and previous literature.

Ethical Statement

The present study was conducted exclusively on plant material and microbial cultures and did not involve the use of human participants or animal subjects. Therefore, formal ethical approval was not required for this study.

RESULTS

Antimicrobial activity of *N. sativa* seeds extracts

The antimicrobial activity of *N. sativa* seed extracts was evaluated against five food spoilage bacteria and five fungi using the agar well diffusion method, and the results are detailed in Tables 1 and 2, respectively. Among all the extracts tested at a concentration of 10 mg/mL, the aqueous extract had the highest antibacterial activity, with inhibition zones ranging from 15.7 to 21.67 mm against all five tested spoilage bacteria, including

both Gram-positive (*Bacillus subtilis* and *Bacillus inaquosorum*) and Gram-negative (*Klebsiella oxytoca*, *Enterobacter mori*, and *Serratia marcescens*) isolates (Table 1).

The ethanolic extract exhibited the highest antifungal activity against four of the five tested fungal isolates, with inhibition zones ranging from 13.17 to 17.6 mm, while *Alternaria alstroemeriae* was found resistant to all tested extracts (Table 2). Both acetone and hexane extracts showed less efficacy against the tested bacterial isolates. However, the hexane extract showed the lowest antifungal activity against *Aspergillus awamori* (9.3±0.33 mm) and *Aspergillus aflatoxiformans* (11.3±0.33 mm), while acetone extract had no antifungal activity against any of the tested isolates. Acetic acid (5%) used as antibacterial reference produced inhibition zones ranging from 38.0 to 52.7 mm, while sodium benzoate (5%) used as antifungal reference, had antimicrobial activity against selected fungal isolates. Therefore, experiments were carried out to determine the Minimum Inhibitory Concentration (MIC) of the extracts against food spoilage microorganisms.

Minimum inhibitory concentration

The MIC of the best-performed extracts, *i.e.*, aqueous extract for spoilage bacteria and ethanolic extract for spoilage fungi, was determined by the broth microdilution method across a concentration range of 0.1 to 10.0 mg/mL and the results are elucidated in Table 1 and Table 2. The MIC values for food spoilage bacteria ranged from 2.5 to 7.5 mg/mL, with *Bacillus subtilis* being the most susceptible (MIC=2.5 mg/mL) and *Klebsiella oxytoca*, *Enterobacter mori*, and *Serratia marcescens* showing MIC values of 7.5 mg/mL (Table 1). For fungal isolates, MIC values of the ethanolic extract ranged from 0.5 to 2.5 mg/mL, with *Aspergillus awamori* being most susceptible (MIC=0.5 mg/mL), followed by *Aspergillus welwitschiae* (MIC=1.0 mg/mL), *Penicillium citrinum* (MIC=0.75 mg/mL), and *Aspergillus aflatoxiformans* (MIC=2.5 mg/mL), while *Alternaria alstroemeriae* showed no susceptibility (Table 2). The results indicated that the MIC values for fungi were considerably lower than those obtained for bacteria, suggesting that food spoilage fungi were more susceptible to *N. sativa* seed extracts than food spoilage bacteria under the tested conditions.

Phytochemical screening

GC-MS analysis of the aqueous and ethanolic extracts of *N. sativa* seeds revealed the presence of seven and nineteen phytochemical compounds, respectively, as represented by corresponding peaks in the chromatograms (Figures 1 and 2). The mass spectra of each detected compound were compared against the NIST and Wiley GC-MS libraries for identification and characterization (Tables 3 and 4). The nature and biological activities of the identified compounds were further documented with reference to Dr. Duke's Phytochemical and Ethnobotanical Databases and relevant published literature (Tables 3 and 4).

In the aqueous extract, seven compounds were identified, with the most abundant being n-hexadecanoic acid (palmitic acid, 27.42%), followed by p-cymene-2,5-diol (thymoquinol, 24.06%), linoleic acid (15.26%), and thymoquinone (14.95%). The remaining compounds included oleic acid (8.15%), myristic acid (6.22%), and 2,4-di-tert-butylphenol (2.45%). In the ethanolic extract, nineteen compounds were identified, with ethyl oleate as the predominant compound (50.97%), followed by glyceryl monooleate (22.61%), ethyl palmitate (11.10%), 1-(+)-ascorbic acid 2,6-dihexadecanoate (5.59%), ethyl stearate (4.17%), and glyceryl monopalmitate (2.18%). Other minor compounds detected in the ethanolic extract included thymoquinone, carvacrol, 2,4-di-tert-butylphenol, p-cymene-2,5-diol, ethyl myristate, ethyl arachidate, ethyl behenate, glyceryl monostearate, propyl linoleate, tetradecanoic acid, and pentadecanoic acid ethyl ester. Across both extracts, fatty acids and their ester derivatives constituted the dominant chemical class, followed by terpenoids and phenolic compounds.

Table 1: Determination of Zone of inhibition (mm) and Minimum Inhibitory Concentration (mg/mL) of Nigella sativa seed extracts exhibiting antibacterial activity against food spoilage bacteria.

Extracts	*Klebsiella oxytoca*		*Enterobacter mori*		*Serratia marcesens*		*Bacillus subtilis*		*Bacillus inaquosorum*	
	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC
Hexane	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Acetone	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Ethanol	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Aqueous	21.67± 0.33	7.5	19.0±1.15	7.5	18.75 ±0.63	7.5	15.7±0.67	2.5	19.83 ±1.09	5.0
DMSO	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Acetic acid (5 %)	46.3±0.88	-	38.0±1.73	-	52.7±1.45	-	42.7±1.45	-	48.0±0.58	-

Data are means of three replicates (n=3)±standard error.

Table 2: Determination of Zone of inhibition (mm) and Minimum Inhibitory Concentration (mg/mL) of Nigella sativa seed extracts exhibiting antifungal activity against food spoilage fungi.

Extracts	*Penicillium citrinum*		*Alternaria alstroemeriae*		*Aspergillus welwitschiae*		*Aspergillus awamori*		*Aspergillus aflatoxiformans*	
	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC	Inhibition zone (mm)	MIC
Hexane	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	9.3±0.33	-	11.3±0.33	-
Acetone	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Ethanol	17.6±0.44	0.75	0.0±0.0	-	14.33 ±1.45	1.0	13.17±0.6	0.5	16.33 ±1.45	2.5
Aqueous	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
DMSO	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-	0.0±0.0	-
Sodium benzoate (5 %)	0.0±0.0	-	0.0±0.0	-	14.3±0.88	-	8.0±0.0	-	0.0±0.0	-

Data are means of three replicates (n=3)±standard error.

Table 3: GC-MS analysis of phytochemicals in the aqueous extract of *Nigella sativa* seeds and their biological activity.

Sl. No.	RT	Name of compound	Mol. formula	MW	Peak Area %	Nature of Compound	Biological activity	References
1.	8.658	Thymoquinone	$C_{10}H_{12}O_2$	164.204	14.95	Monoterpene	Antibacterial, antioxidant, anti-inflammatory, anticancer, antidiabetic.	(Gali-Muhtasib *et al.*, 2008; Gali-Muhtasib *et al.*, 2006; Khader and Eckl, 2014)
2.	12.642	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	2.45	Phenolic	Antioxidant activity, Antibacterial, Antifungal, Anti-inflammatory, Antiviral, Cytotoxic, Insecticidal, Nematicidal.	(Zhao *et al.*, 2020)
3.	13.258	p-Cymene-2,5-diol (Thymoquinol)	$C_{10}H_{14}O_2$	166.22	24.06	Monoterpene	Antimicrobial, antioxidant, anti-inflammatory.	(Kokosaka *et al.*, 2008; Halawani, 2009)
4.	15.722	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228.37	6.22	Fatty acid	Antioxidant, Anticancer, Hypocholesterolemic	(Arora and Kumar, 2018)
5.	18.276	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256.42	27.42	Fatty acid	Antioxidants, anti-inflammatory, antimicrobial.	(Aparna *et al.*, 2012; Purushothaman *et al.*, 2024)
6.	20.692	9,12-Octadecadienoic acid (Linoleic acid)	$C_{18}H_{32}O_2$	280.45	15.26	Fatty acid	Antimicrobial, Antioxidant, AntiInflammatory, Anticarcinogenic, Antiarthritic, Hepatoprotectiv, Anti-histaminic, Anticoronary.	(Arora and Kumar, 2018; Chirumamilla *et al.*, 2022; Singh *et al.*, 2014; Arora and Meena, 2017)
7.	20.776	9-Octadecanoic acid, (E) (Oleic acid)	$C_{18}H_{34}O_2$	282.46	8.15	Fatty acid	AntiInflammatory, Antibacterial, Antiarthritic, Hepatoprotectiv, Anti-histaminic, Anticoronary.	(Arora and Kumar, 2018)

RT- Retention Time, MW- Molecular Weight of the compound.

Table 4: GC-MS analysis of phytochemicals in the ethanolic extract of *Nigella sativa* seeds and their biological activity.

Sl. No.	RT	Name of compound	Mol. formula	MW	Peak Area %	Nature	Biological activity	References
1.	8.661	Thymoquinone	$C_{10}H_{12}O_2$	164.204	0.16	Monoterpenoid	Antibacterial, antioxidant, anti-inflammatory, anticancer, antidiabetic.	(Gali-Muhtasib *et al.*, 2008; Gali-Muhtasib *et al.*, 2006; Mathur *et al.*, 2011; Khader and Eckl, 2014)
2.	9.502	Phenol,2-methyl-5-(1-methylethyl) (Carvacrol)	$C_{10}H_{14}O$	150.22	0.09	Phenolic	Antimicrobial, antioxidant, anti-inflammatory, antiviral, insecticidal, antitumor, antimutagenic, neuroprotective.	(Mohammedi, 2017; Maczka *et al.*, 2023)
3.	12.645	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	0.10	Phenolic	Antioxidant activity, Antibacterial, Antifungal, Anti-inflammatory, Antiviral, Cytotoxic, Insecticidal, Nematicidal.	(Zhao *et al.*, 2020)
4.	13.262	p-Cymene-2,5-diol (Thymoquinol)	$C_{10}H_{14}O_2$	166.22	0.07	Phenolic	Antimicrobial, antioxidant, anti-inflammatory.	(Kokosaka *et al.*, 2008; Halawani, 2009)
5.	15.744	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228.37	0.26	Fatty acid	Antioxidant, Anticancer, Hypocholesterolemic.	(Arora and Kumar, 2018)
6.	16.134	Tetradecanoic acid, ethyl ester (Ethyl myristate)	$C_{16}H_{32}O_2$	256.42	0.55	Fatty acid ethyl ester	No activity reported.	-
7.	17.389	Pentadecanoic acid, ethyl ester (Ethyl pentadecanoate)	$C_{17}H_{34}O_2$	270.45	0.09	Fatty acid ethyl ester	No activity reported.	-
8.	18.450	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{44}H_{74}O_{14}$	772.98	5.59	Fatty acid	Antioxidant, Antimicrobial.	(Dinde *et al.*, 2018)
9.	18.813	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	284.48	11.10	Fatty acid ethyl ester	Antioxidant, Nematicide, Insecticide, Lubricant, Antiandrogenic, Hemolytic, Hypo -cholesterolemic.	(Arora and Kumar, 2018)
10.	19.788	(Z)-Ethyl heptadec-9-enoate	$C_{19}H_{36}O_2$	296.49	0.19	Fatty acid ester	No activity reported.	-
11.	21.443	Ethyl Oleate	$C_{20}H_{38}O_2$	310.52	50.97	Fatty acid ester	Antibacterial.	(Akin-Osanaiye *et al.*, 2011)
12.	21.682	Octadecanoic acid, ethyl ester (Ethyl stearate)	$C_{20}H_{40}O_2$	312.53	4.17	Fatty acid ester	No activity reported.	-

Sl. No.	RT	Name of compound	Mol. formula	MW	Peak Area %	Nature	Biological activity	References
13.	22.549	Trans, trans-9,12-Octadecadienoic acid, propyl ester (Propyl linoleate)	$C_{21}H_{38}O_2$	322.53	0.17	Fatty acid ester	No activity reported.	-
14.	24.549	Eicosanoic acid, ethyl ester (Ethyl arachidate)	$C_{22}H_{44}O_2$	340.59	0.77	Fatty acid ester	No activity reported.	
15.	26.297	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Glyceryl monopalmitate)	$C_{19}H_{38}O_4$	330.50	2.18	Fatty acid ester	Antioxidant.	(Arora and Kumar, 2018)
16.	27.473	Docosanoic acid, ethyl ester (Ethyl behenate)	$C_{24}H_{48}O_2$	368.64	0.17	Fatty acid ester	No activity reported	-
17.	29.053	9-Octadecanoic acid (Z)-2,3-dihydroxypropyl ester (Glyceryl monooleate)	$C_{21}H_{40}O_4$	356.54	22.61	Fatty acid	No activity reported	-
18.	29.283	Octadecanoic acid, 2,3-dihydroxypropyl ester (Glyceryl monostearate)	$C_{21}H_{42}O_4$	358.57	0.67	Fatty acid	Anticancer, Antimicrobial.	(Arora and Kumar, 2018)

RT- Retention Time, MW- Molecular weight of the compound.

DISCUSSION

N. sativa seed extracts demonstrated significant efficacy in inhibiting the growth of food spoilage microorganisms, with the inhibitory effect varying according to the solvent polarity used for extraction. The aqueous extract exhibited broad-spectrum antibacterial activity against all tested Gram-positive and Gram-negative bacteria, while the ethanolic extract showed selective antifungal activity against four of five tested fungal isolates. The differential antimicrobial activity observed across solvent extracts can be attributed to the varying polarity of solvents. Polar solvents such as water preferentially extract hydrophilic compounds including phenolic acids, flavonoids, and polysaccharides that contribute to antibacterial activity, while ethanol, being intermediate in polarity, efficiently extracts terpenoids, fatty acid esters, and lipophilic phenolics that are more effective against fungal cell membranes (De *et al.*, 2019). The absence of antimicrobial activity in acetone and hexane extracts, with the exception of limited antifungal activity observed in the hexane extract against *Aspergillus awamori* and *Aspergillus aflatoxiformans*, suggests that the non-polar fraction of *N. sativa* seeds contain fewer bioactive compounds relevant to food spoilage microorganisms under the tested conditions. The tested microorganisms were isolated from spoiled fruits and oilseeds and are commonly associated with food-borne diseases and the production of harmful metabolites and mycotoxins that pose significant risks to human health (Rashmi *et al.*, 2024). The results of the present study are consistent with previous reports attributing the broad-spectrum antimicrobial activity of *N. sativa* seed extracts against Gram-positive and Gram-negative bacteria, human pathogens, phytopathogenic fungi, and dermatophytes to their constituent bioactive compounds (Singh *et al.*, 2014; Elgorban *et al.*, 2014; Shohayeb and Halawani, 2012; Aljabre *et al.*, 2005).

GC-MS analysis revealed a diverse array of chemical constituents including terpenoids, phenolics, fatty acids, and their derivatives in both aqueous and ethanolic extracts of *N. sativa* seeds, findings that are consistent with previous literature on phytochemical profiles of *N. sativa* (Javed *et al.*, 2012; Bawani and Anandhi, 2021; Singh *et al.*, 2014). The dominance of fatty acids and their ethyl ester derivatives in both extracts, particularly in the ethanolic extract, suggests that lipid-soluble bioactive compounds play a significant role in the observed antimicrobial properties of *N. sativa* seeds.

Thymoquinone, identified in both aqueous and ethanolic extracts, is the major bioactive monoterpenoid constituent of *N. sativa* essential oil and has been reported for its antibacterial and antifungal activity against a broad spectrum of pathogens, as well as its capacity to inhibit biofilm formation (Gali-Muhtasib *et al.*, 2008; Chaeib *et al.*, 2011; Nainawat *et al.*, 2025). Carvacrol, a monoterpenoid phenol identified in the ethanolic extract and known to co-occur with its isomer thymol in the essential oils of several aromatic plants including *N. sativa*, possesses potent antimicrobial activity against a wide range of disease-causing bacteria and fungi, likely through membrane disruption and inhibition of cellular respiration (Maczka *et al.*, 2023).

2,4-Di-tert-butylphenol, a phenolic compound widely identified in various plants and microorganisms, has demonstrated efficacy against Gram-positive bacteria including *Streptococcus* spp. and *Bacillus* spp., as well as root rot-causing fungi such as *Phytophthora* spp., *Fusarium* spp., and *Verticillium* spp., in addition to exhibiting antioxidant, antiviral, anti-inflammatory, insecticidal, nematicidal, and cytotoxic properties (Zhao *et al.*, 2020).

Among the identified fatty acid compounds, n-hexadecanoic acid (palmitic acid), previously reported in the medicinal plant *Excoecaria agallocha*, has demonstrated inhibitory activity against *Bacillus subtilis, Aeromonas hydrophila, Escherichia coli, Vibrio harveyi*, and *Klebsiella pneumoniae*, along with antioxidant and anti-inflammatory properties (Aparna *et al.*, 2012; Purushothaman *et al.*, 2024). 9,12-Octadecadienoic acid (linoleic acid), identified in the aqueous extract and also reported in other medicinal plants, exhibits antioxidant, antimicrobial, anti-carcinogenic, antiarthritic, hepatoprotective, anti-histaminic, and anti-coronary activities (Kapoor *et al.*, 2014; Arora and Kumar, 2018; Chirumamilla *et al.*, 2022). Ethyl oleate (9-octadecanoic acid ethyl ester), the dominant compound in the ethanolic extract (50.97%), has been identified in the essential oil of *Phyllanthus amarus* and other medicinal plants and reported to exhibit antibacterial activity against *Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Micrococcus luteus*, and *Klebsiella pneumoniae* (Akin-Osanaiye *et al.*, 2011). Glyceryl monopalmitate, a fatty acid ester, has been reported to possess antioxidant, antiandrogenic, hypocholesterolemic, hemolytic, and alpha reductase inhibitor properties (Arora and Kumar, 2018), while glyceryl monostearate, a monoacylglycerol widely used as a food additive, exhibits

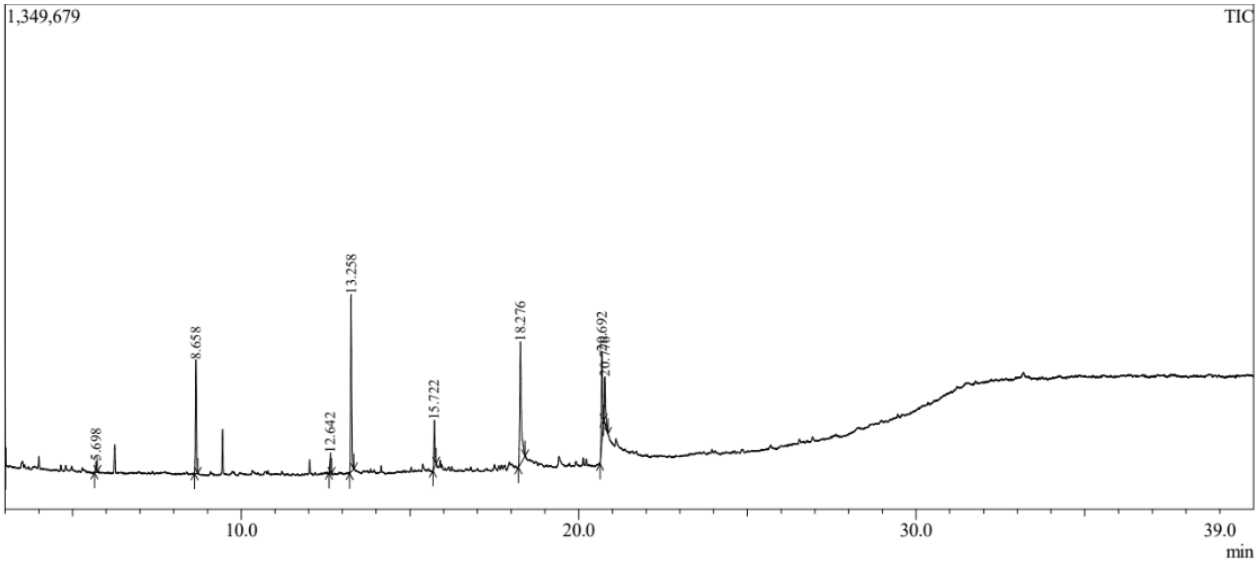

Figure 1: GC-MS Chromatogram of Aqueous Extract of *Nigella sativa* seeds.

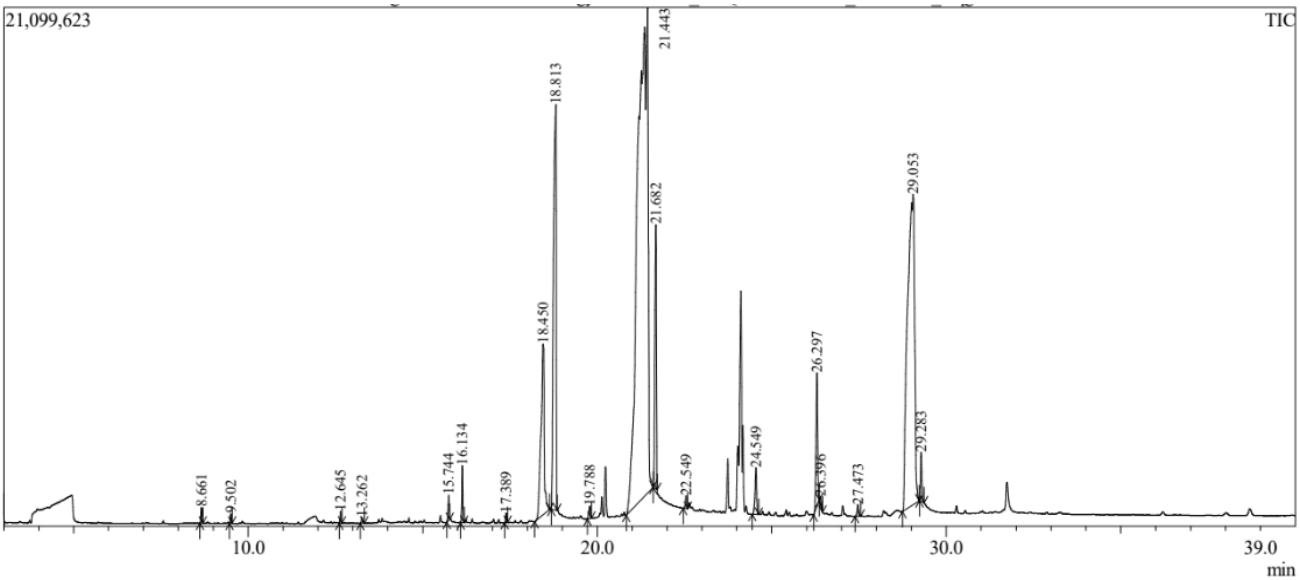

Figure 2: GC-MS Chromatogram of Ethanolic Extract of *Nigella sativa* seeds.

antimicrobial and anticancer activities (Arora and Kumar, 2018). 1-(+)-Ascorbic acid 2,6-dihexadecanoate, a lipophilic ascorbic acid derivative identified in the ethanolic extract, has been reported as an effective antimicrobial agent (Dinde *et al.*, 2018).

The antimicrobial efficacy of these phytocompounds can be attributed to their ability to interfere with several microbial cellular processes, including disruption of cell membrane integrity and permeability, inhibition of cell wall biosynthesis, impairment of protein synthesis, and alteration of structural composition, collectively leading to cell lysis and death (Jesmin-Akter *et al.*, 2019; Mohammed *et al.*, 2019; Maczka *et al.*, 2023; Rashmi *et al.*, 2025). The synergistic interaction among multiple bioactive compounds present in the extracts may further potentiate their overall antimicrobial efficacy, as has been demonstrated for essential oil components of *N. sativa* in previous studies.

CONCLUSION

The present study demonstrates that the aqueous and ethanolic extracts of *Nigella sativa* seeds possess significant antimicrobial activity against food spoilage bacteria and fungi, respectively, with MIC values ranging from 2.5 to 7.5 mg/mL for bacteria and 0.5 to 2.5 mg/mL for fungi. GC-MS analysis confirmed the presence of diverse bioactive phytochemicals in both extracts, including thymoquinone, carvacrol, 2,4-di-tert-butylphenol, n-hexadecanoic acid, linoleic acid, ethyl oleate, and glyceryl monooleate, which are well-documented for their antimicrobial, antioxidant, and anti-inflammatory properties. The lower MIC values recorded for fungal isolates suggest that *N. sativa* seed extracts are particularly effective against food spoilage fungi. The natural origin of these bioactive compounds supports their potential as safe alternatives to synthetic preservatives in the food industry. Based on these findings, *N. sativa* seed extracts, particularly the aqueous and ethanolic fractions, is a promising alternative for application in natural food preservation against spoilage microorganisms. These extracts can be further isolated and purified to determine their synergistic interactions, evaluation of their safety through cytotoxicity and *in vivo* studies and assessment of their efficacy in food biopreservation.

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ABBREVIATIONS

NSS: *Nigella sativa* seeds; **DMSO:** Dimethyl sulfoxide; **MIC:** Minimum Inhibitory Concentration; **SEM:** Standard Error Mean; **GC-MS:** Gas Chromatography- Mass Spectrometry; **MW:** Molecular weight; **RT:** Retention Time; **NIST:** National Institute Standard and Technology; **HPLC:** High-Performance Liquid Chromatography; **CLSI:** Clinical and Laboratory Standards Institute.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Nigella sativa, also known as black cumin, and a member of the Ranunculaceae family have been valued both as a traditional medicine as well as flavoring agents for many years. Despite its diverse therapeutic properties, the potential of *N. sativa* seeds in controlling food spoilage pathogens is still an ongoing investigation. This study confirmed the antimicrobial properties of *N. sativa* seeds extract against food spoilage bacteria and fungi isolated from different spoiled foods. The bioactive compounds responsible for inhibition of spoilage microorganisms was further identified using GC-MS analysis. These phytochemicals can be purified from *N. sativa* seeds to evaluate their antimicrobial activity against food pathogens. It can also be applied as natural preservatives to reduce post-harvest losses and provide crop protection against phytopathogens.

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