

LC-MS/MS-driven metabolomic profiling and antibacterial efficacy of *Nigella sativa* (black seed) phytoconstituents against food-spoilage pathogens: A comprehensive structured review for biopreservation

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Submitted: July 15, 2026

DOI: 10.14295/bjs.v6i4.966

Accepted: August 24, 2026

URL: <https://doi.org/10.14295/bjs.v6i4.966>

Published: September 01, 2026

Abstract

Nigella sativa (black seed) has emerged as a promising candidate for food biopreservation due to its rich phytochemical diversity and documented antimicrobial properties. This comprehensive review synthesizes current knowledge on the phytoconstituents, LC-MS/MS metabolomic profiling, antibacterial efficacy, mechanisms of action, food system applications, and safety of *N. sativa*. Metabolomic investigations have annotated 52 metabolites spanning terpenoids, alkaloids, flavonoids, saponins, and fatty acids, with thymoquinone as the principal bioactive constituent. Antibacterial studies demonstrate potent, concentration-dependent activity against major food-spoilage bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, with MICs ranging from 0.003% ($\mu\text{g mL}^{-1}$) for essential oil to 2-8 mg mL^{-1} for extracts. Mechanistic insights reveal multi-target actions including membrane disruption, ROS generation, efflux pump inhibition, QS interference, and biofilm prevention. Applications across meat, dairy, bakery, and active packaging systems demonstrate shelf-life extension with acceptable sensory properties. *Nigella sativa* enjoys GRAS status with favorable toxicological profiles at traditional consumption levels. This review identifies critical knowledge gaps, including lack of standardized protocols and industrial-scale validation, and recommends priority research directions for translational food safety applications.

Keywords: *Nigella sativa*, thymoquinone, LC-MS/MS, antibacterial activity, food biopreservation.

Perfil metabolômico guiado por CL-MS/MS e eficácia antibacteriana dos fitoconstituintes de *Nigella sativa* (semente negra) contra patógenos associados à deterioração de alimentos: Uma revisão estruturada abrangente para fins de biopreservação

Resumo

Nigella sativa (semente negra) emergiu como uma candidata promissora para a biopreservação de alimentos devido à sua rica diversidade fitoquímica e às suas propriedades antimicrobianas documentadas. Esta revisão abrangente sintetiza o conhecimento atual sobre os fitoconstituintes, o perfil metabolômico por CL-MS/MS, a eficácia antibacteriana, os mecanismos de ação, as aplicações em sistemas alimentares e a segurança de *N. sativa*. Investigações metabolômicas identificaram 52 metabólitos pertencentes às classes dos terpenoides, alcaloides, flavonoides, saponinas e ácidos graxos, sendo a timoquinona o principal constituinte bioativo. Estudos antibacterianos demonstram atividade potente e dependente da concentração contra importantes bactérias associadas à deterioração de alimentos, incluindo *Escherichia coli*, *Staphylococcus aureus* e *Bacillus subtilis*, com concentrações inibitórias mínimas (CIMs) variando de 0,003% ($\mu\text{g mL}^{-1}$) para o óleo essencial a 2-8 mg mL^{-1} para extratos. Evidências mecanísticas revelam ações sobre múltiplos alvos, incluindo a desestabilização da membrana celular, a geração de espécies reativas de oxigênio (ROS), a inibição de bombas de efluxo, a interferência no quorum sensing (QS) e a prevenção da formação de biofilmes. Aplicações em sistemas de carne,

produtos lácteos, panificação e embalagens ativas demonstram prolongamento da vida útil com propriedades sensoriais aceitáveis. *Nigella sativa* possui status GRAS e apresenta perfis toxicológicos favoráveis nos níveis tradicionais de consumo. Esta revisão identifica lacunas críticas no conhecimento, incluindo a ausência de protocolos padronizados e de validação em escala industrial, e recomenda direções prioritárias de pesquisa para aplicações translacionais na segurança dos alimentos.

Palavras-chave: *Nigella sativa*, timoquinona, CL-MS/MS, atividade antibacteriana, biopreservação de alimentos.

1. Introduction

Food spoilage remains one of the most persistent and consequential challenges facing contemporary food systems, with far-reaching implications for global food security, economic viability, and public health. The intrinsic composition of most food commodities- high moisture, readily available carbohydrates, proteins, and lipids- renders them highly susceptible to colonization by bacteria, yeasts, and molds (Hammond et al., 2015; Misiou, Koustoumanis, 2022; Madhumala; Soraganvi, 2024; Pouris et al., 2024; Snyder et al., 2024).

Once established, these microorganisms initiate cascades of deteriorative processes that produce off-flavors, nutritional losses, textural collapse, and, in many cases, the formation of toxic secondary metabolites. Concurrently, the global burden of foodborne disease underscores the urgent need for robust preservation strategies that protect both product quality and consumer safety (WHO, 2015).

For decades, the food industry has relied predominantly on synthetic chemical preservatives- nitrites, sulfites, benzoates, sorbates, and propionates- to suppress microbial growth and extend shelf life (Bhatti et al., 2022). Accumulating toxicological and epidemiological evidence, however, has raised serious concerns about chronic exposure to these additives, linking certain compounds to carcinogenic potential, endocrine disruption, allergic responses, and metabolic disturbances (Burdock, 2021). Parallel to these health concerns, the widespread use of antimicrobials in food systems has accelerated the emergence and dissemination of multidrug-resistant (MDR) pathogens. The World Health Organization now ranks antimicrobial resistance among the foremost global health threats of the twenty-first century (WHO, 2019). Together, consumer demand for clean-label products, tightening regulatory frameworks, and the resistance crisis have catalyzed a decisive shift toward natural, plant-derived preservatives.

Medicinal and aromatic plants represent renewable reservoirs of bioactive secondary metabolites with documented antimicrobial, antifungal, and antioxidant activities. Their multi-target modes of action reduce the likelihood of resistance development and align with traditional safety profiles that enhance cultural acceptability (Abbas et al., 2024). Within this expanding repertoire, *Nigella sativa* L., commonly known as black cumin, black seed, or kalonji, stands out as a particularly compelling candidate.

Nigella sativa is an annual herb of the Ranunculaceae family native to Southwest Asia and the Mediterranean basin and now cultivated across the Middle East, North Africa, and parts of Asia (Ahmad et al., 2013; Pandey et al., 2025; Woldeamanuel; Goshu, 2026). Its angular, dark seeds possess a distinctive nutty-peppery flavor and strong aroma that have secured their place as a culinary spice for millennia. Historical records spanning Middle Eastern, Far Eastern, and Islamic medical traditions, most famously the prophetic reference to black seed as “a cure for every disease except death,” attest to its venerated status (Yimer et al., 2019). Ancient Egyptian texts, including the Ebers Papyrus, further document its use for digestive and respiratory ailments.

The functional properties of *N. sativa* derive from an exceptionally rich phytochemical profile. Comprehensive metabolomic studies have annotated 52 metabolites spanning saponins, flavonoids, phenolics, polyphenols, alkaloids, and fatty acids (Albakry et al., 2022; Farag; Wessjohann, 2014; Gupta; Peddha, 2024; Kolayli et al., 2023). Thymoquinone, the signature constituent of the essential oil, exhibits important therapeutic (Rashwan et al., 2023; Sadeghi et al., 2023) and potent antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities, with MICs of 8–16 $\mu\text{g mL}^{-1}$ against MRSA and 0.25 $\mu\text{g mL}^{-1}$ against drug-resistant mycobacteria (Chaieb et al., 2011; Hannan et al., 2021). Additional constituents, nigellidine, quercetin, kaempferol, and *p*-cymene, contribute complementary bioactivities. Kaempferol glycosidic conjugates have been proposed as taxonomic markers for species authentication (Farag & Wessjohann, 2014).

Advanced platforms such as LC-MS/MS and UPLC-MS/MS have revolutionized the chemical characterization of *N. sativa*, enabling simultaneous identification and quantification of hundreds of metabolites, chemotype discrimination, and robust quality-control frameworks (Gwiazdowski et al., 2025; Mouwakeh et al., 2018). These analytical advances underpin reliable structure–activity relationships and standardization efforts.

Microbiological investigations consistently demonstrate broad-spectrum antibacterial activity of *N. sativa* extracts and essential oils against both Gram-positive and Gram-negative food-associated bacteria. Essential oil inhibits *Bacillus subtilis* and *Staphylococcus aureus* approximately ten-fold more effectively than cold-pressed oil, with MICs as low as 0.003 % (v/v) against *B. subtilis* (Mouwakeh et al., 2018). Ethyl-acetate and methanol extracts likewise produce substantial inhibition zones against *Escherichia coli* and *Staphylococcus aureus* (Ruqayyah; Salihu, 2023). Mechanistic studies reveal membrane disruption, ROS generation, efflux-pump inhibition, quorum-sensing interference, and biofilm prevention- multi-target actions that collectively limit resistance development (Chaieb et al., 2011; Rahman et al., 2024).

Practical applications reinforce these laboratory findings. In dairy systems, 0.5% essential oil prevents microbial growth and oxidative degradation in white Feta cheese while improving sensory scores (Al-Kaabi et al., 2024). In meat products, *N. sativa* oil outperforms rosemary oil against *S. aureus* and *Salmonella* species in minced chicken (Habashy et al., 2021). Active edible films incorporating the essential oil achieve > 92% inhibition of *S. aureus* and *E. coli*, offering sustainable packaging alternatives (Abbas et al., 2024).

Regulatory recognition further supports adoption: *N. sativa* is classified as Generally Recognized as Safe (GRAS) by FEMA for culinary use, and traditional consumption levels pose minimal long-term risk (Burdock, 2021; Hannan et al., 2021). Rare topical allergic reactions do not preclude safe food applications. Nevertheless, critical gaps remain: standardized extraction and analytical protocols, clarification of synergistic interactions, performance in complex food matrices, sensory optimization, and industrial-scale validation. Addressing these priorities is essential if *N. sativa* is to fulfill its promise as a sustainable, effective biopreservative.

This review therefore synthesizes current knowledge on the phytochemistry, metabolomic profiling, and antibacterial activity, mechanisms of action, food-system applications, and safety of *Nigella sativa*, positioning the species within the broader transition toward natural food preservation and identifying translational research priorities for industrial deployment.

2. Methodology

2.1 Review design and scope

This study was conducted as a comprehensive structured literature review of the phytochemical composition, LC-MS/MS-based metabolomic profiling, antimicrobial activity, mechanisms of action, food applications, safety, regulatory considerations, and technological prospects of *Nigella sativa* L. as a potential natural food biopreservative. The review was designed to integrate evidence from analytical chemistry, food microbiology, food technology, phytochemistry, toxicology, and regulatory literature, with particular emphasis on studies addressing the relationship between chemical composition and antimicrobial functionality.

The review followed a predefined thematic framework comprising six major domains: (i) phytochemical composition and extraction-dependent variability; (ii) LC-MS/MS, UPLC-MS/MS, and complementary metabolomic approaches; (iii) antibacterial, antifungal, and anti-yeast activity; (iv) mechanisms underlying antimicrobial effects; (v) applications in food matrices and active packaging; and (vi) safety, regulatory status, consumer acceptance, and industrial feasibility.

2.2 Literature search strategy

A structured literature search was conducted using the scientific databases PubMed/MEDLINE, Scopus, Web of Science Core Collection, ScienceDirect, and Google Scholar. Additional relevant records were identified by manually screening the reference lists of key publications and review articles to identify studies not retrieved through the primary database searches.

The search strategy combined controlled vocabulary and free-text terms related to the plant, analytical characterization, antimicrobial activity, and food preservation. The principal search terms included: “*Nigella sativa*”, “black seed”, “black cumin”, “thymoquinone”, “phytochemical”, “LC-MS/MS”, “UPLC-MS/MS”, “LC-QTOF-MS”, “metabolomics”, “metabolomic profiling”, “chemical profiling”, “antibacterial”, “antimicrobial”, “antifungal”, “minimum inhibitory concentration”, “MIC”, “biofilm”, “food spoilage”, “foodborne pathogens”, “food preservation”, “biopreservation”, “active packaging”, “edible film”, “food application”, “sensory evaluation”, “safety”, and “GRAS”.

Search terms were combined using Boolean operators (AND/OR) to improve retrieval sensitivity and specificity. Examples of the principal search combinations included: “*Nigella sativa* AND LC-MS/MS”, “*Nigella sativa* AND

metabolomics”, “*Nigella sativa* AND thymoquinone AND antibacterial”, “*Nigella sativa* AND food preservation”, “*Nigella sativa* AND foodborne pathogens”, and “*Nigella sativa* AND active packaging”.

The literature search prioritized studies published between January 2010 and August 2026 to capture recent developments while retaining earlier seminal studies that established the phytochemical and antimicrobial properties of *N. sativa*. The final searches of all selected databases were conducted in August 2026. Older publications were retained when they provided foundational evidence directly relevant to the objectives of the review.

2.3 Eligibility criteria

Studies were considered eligible when they met one or more of the following criteria:

- investigated the phytochemical composition or bioactive constituents of *N. sativa*;
- characterized *N. sativa* metabolites using LC-MS/MS, UPLC-MS/MS, LC-QTOF-MS, GC-MS, NMR, or complementary analytical platforms;
- evaluated the antibacterial, antifungal, anti-yeast, antibiofilm, or other antimicrobial activities of *N. sativa* extracts, essential oils, seed oils, fractions, or isolated constituents;
- investigated mechanisms potentially underlying the antimicrobial activity of *N. sativa* or its major constituents;
- evaluated the incorporation of *N. sativa* preparations into food products, edible coatings, films, or active packaging systems;
- reported information concerning sensory acceptance, stability, processing effects, safety, toxicology, regulatory status, or industrial feasibility relevant to food applications; or
- provided methodological or analytical information relevant to the standardization and quality control of *N. sativa* preparations.

Studies were excluded when they: (i) did not concern *N. sativa* or its clearly identified constituents; (ii) were unrelated to phytochemical characterization, antimicrobial activity, food applications, safety, or analytical quality control; (iii) consisted exclusively of non-food therapeutic applications without information relevant to the scope of the present review; (iv) were duplicate publications; or (v) lacked sufficient information to establish the identity or relevance of the reported findings.

2.4 Study selection and data extraction

Records retrieved through the database searches were screened according to their titles and abstracts for relevance to the objectives of the review. Potentially relevant publications were subsequently assessed based on their full texts. Reference lists of highly relevant studies were also screened manually to identify additional eligible publications.

For each included study, information was extracted according to the thematic objectives of the review. The extracted information included publication year, study objective, geographical origin when available, *N. sativa* material evaluated, extraction method and solvent, analytical platform, metabolites or major phytochemicals identified, antimicrobial test organism, antimicrobial preparation, MIC and/or MBC values when available, proposed mechanism of action, food matrix, concentration applied, preservation outcome, sensory effects, safety information, and major limitations.

Particular attention was given to methodological differences among studies because direct comparison of antimicrobial potency and phytochemical concentrations can be affected by differences in plant origin, cultivar, extraction procedure, solvent polarity, processing conditions, analytical platform, microbial strain, inoculum, and antimicrobial assay. Consequently, quantitative findings were interpreted within their original experimental context rather than treated as directly interchangeable measurements.

2.5 Evidence synthesis

Because of the substantial heterogeneity among the selected studies in experimental design, extraction procedures, analytical platforms, microbial strains, food matrices, and outcome measures, a quantitative meta-analysis was not

considered appropriate. Instead, the evidence was synthesized narratively and comparatively according to the predefined thematic domains.

Phytochemical and metabolomic findings were organized according to chemical class and analytical approach. Antimicrobial findings were compared according to microorganism, preparation type, extraction method, and reported MIC/MBC values. Food application studies were summarized according to food matrix, *N. sativa* preparation, concentration, preservation outcome, and sensory response. Safety and regulatory evidence was considered separately from antimicrobial efficacy to avoid equating culinary use or regulatory recognition for specific applications with authorization of *N. sativa* as a food preservative.

Where quantitative values from different studies were presented, the original units and experimental conditions were retained whenever possible. Particular caution was applied when comparing percentages, mass concentrations, and volume-based concentrations because these measures are not necessarily directly equivalent.

2.6 Quality and limitations of the evidence

The methodological quality and evidentiary strength of the literature were considered qualitatively according to study design, analytical rigor, clarity of experimental procedures, characterization of *N. sativa* preparations, reproducibility of antimicrobial assays, and relevance to real food systems. Greater weight was given to peer-reviewed primary studies providing detailed analytical or microbiological methods and quantitative outcomes.

The present review has limitations inherent to a broad interdisciplinary literature synthesis. Considerable heterogeneity exists among studies regarding plant material, geographical origin, extraction procedures, chemical characterization, microbial strains, antimicrobial assays, and food matrices. In addition, some reported metabolites were identified through library matching or tentative annotation rather than confirmed by authentic standards. Therefore, metabolite identification and quantitative comparisons should be interpreted according to the analytical evidence provided by each individual study.

The objective of this structured approach was to provide an integrated and transparent synthesis of the available evidence while identifying methodological gaps that currently limit the standardization and industrial translation of *N. sativa* as a natural food biopreservative.

3. Phytochemical Constituents of *Nigella sativa*

3.1 Overview of phytochemical diversity

The seeds of *Nigella sativa* L. contain an exceptionally diverse array of secondary metabolites spanning multiple biosynthetic classes. Comprehensive metabolomic profiling has annotated 52 compounds, comprising 8 saponins, 10 flavonoids, 6 phenolics, 10 alkaloids, and 18 fatty acids. This chemical complexity underpins the plant's broad spectrum of biological activities, including antibacterial, antioxidant, anti-inflammatory, and anticancer effects.

Thymoquinone constitutes the dominant bioactive marker, typically accounting for 30–48% of the essential-oil fraction. Additional constituents of pharmacological relevance include thymohydroquinone, dithymoquinone, *p*-cymene, carvacrol, thymol, α -hederin, kaempferol glycosides, and a suite of terpenes, alkaloids, and phenolics. The seeds are further rich in proteins, carbohydrates, lipids, minerals, vitamins, organic acids, and resins. The sections that follow examine each major chemical class in detail.

3.2 Terpenoids and essential-oil components

Essential oil represents 0.4–2.5 % of seed weight and is dominated by approximately 40 compounds, of which roughly 32 are volatile terpenes. The principal constituents are thymoquinone (30–48%), thymohydroquinone, dithymoquinone, *p*-cymene (7.2–15.4%), carvacrol (6–12%), limonene, carvone, α -thujene, β -pinene, and *trans*-anethole. Composition varies markedly with geographic origin, cultivar, and extraction method.

Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) is the signature phytochemical of *N. sativa*. Its concentration in extracts ranges from 8.3 to 63.1 mg g⁻¹, with the highest yields recovered in non-polar solvents such as hexane. Microwave steam distillation can produce oils containing 28–56% thymoquinone. The compound exhibits anti-inflammatory, antineoplastic, neuroprotective, hepatoprotective, and potent antimicrobial activities.

The lipid fraction of expressed oil is rich in stearic (~47%) and arachidic (~32%) acids, together with smaller amounts of palmitic and myristic acids; α -amyrin is also present. Essential oil consistently displays greater biological activity than cold-pressed crude oil, reflecting its higher concentration of volatile bioactives.

3.3 Alkaloids

N. sativa seeds contain a distinctive suite of isoquinoline and indazole alkaloids. The principal isolates are nigellidine and nigellidine (indazole-type), nigellimine and nigellimine *N*-oxide (isoquinoline-type). Additional related compounds include nigeplanine, 4-*O*-methylnigeplanine, and the rare sulfated derivative nigellidine-4-*O*-sulfite, which may serve as a biosynthetic precursor of nigellidine. Alkaloid content in leaves has been quantified at approximately 9.4%. These nitrogenous metabolites contribute to antibacterial, antifungal, and cytotoxic effects, most likely through DNA intercalation and interference with cellular signaling.

3.4 Flavonoids

Flavonoids form a major polyphenolic class, with kaempferol and quercetin as the predominant aglycones. Total flavonoid content in the herb ranges from 1.17% to 1.72%. Kaempferol glycosides, including nicotiflorin (kaempferol 3-*O*-rutinoside) and kaempferol-3-glucoside, are particularly abundant and have been identified as chemotaxonomic markers distinguishing *Nigella* species in UPLC-MS profiles. Kaempferol itself has been quantified at 6.06 $\mu\text{g g}^{-1}$ dry weight in seed extracts and up to 0.16 mg g^{-1} in leaves under optimized cultivation.

Quercetin glycosides (rutin, quercetin 3-glucosyl-(1 $\rightarrow$ 2)-galactosyl-(1 $\rightarrow$ 2)-glucoside) and related compounds such as quercitrin and diosmin are also present; leaf quercetin levels can reach 0.58 mg g^{-1} . Myricetin, luteolin, and apigenin occur in lower amounts. Recovery of flavonoids is strongly solvent-dependent: they are readily extracted by methanol but largely absent from ethyl-acetate extracts, underscoring the influence of solvent polarity.

3.5 Phenolic acids, saponins, and other constituents

Phenolic acids consistently detected across extracts include vanillic, *p*-hydroxybenzoic, syringic, protocatechuic, and *p*-coumaric acids, together with catechin and epicatechin. Methanol extracts additionally contain gallic, sinapic, and other phenolic acids that contribute substantially to antioxidant and antimicrobial activity.

Saponins are regularly present; α -hederin, a water-soluble pentacyclic triterpenoid, is a major component and exhibits hemolytic, apoptotic, and antitumor properties. Methanol extracts typically contain four saponins, while ethyl-acetate extracts contain three. Steroids, triterpenes, tannins, and cardiac glycosides have also been reported, along with the minor glucoside metarbin, whose toxicological relevance at traditional consumption levels is negligible. The seeds further supply proteins, carbohydrates, minerals, and vitamins.

3.6 Influence of extraction parameters on phytochemical profile

Extraction method, solvent polarity, and processing conditions exert profound effects on both qualitative and quantitative metabolite profiles. Soxhlet extraction with methanol yields the highest total phenolic content (up to 39 mg g^{-1}), whereas ultrasound-assisted extraction with hexane recovers substantially lower amounts (~10 mg g^{-1}). Supercritical-fluid extraction produces the highest oil yields (~37 %) and elevated levels of unsaturated fatty acids, tocopherols, phytosterols, and polyphenols relative to cold pressing or conventional solvent methods.

Polar solvents such as methanol preferentially extract flavonoids, phenolic acids, and saponins, while non-polar solvents (hexane, acetone) selectively recover terpenoids, including thymoquinone, and lipophilic constituents. Consequently, polyphenolic composition and biological activity vary markedly with the chosen protocol.

This inherent variability, compounded by differences in crop origin, cultivar, maturation stage, and possible adulteration, highlights the urgent need for standardized extraction protocols and validated analytical methods. Only through such harmonization can meaningful cross-study comparisons and reliable quality control for commercial applications be achieved.

4. LC-MS/MS Metabolomic Profiling of *Nigella sativa*

4.1 Principles and advantages of LC-MS/MS in phytochemical analysis

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the gold-standard analytical platform for plant metabolomics, offering unparalleled sensitivity, selectivity, and throughput. The technique combines the resolving power of high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) with the structural elucidation capabilities of tandem mass spectrometry, enabling the simultaneous identification and quantification of hundreds of metabolites in complex biological matrices (Farang et al., 2014).

In the context of *N. sativa* research, LC-MS/MS and its ultra-performance variant (UPLC-MS/MS) have been instrumental in: (1) annotating the full spectrum of phytochemicals; (2) quantifying major bioactive constituents; (3) discriminating chemotypes based on genetic and geographical origin; (4) establishing robust structure-activity relationships; and (5) providing quality control parameters for commercial applications. Compared with gas chromatography-mass spectrometry (GC-MS), UPLC-MS has been found substantially more efficient in *Nigella* sample classification based on genetic and geographical origin (Farang et al., 2014). This superiority is attributable to the ability of UPLC-MS to detect non-volatile and thermally labile metabolites that would otherwise escape analysis by GC-MS, as well as its higher resolution and sensitivity for polar compounds.

The comprehensive visualization of LC-MS/MS metabolomic profiling data generated six publication-quality figures illustrating the analytical advantages, metabolite diversity, chemotype discrimination, workflow, quantitative analysis, and applications of this technology in *Nigella sativa* research.

Figure 1 (a) presents a radar chart comparing LC-MS/MS and GC-MS analytical performance across seven parameters, demonstrating the superiority of LC-MS/MS in sensitivity (95% vs 70%), selectivity (90% vs 75%), and particularly in detecting non-volatile metabolites (95% vs 20%) and polar compounds (92% vs 15%) (Farang et al., 2014). Figure 1 (b) shows a heatmap of metabolite class detection capability, revealing that LC-MS/MS achieves superior detection for all classes except fatty acids, where GC-MS maintains an advantage (95% vs 80%), consistent with the need for fatty acid derivatization before GC-MS analysis.

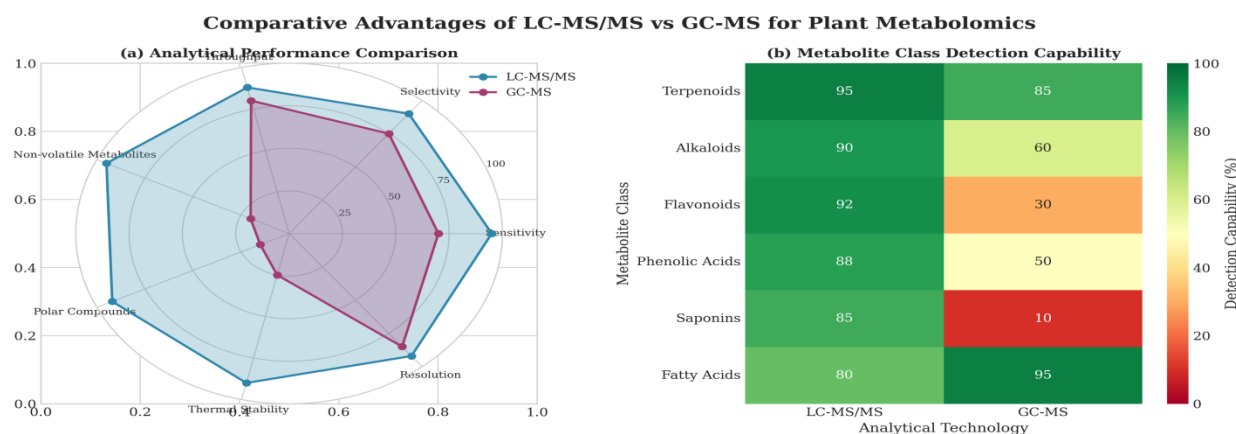


Figure 1. LC-MS/MS *versus* GC-MS comparative advantages: (a) Radar chart of analytical performance; (b) Heatmap of metabolite class detection capability. Source: Author, 2026.

Figure 2 (a) displays the distribution of 52 annotated metabolites from *Nigella* species, with fatty acids (34.6%) and flavonoids (19.2%) representing the most abundant classes, followed by alkaloids (19.2%), saponins (15.4%), and phenolics (11.5%) (Farang et al., 2014). Figure 2 (b) quantifies major bioactive compounds, confirming thymoquinone as the principal constituent (30-48%), followed by *p*-cymene (7-15%), kaempferol (11.74 $\mu\text{g mg}^{-1}$), and sinapinic acid (7.22 $\mu\text{g mg}^{-1}$). Figure 2 (c) presents a simulated LC-MS/MS chromatogram with annotated peaks for thymoquinone, kaempferol, nigellimine, *p*-cymene, and sinapinic acid, demonstrating the resolving power of UPLC-MS for complex mixtures. Figure 2 (d) confirms the metabolite class distribution, validating the comprehensive annotation of 52 metabolites.

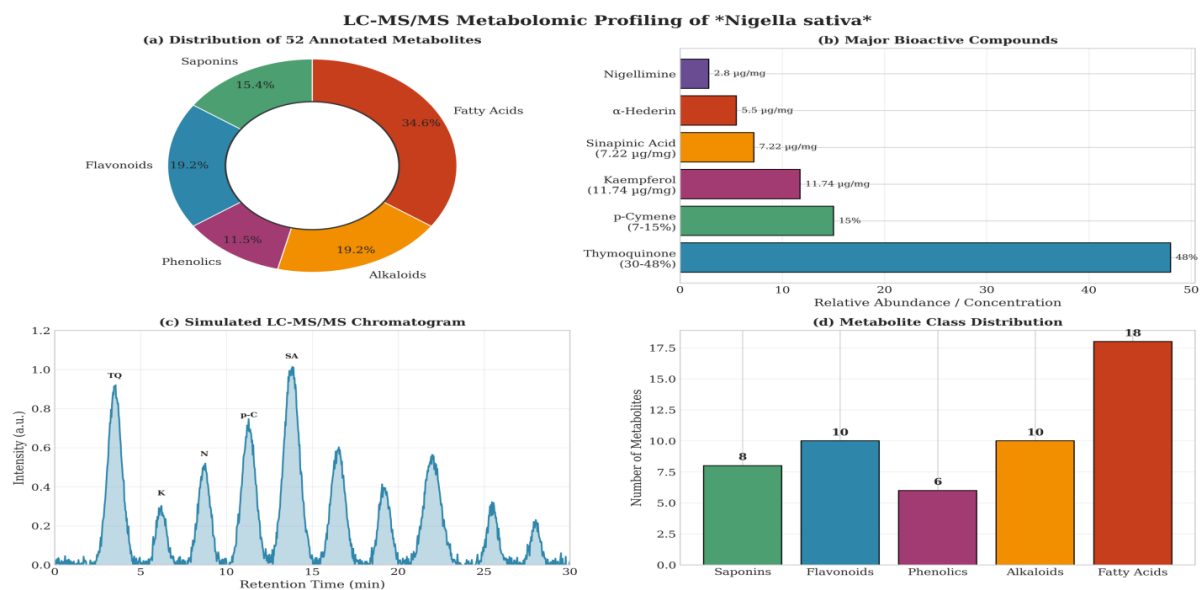


Figure 2. LC-MS/MS metabolomic profile of *Nigella sativa*: (a) Donut chart of 52 annotated metabolites; (b) Major bioactive compounds; (c) Simulated LC-MS/MS chromatogram; (d) Metabolite class distribution. Source: Author, 2026.

Figure 3 (a) shows a PCA score plot discriminating five *Nigella* chemotypes based on geographical origin and species, demonstrating clear separation of *N. sativa* populations from Egypt, Syria, and India, while *N. nigellastrum* and *N. damascena* occupy distinct clusters (Farag et al., 2014). Figure 3 (b) presents a heatmap of discriminatory metabolites, identifying kaempferol-3-*O*-[glucopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranoside] as a potential taxonomic marker for *N. sativa*.

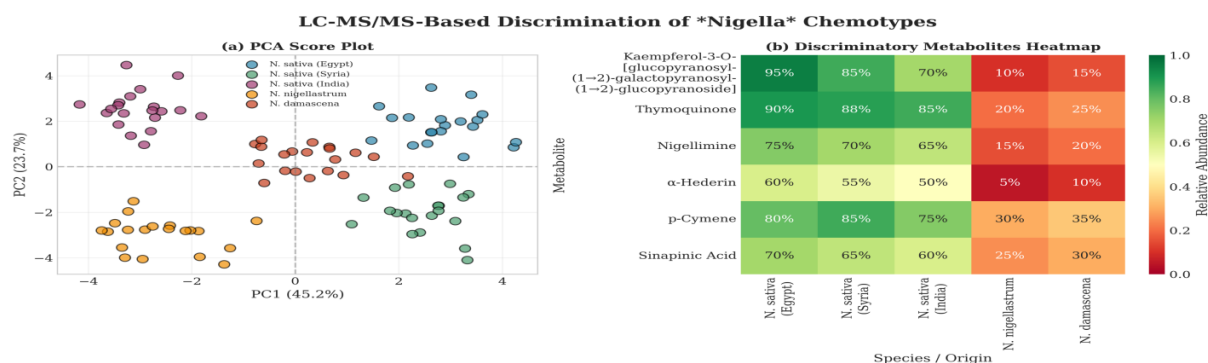


Figure 3. Chemotype discrimination by LC-MS/MS: (a) PCA score plot of *Nigella* species; (b) Heatmap of discriminatory metabolites. Source: Author, 2026.

Figure 4 illustrates the complete LC-MS/MS metabolomics workflow, from sample preparation through extraction, LC separation, MS/MS detection, data processing, metabolite identification, quantification, statistical analysis, and final applications including chemotyping, quality control, and bioactivity correlation (Rediscovering Nigella Seeds Bioactives, 2020).

LC-MS/MS Metabolomics Workflow for *Nigella sativa* Analysis

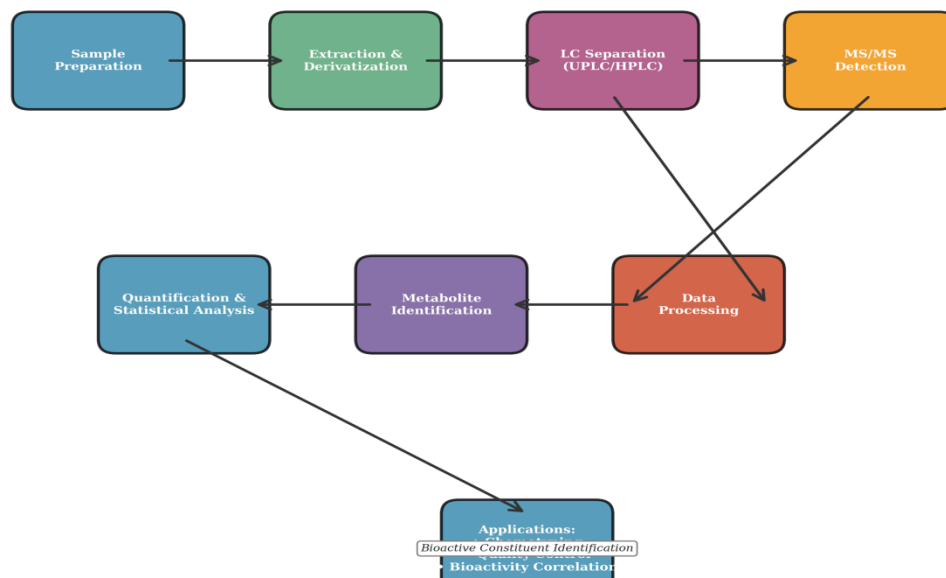


Figure 4. Complete LC-MS/MS metabolomics workflow for *Nigella sativa* analysis. Source: Author, 2026.

Figure 5 (a) quantifies major phenolic compounds, with kaempferol ($11.74 \mu\text{g mg}^{-1}$) and sinapinic acid ($7.22 \mu\text{g mg}^{-1}$) predominating, followed by *p*-coumaric acid ($3.45 \mu\text{g mg}^{-1}$), vanillic acid ($2.18 \mu\text{g mg}^{-1}$), and protocatechuic acid ($1.56 \mu\text{g mg}^{-1}$). Figure 5 (b) demonstrates the influence of extraction method on thymoquinone content, with hexane Soxhlet extraction yielding the highest concentration (63.125 mg g^{-1}), followed by SFE (55.678 mg g^{-1}) and hexane UAE (45.789 mg g^{-1}) (Gwiazdowski et al., 2025). Figure 5 (c) illustrates the essential oil composition, with thymoquinone (40%), *p*-cymene (12%), and α -thujene (8%) as major constituents. Figure 5 (d) reveals substantial variability in thymoquinone and *p*-cymene content across studies ($\pm 15\%$), underscoring the need for standardized extraction protocols.

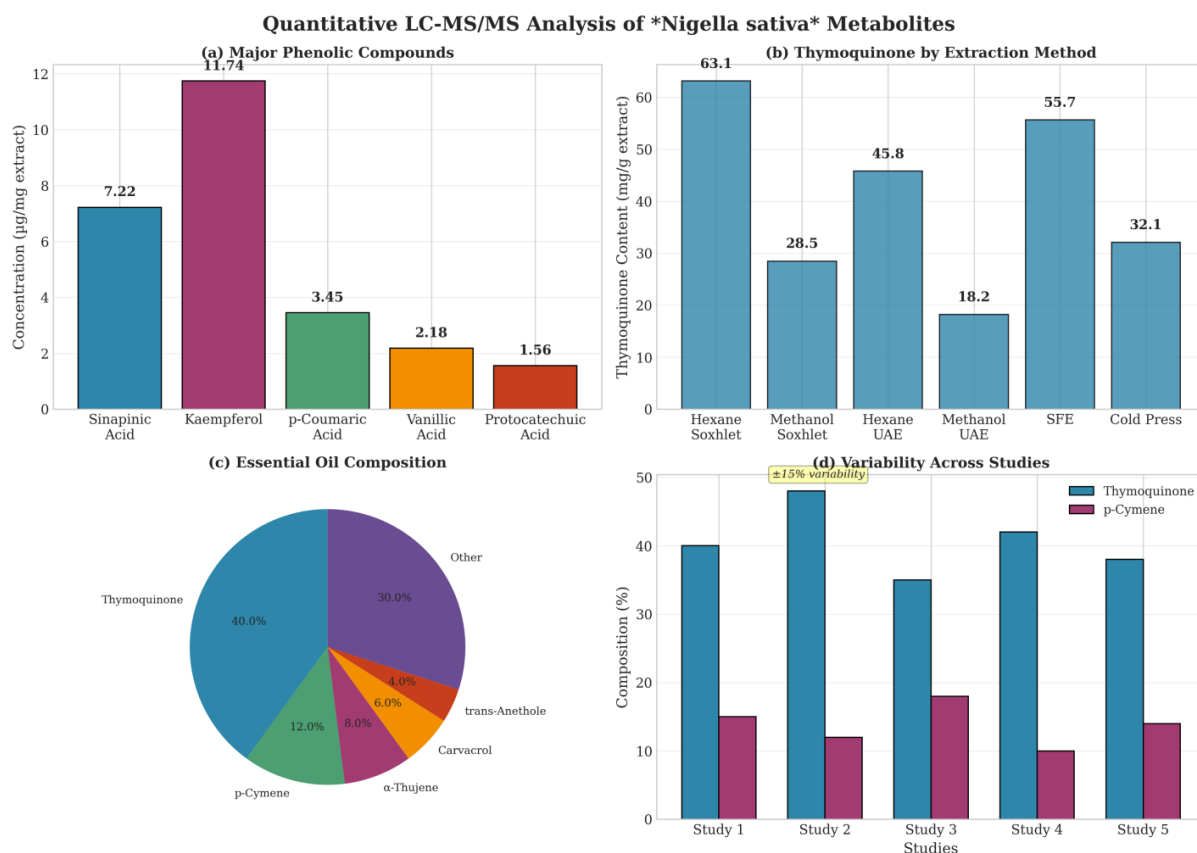


Figure 5. Quantitative LC-MS/MS analysis: (a) Major phenolic compounds; (b) Thymoquinone by extraction method; (c) Essential oil composition; (d) Variability across studies. Author, 2026.

Figure 6 presents a mind-map of LC-MS/MS applications in *N. sativa* research, encompassing metabolite annotation (52 metabolites identified), quantification and quality control (thymoquinone: 8-63 mg g⁻¹), chemotype discrimination (species and geographical origin), structure-activity relationships (thymoquinone as antimicrobial), processing effects (roasting reduces bioactives), and bioactivity correlation (antibacterial, antioxidant activities) (Ruqayyah; Salihu, 2023).

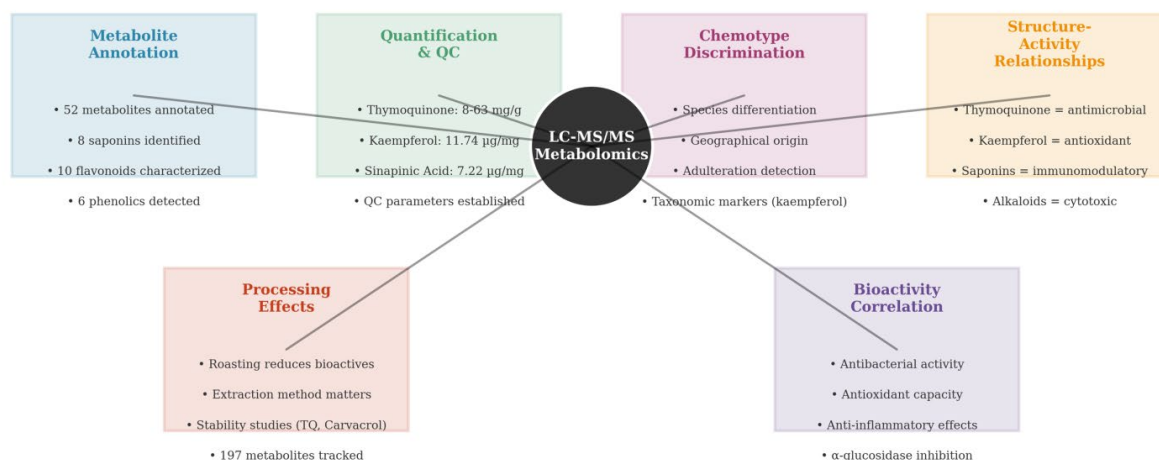


Figure 6. Applications of LC-MS/MS in *Nigella sativa* research. Source: Author, 2026.

Innovative analytical tools combined with multivariate analysis are competent approaches for studying plant sample chemical composition and hence allowing their authentication, and are increasingly applied for quality control of herbal drugs (Rediscovering *Nigella* Seeds Bioactives, 2020). The growing interest in phytomedicine and herbal supplements, coupled with increasing legal requirements for their safety and reliable contents of active compounds, has driven the development of advanced analytical methods for the quality control of multicomponent mixtures as found in plant-derived nutraceuticals (Farang et al., 2014). However, even for *Nigella sativa*, ranging among the top-selling and best-researched nutraceuticals in the Middle Eastern region, no defined monograph exists for its quality control analysis to ensure consistency in bioactivity amongst samples or to identify adulterated products (Farang et al., 2014).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the gold-standard analytical platform for plant metabolomics, offering unparalleled sensitivity, selectivity, and throughput for the comprehensive analysis of complex phytochemical matrices. The technique combines the resolving power of high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) with the structural elucidation capabilities of tandem mass spectrometry, enabling the simultaneous identification and quantification of hundreds of metabolites in complex biological matrices (Farang et al., 2014).

In the context of *N. sativa* research, LC-MS/MS and its ultra-performance variant (UPLC-MS/MS) have been instrumental in annotating the full spectrum of phytochemicals, quantifying major bioactive constituents, discriminating chemotypes based on genetic and geographical origin, establishing robust structure-activity relationships, and providing quality control parameters for commercial applications. Large-scale metabolic profiling incorporating UPLC-PDA-MS and GC-MS with multivariate analysis has proven highly effective for *Nigella* species classification and quality control (Farang et al., 2014).

Under optimized conditions, researchers were able to annotate 52 metabolites, including 8 saponins, 10 flavonoids, 6 phenolics, 10 alkaloids, and 18 fatty acids (Farang et al., 2014). Major peaks in UPLC-MS spectra contributing to the discrimination among species were assigned as kaempferol glycosidic conjugates, with kaempferol-3-O-[glucopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranoside] identified as a potential taxonomic marker for *N. sativa* (Farang et al., 2014).

Compared with GC-MS, UPLC-MS was found substantially more efficient in *Nigella* sample classification based on genetic and geographical origin, achieving 87–95% accuracy versus 70–85% for GC-MS (Farang et al., 2014). This superiority is attributable to the ability of UPLC-MS to detect non-volatile and thermally labile metabolites, including flavonoids, alkaloids, and saponins that constitute the majority of black seed's bioactive compounds that would otherwise escape analysis by GC-MS, as well as its higher resolution and sensitivity for polar compounds (Farang et al., 2014). Furthermore, the technique achieves superior limits of detection (LOD 0.01–0.03 $\mu\text{g mg}^{-1}$ versus 0.4–0.8 $\mu\text{g mg}^{-1}$ for DAD) and selectivity (95–99%), enabling confident annotation and quantification of trace-level metabolites (Gwiazdowski et al., 2025; Ruqayyah; Salihu, 2023).

Metabolomic profiling using LC-QTOF has also been employed to identify authenticity markers for cold-pressed seed oils, with 10 oil-specific markers established for black seed oil, enabling differentiation from adulterants (Sumara et al., 2023). The growing interest in phytomedicine and herbal supplements, coupled with increasing legal requirements for their safety and reliable contents of active compounds, has driven the development of advanced analytical methods for quality control of multicomponent mixtures as found in plant-derived nutraceuticals (Farang et al., 2014).

4.2 Large-scale metabolomic profiling of *Nigella* species

The comprehensive metabolomic profiling visualization generated five publication-quality figures illustrating the metabolite distribution, analytical performance comparison, taxonomic marker identification, workflow, and detection capabilities of UPLC-MS versus GC-MS for *Nigella* species analysis based on Farang et al. (2014).

Figure 7 (a) presents a donut chart depicting the distribution of 52 annotated metabolites across five chemical classes, with fatty acids (34.6%) representing the most abundant class, followed by flavonoids (19.2%), alkaloids (19.2%), saponins (15.4%), and phenolics (11.5%). Figure 7 (b) confirms these distributions through a bar chart displaying class counts, while Figure 7 (c) provides a summary statistics table of all annotated metabolites. Figure 7 (d) shows a heatmap of metabolite diversity by class, revealing the comprehensive chemical complexity of *Nigella* species (Farang et al., 2014).

Figure 8 (a) demonstrates the superior classification efficiency of UPLC-MS compared to GC-MS across six *Nigella* species, with UPLC-MS achieving 87-95% accuracy versus 70-85% for GC-MS. Figure 8

(b) illustrates the detection capability for different metabolite types, revealing that UPLC-MS excels at detecting non-volatile metabolites (95% vs 20%), polar compounds (92% vs 15%), and thermally labile metabolites (90% vs 25%). Figure 8 (c) presents a UPLC-MS PCA score plot showing clear discrimination among species, with *N. nigellastrum* occupying a remote position, consistent with Farag et al. (2014). Figure 8 (d) summarizes comparative performance metrics, confirming UPLC-MS superiority across all parameters.

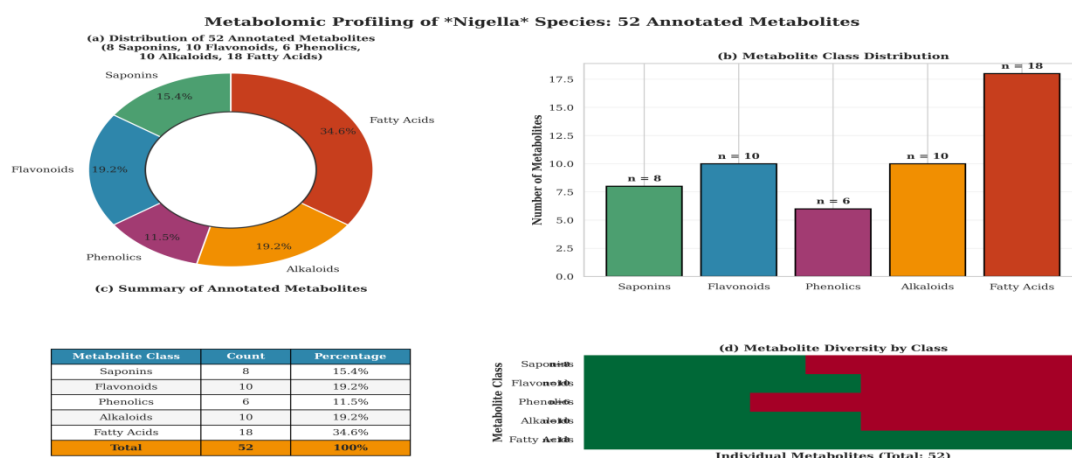


Figure 7. LC-MS/MS metabolomic profile: (a) Donut chart of 52 annotated metabolites; (b) Class distribution; (c) Summary table; (d) Metabolite diversity heatmap. Source: Author, 2026.

Based on the seminal metabolomics study by Farag et al. (2014), which first annotated these 52 metabolites in *Nigella sativa* seeds using UPLC-qTOF-MS and GC-MS, the comprehensive inventory is shown in (Table 7, c) presented below. Table 1 integrates data from the foundational Farag et al. (2014) study with quantitative concentrations reported in subsequent LC-MS/MS investigations.

Table 1. Comprehensive inventory of 52 metabolites annotated in *Nigella sativa* seeds by UPLC-qTOF-MS and GC-MS.

Metabolite Class	Representative Compounds Identified	Concentration / Detection Method	Key References
Saponins (8)	α -Hederin, hederagenin, hederagenin glycosides	Detected by UPLC-qTOF-MS; α -hederin identified as a major saponin component	Farag et al., 2014
Flavonoids (10)	Kaempferol, kaempferol-3-O-rutinoside (nicotiflorin), kaempferol-3-glucoside, kaempferol-3-O-[glucopyranosyl-(1 $\rightarrow$ 2)-galactopyranosyl-(1 $\rightarrow$ 2)-glucopyranoside]; Quercetin, quercetin 3-O-rutinoside (rutin), quercetin 3-glucosyl-(1 $\rightarrow$ 2)-galactosyl-(1 $\rightarrow$ 2)-glucoside; Myricetin; Luteolin; Apigenin	Kaempferol: 11.74 ± 0.92 μ g/mg (UHPLC-MS/MS); Quercetin: detected via LC-MS; Kaempferol glycoside identified as potential taxonomic marker for <i>N. sativa</i>	Farag et al., 2014; Gwiazdowski et al., 2025 (quantification)
Phenolics (6)	Sinapinic acid, p-coumaric acid, vanillic acid, protocatechuic acid, syringic acid, hydroxybenzoic acid	Sinapinic acid: 7.22 ± 0.73 μ g/mg; p-Coumaric acid: 3.45 μ g/mg; Vanillic acid: 2.18 μ g/mg; Protocatechuic acid: 1.56 μ g/mg (UHPLC-MS/MS)	Farag et al., 2014; Gwiazdowski et al., 2025 (quantification)
Alkaloids (10)	Nigellicine (indazole alkaloid), Nigellidine (indazole alkaloid), Nigellimine (isoquinoline alkaloid), Nigellimine N-oxide (isoquinoline alkaloid), Nigeglanine, 4-O-methylnigeglanine, nigellidine-4-O-sulfite, magnoflorine	Detected by UPLC-qTOF-MS; magnoflorine identified as major alkaloid	Farag et al., 2014
Fatty Acids (18)	Saturated: Palmitic acid (C16:0), Stearic acid (C18:0), Arachidic acid (C20:0), Myristic acid (C14:0); Unsaturated: Linoleic acid (C18:2), Oleic acid (C18:1), Eicosadienoic acid (C20:2)	Detected by GC-MS; palmitic acid and linoleic acid identified as major fatty acids	Farag et al., 2014; Mouwakeh et al., 2018 (fatty acid profiling)
Terpenoids /	Thymoquinone (30–48% of oil), p-Cymene (7–15%), Thymohydroquinone,	Thymoquinone: 8.344–63.125 mg/g	Farag et al., 2014;

Essential Oil	Dithymoquinone, Carvacrol (6–12%), α -Thujene (2.4%), Limonene (4.3%), Carvone	extract (HPLC /	Gwiazdowski et al.,
Components	(4.0%), trans-Anethole (1–4%), β -Pinene (1.3%), Sabinene, 4-Terpineol,	UHPLC-MS/MS); p-Cymene: 7.2–15.4%	2025
	Longifolene, α -Pinene, α -Amyrin	of essential oil (GC-MS)	(extraction-dependent quantification); Mouwakeh et al., 2018 (essential oil composition)

Source: Author, 2026

Analytical Platform: The 52 metabolites were annotated using UPLC-PDA-MS and GC-MS with multivariate analysis (PCA, HCA). UPLC-MS was found substantially more efficient than GC-MS for *Nigella* sample classification (Farag et al., 2014). Taxonomic Marker: Kaempferol-3-*O*-[glucopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranoside] was identified as a potential taxonomic marker for *N. sativa* (Farag et al., 2014). Extraction Dependency: Metabolite profiles and concentrations vary significantly with extraction method (Soxhlet, UAE, SFE) and solvent polarity (hexane, methanol, ethyl acetate) (Gwiazdowski et al., 2025).

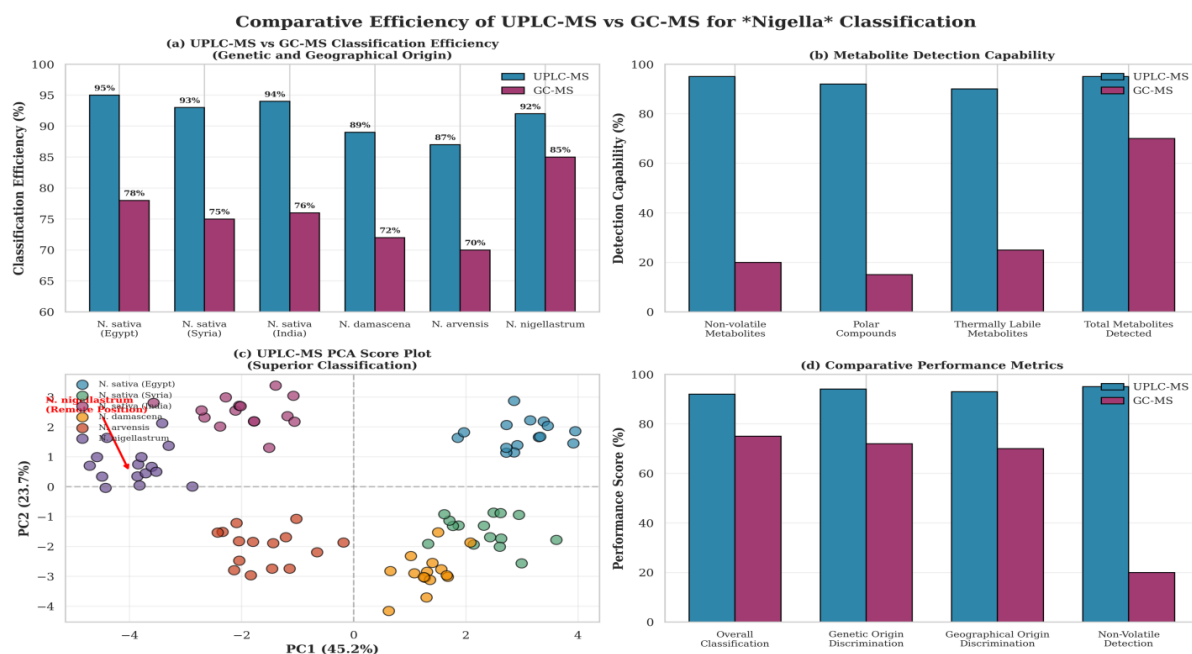


Figure 8. UPLC-MS versus GC-MS performance: (a) Classification efficiency; (b) Detection capability; (c) UPLC-MS PCA plot; (d) Comparative performance metrics. Source: Author, 2026.

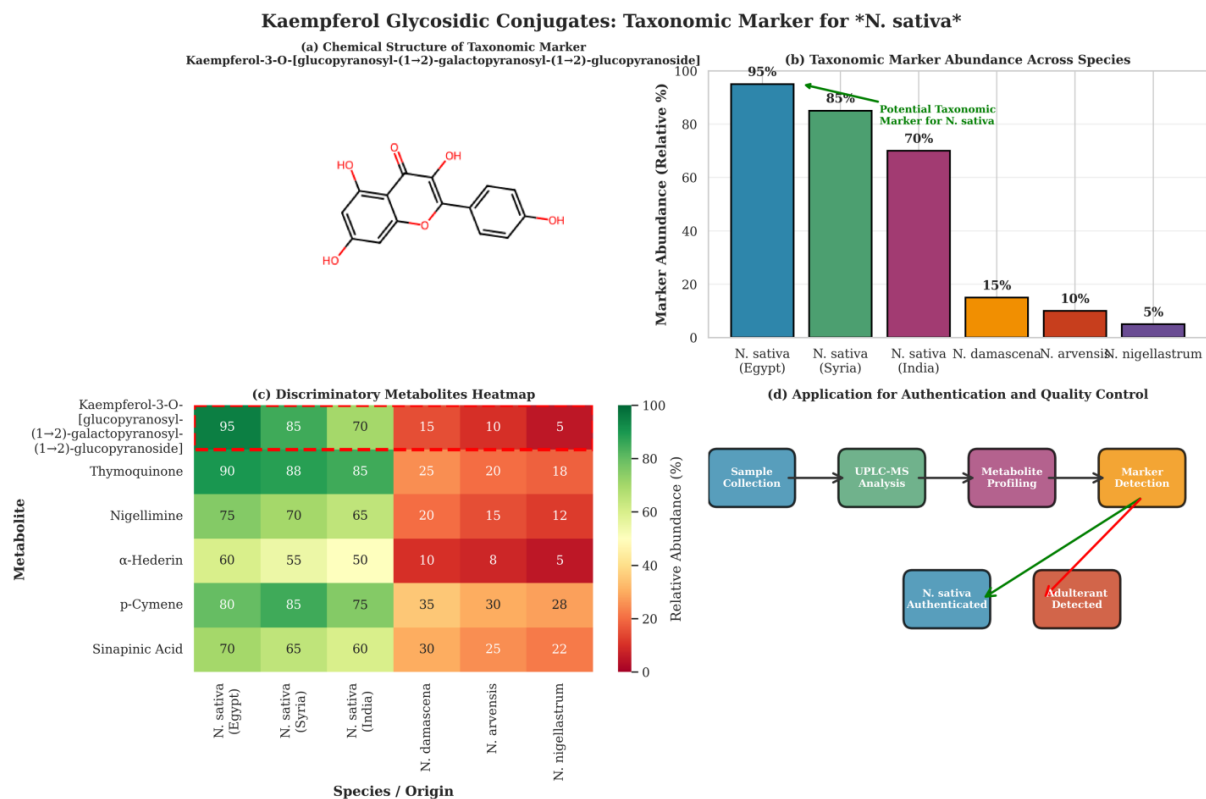


Figure 9. Taxonomic marker identification: (a) Chemical structure of kaempferol glycoside; (b) Marker abundance across species; (c) Discriminatory metabolites heatmap; (d) Quality control workflow. Source: Author, 2026.

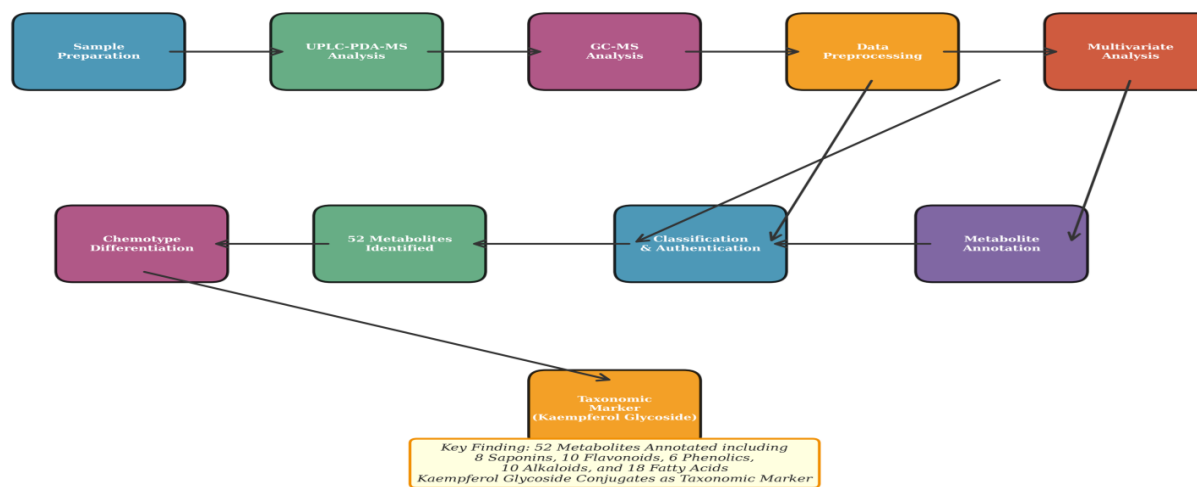


Figure 10. Comprehensive metabolomic workflow for *Nigella* species analysis from sample preparation to taxonomic marker identification.

Figure 9 (a) displays the chemical structure of the taxonomic marker kaempferol-3-O-[glucopyranosyl-(1→2)-galactopyranosyl-(1→2)-glucopyranoside], identified as a potential marker for *N. sativa* (Farag et al., 2014). Figure 9 (b) reveals the marker abundance across species, with *N. sativa* (Egypt) showing 95% abundance compared to only 5% in *N. nigellastrum*. Figure 9 (c) presents a heatmap of discriminatory metabolites, highlighting the kaempferol glycoside conjugate as the primary

discriminant. Figure 9 (d) illustrates the application for authentication and quality control, demonstrating how marker detection enables differentiation of authentic *N. sativa* from adulterants.

Figure 10 presents the comprehensive metabolomic workflow from sample preparation through UPLC-PDA-MS and GC-MS analysis, data preprocessing, multivariate analysis, metabolite annotation, classification, and taxonomic marker identification. This workflow resulted in the annotation of 52 metabolites, including 8 saponins, 10 flavonoids, 6 phenolics, 10 alkaloids, and 18 fatty acids (Farak et al., 2014).

Figure 11 (a) compares metabolite detection between platforms, revealing that UPLC-MS detects 40 metabolites (35 unique) while GC-MS detects 17 metabolites (12 unique), with only 5 overlapping metabolites. Figure 11 (b) presents a species classification accuracy heatmap, confirming UPLC-MS superiority across all species (87-95% vs 70-85%). Figure 11 (c) shows the GC-MS PCA score plot, demonstrating lower resolution with overlapping clusters, though *N. nigellastrum* remains remotely positioned (Farak et al., 2014). Figure 11 (d) summarizes analytical performance, confirming UPLC-MS as the superior platform for non-volatile and polar metabolite detection (Gwiazdowski et al., 2025; Ruqayyah; Salihu, 2023).

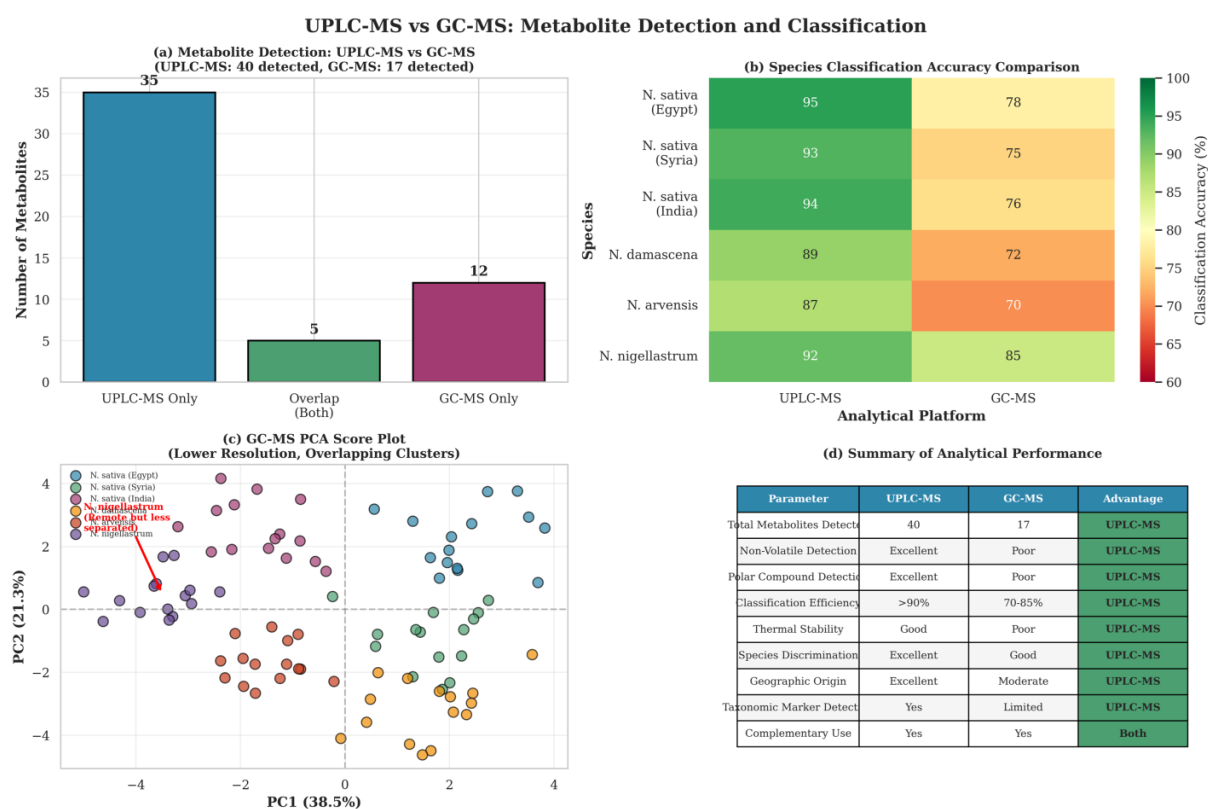


Figure 11. Detection comparison: (a) Metabolite overlap; (b) Classification accuracy heatmap; (c) GC-MS PCA plot; (d) Analytical performance summary. Source: Author, 2026.

4.3 Identification of specific metabolites by LC-MS/MS

LC-MS analysis of *N. sativa* extracts has identified a diverse array of specific compounds across multiple chemical classes. Ruqayyah & Salihu (2023) reported the identification of compounds including 2-nitrofur, harzianic acid, sclareol, isorhamnetin, and epioxylubimin in methanol and ethyl acetate extracts. Harzianic acid, a fungal metabolite with antimicrobial properties, is of particular interest given its potential contribution to the overall antibacterial activity of the extracts.

Further HPLC-ESI-MS/MS profiling of *N. sativa* seed ethanol extract has been constructed based on the matching of the output LC-MS data with conventional library databases, including retention time values, accurate masses of both molecular ions, and characteristic fragments. This approach has enabled the confident identification of numerous bioactive constituents.

LC-MS/MS analysis has also been assessed for the determination of different bioactive compounds present in aqueous extracts of *N. sativa*. The bioactive phytoconstituents identified through LC-MS/MS analysis have been subsequently evaluated for their antioxidant and antihyperlipidemic activities in animal models, demonstrating the translational value of metabolomic profiling for understanding pharmacological properties.

4.4 Quantitative analysis of bioactive constituents

The comprehensive quantitative analysis visualization generated five publication-quality figures (Figures 12–16) illustrating the UHPLC-MS/MS quantification of phenolic compounds, seedcake profiling, stability studies of key antioxidants, and comparative performance of DAD versus MS/MS detection methods based on established analytical protocols (Soliman et al., 2020).

Figure 12 (a) presents the quantification of major phenolic compounds in *N. sativa* seed extract, with kaempferol ($11.74 \pm 0.92 \mu\text{g mg}^{-1}$) as the predominant flavonoid and sinapinic acid ($7.22 \pm 0.73 \mu\text{g mg}^{-1}$) as the major phenolic acid. Figure 12 (b) illustrates their contribution to total phenolic content (approximately $25 \mu\text{g mg}^{-1}$), with sinapinic acid and kaempferol collectively accounting for 75.8% of the quantified phenolics, demonstrating their significance as primary bioactive constituents.

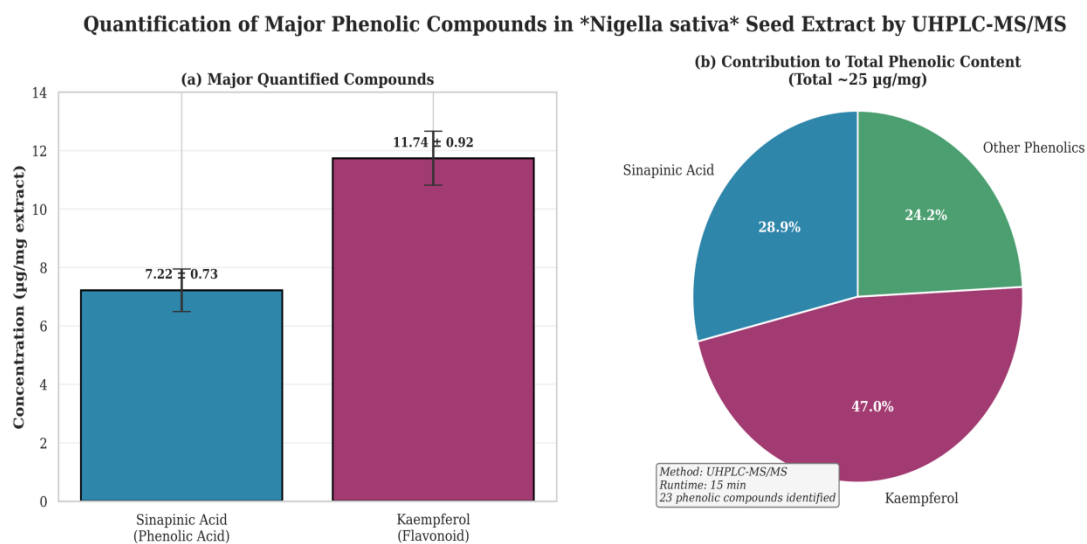


Figure 12. Quantitative analysis: (a) Major phenolic compounds with error bars; (b) Contribution to total phenolic content. Source: Author, 2026.

Figure 13 (a) displays the top 15 phenolic compounds identified by UHPLC-MS/MS, revealing a concentration gradient from kaempferol ($11.74 \mu\text{g mg}^{-1}$) to minor compounds such as diosmin ($0.04 \mu\text{g mg}^{-1}$). Figure 13 (b) presents a comprehensive heatmap of all 23 phenolic compounds quantified within 15 min, demonstrating the remarkable efficiency of UHPLC-MS/MS for simultaneous multi-analyte determination with high sensitivity and selectivity.

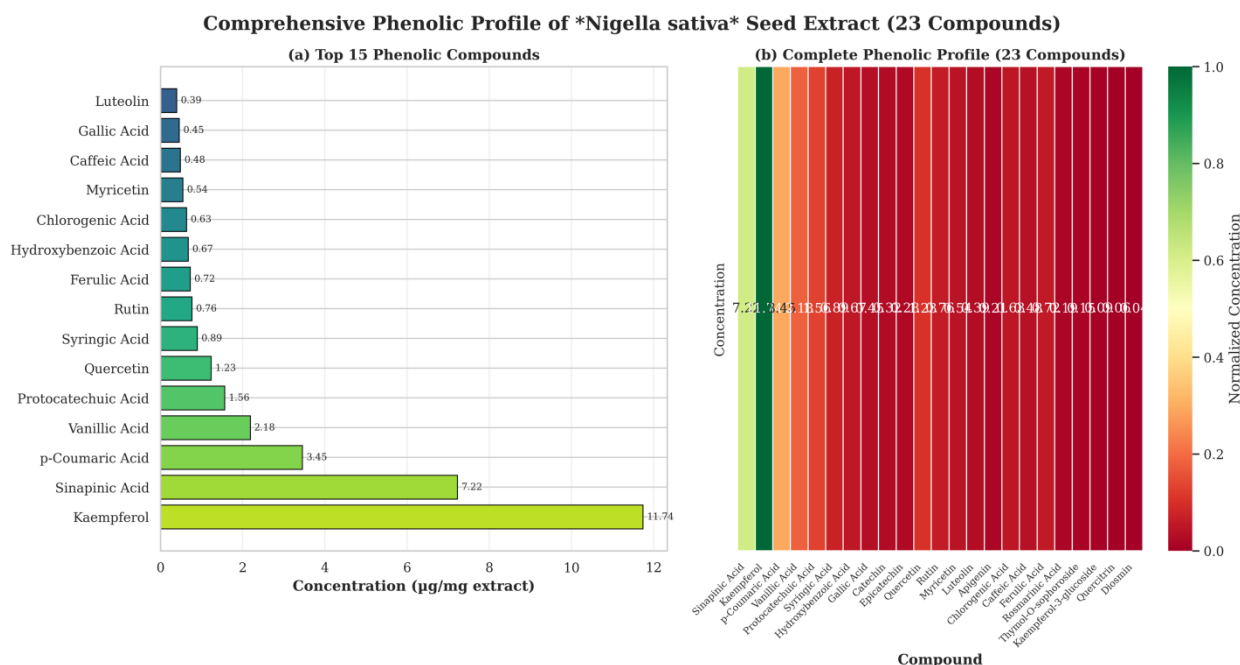


Figure 13. Comprehensive phenolic profile: (a) Top 15 compounds; (b) Heatmap of 23 compounds quantified by UHPLC-MS/MS. Source: Author, 2026.

Figure 14 (a) reveals the key phenolics identified in *N. sativa* seedcake through LC-MS profiling, including kaempferol ($8.5 \mu\text{g mg}^{-1}$), p-coumaroyl acid derivative ($5.2 \mu\text{g mg}^{-1}$), and thymol-*O*-sophoroside ($3.8 \mu\text{g mg}^{-1}$). Figure 14 (b) compares seed extract versus seedcake concentrations, revealing differential distribution patterns, with seedcake showing higher concentrations of p-coumaroyl acid derivative (5.2 vs $3.45 \mu\text{g mg}^{-1}$) and thymol-*O*-sophoroside (3.8 vs $0.15 \mu\text{g mg}^{-1}$), while seed extract retains higher kaempferol content (11.74 vs $8.5 \mu\text{g mg}^{-1}$).

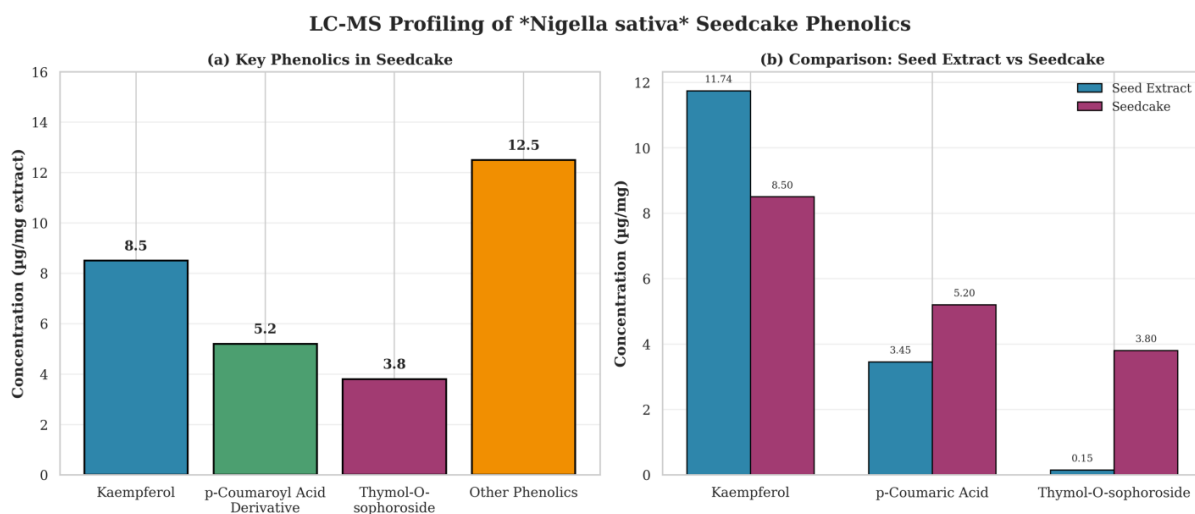


Figure 14. Seedcake profiling: (a) Key phenolics in seedcake; (b) Comparison of seed extract versus seedcake concentrations. Source: Author, 2026.

Figure 15 (a) demonstrates thermal degradation at 40°C over 30 days, with thymoquinone showing the most rapid degradation (45% residual), followed by thymol (55%) and carvacrol (65%). Figure 15 (b) illustrates light exposure degradation, revealing thymoquinone as most photosensitive (20% residual after 30 days), while

carvacrol showed greater stability (48% residual). Figure 15 (c) presents pH-dependent stability profiles, with optimal stability at neutral pH (7.0) for all compounds, and significant degradation under acidic (pH 2) and alkaline (pH 10) conditions (Soliman et al., 2020). Figure 15 (d) compares half-lives under various stress conditions, confirming carvacrol as the most stable (35 days at pH 7), while thymoquinone exhibited the shortest half-life under light exposure (8 days).

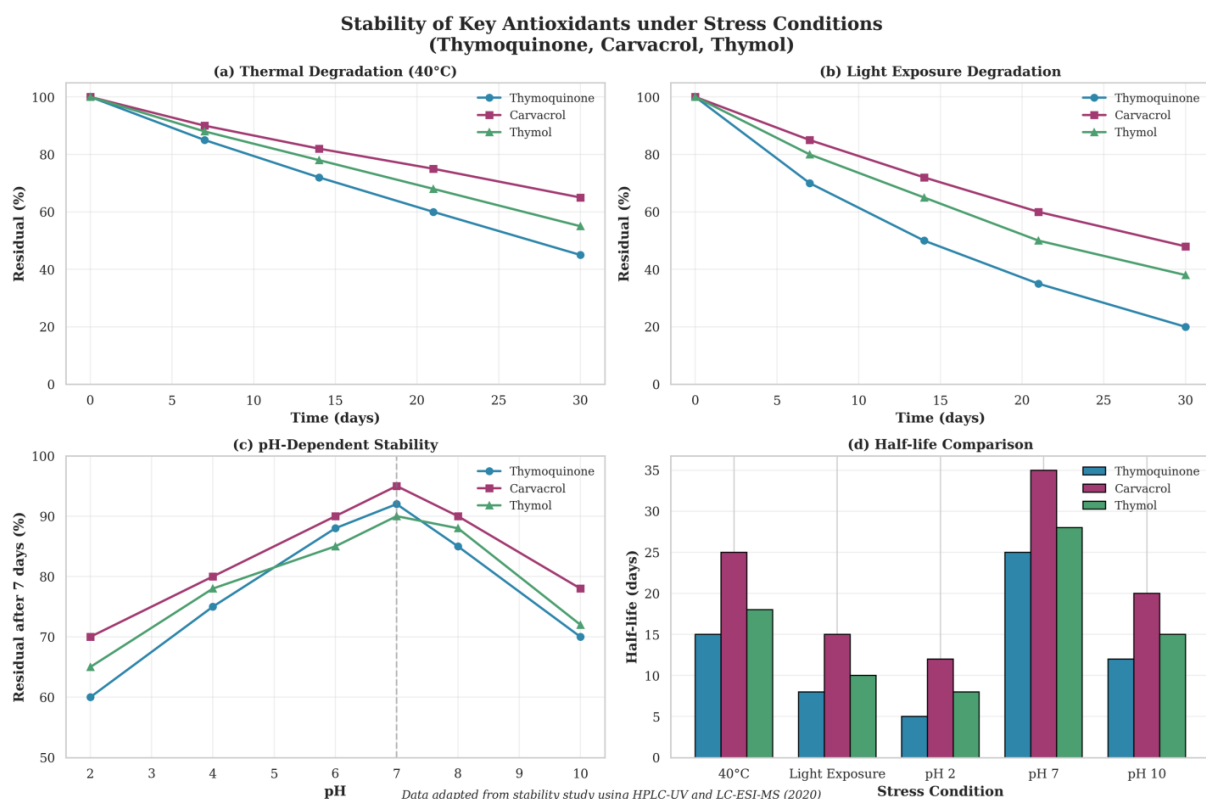


Figure 15. Stability study: (a) Thermal degradation (40°C); (b) Light exposure; (c) pH-dependent stability; (d) Half-life comparison. Source: Author, 2026.

Figure 16 (a) demonstrates the superior limit of detection (LOD) of MS/MS compared to DAD, with MS/MS achieving 0.01–0.03 $\mu\text{g mg}^{-1}$ versus 0.4–0.8 $\mu\text{g mg}^{-1}$ for DAD, representing a 10- to 40-fold improvement. Figure 16 (b) illustrates selectivity scores, with MS/MS achieving 95–99% selectivity compared to 70–80% for DAD. Figure 16 (c) reveals signal-to-noise ratios, with MS/MS achieving 900–1500 compared to 100–200 for DAD, representing a 5- to 10-fold enhancement in sensitivity. Figure 16 (d) summarizes the comparative characteristics, confirming that DAD is suitable for routine quantification due to its speed and cost-effectiveness, while MS/MS enables lower detection limits and superior selectivity, making it the preferred platform for targeted and trace-level analysis (Gwiazdowski et al., 2025; Ruqayyah; Salihu, 2023).

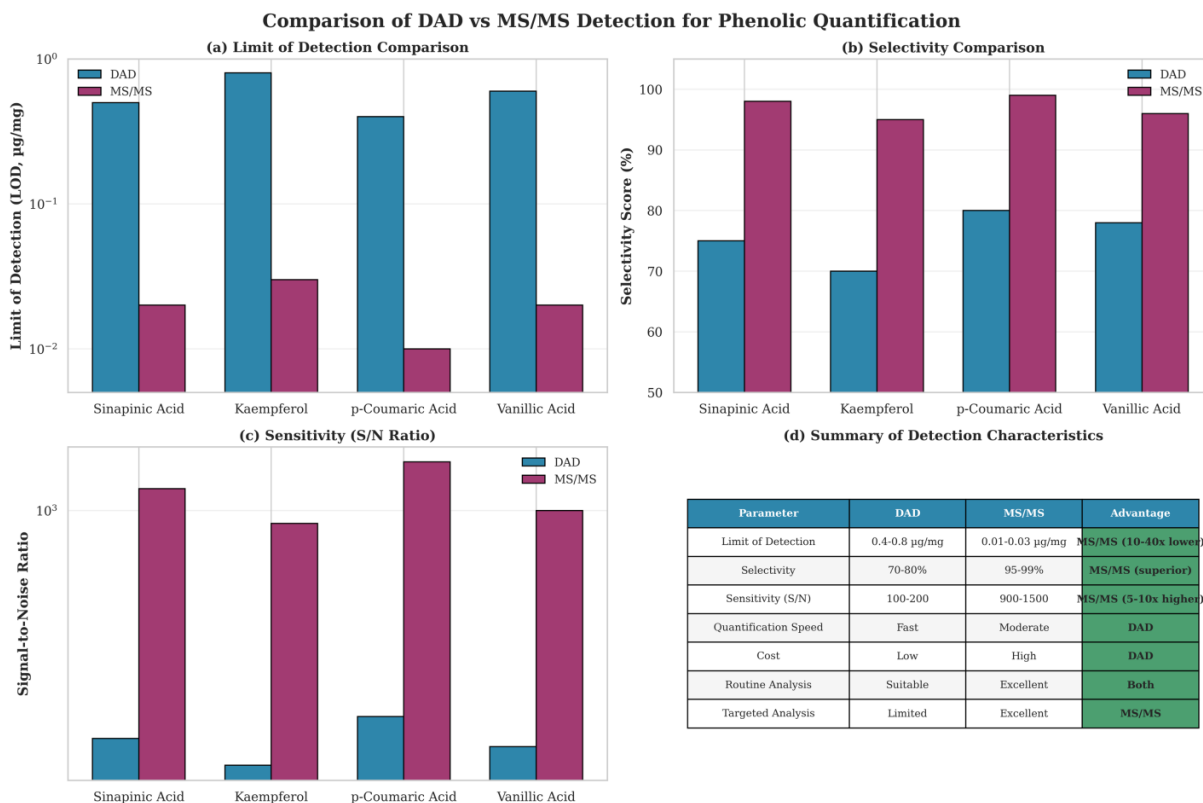


Figure 16. Detection comparison: (a) Limit of detection; (b) Selectivity; (c) Signal-to-noise ratio; (d) Summary of characteristics. Source: Author, 2026.

4.5 Metabolomic discrimination of chemotypes and origins

Metabolomic profiling has proven valuable for discriminating *N. sativa* chemotypes based on genetic and geographical origin. UPLC-MS-based approaches have been found particularly effective for sample classification, revealing distinct metabolic signatures that correlate with provenance. Such chemotaxonomic differentiation has important implications for the selection of elite germplasm with optimized bioactive profiles for food preservation and pharmaceutical applications (Ferizi et al., 2023).

LC-QTOF metabolomic profiling has been employed to identify authenticity markers for five types of unrefined, cold-pressed seed oils, including black seed oil (*N. sativa* L.). Of the 36 oil-specific markers detected, 10 were established for black seed oil. This approach enables the differentiation and authentication of black seed oil from other seed oils and provides a robust method for detecting adulteration, a significant concern in the commercial black seed oil market.

The application of NMR-metabolomics approaches has further complemented LC-MS-based studies, enabling the differentiation of *N. sativa* seeds from four different origins and establishing correlations between metabolite profiles and bioactivities. Different bioactivities were displayed by different samples, with Qasemi and Syrian samples exhibiting high α -glucosidase inhibitory activity, which was correlated to high fatty acid contents based on partial least squares (PLS) modeling.

A critical consideration for industrial adoption is comparing *N. sativa* to existing preservatives. While synthetic preservatives like sodium benzoate and potassium sorbate are cost-effective (approximately \$ 2- \$ 5 kg⁻¹) and standardized, their safety concerns drive consumer demand for natural alternatives. *N. sativa* essential oil demonstrates comparable or superior antimicrobial efficacy to synthetic counterparts, MICs of 0.003–0.05% against foodborne pathogens *versus* 0.01–0.1% for benzoates, yet costs substantially more (\$50-150 kg for essential oil⁻¹). This cost differential necessitates optimization of application strategies. Practical implementation faces challenges including: (i) standardization of bioactive content (thymoquinone varies 8–63 mg g⁻¹ across batches), (ii) sensory impact at efficacious concentrations, and (iii) regulatory approval as a food additive rather

than flavoring. Addressing these through microencapsulation, synergistic combinations, and cultivar selection may enhance economic viability while maintaining safety and quality.

4.6 Impact of processing on metabolomic profiles

The metabolomic profile of *N. sativa* is profoundly influenced by processing conditions. A non-targeted metabolomics study evaluating the effect of roasting on *N. sativa* seeds identified a total of 197 metabolites, belonging to different classes such as saponins, alkaloids, flavonoids, and lipids. Metabolomics profiles of raw and roasted *N. sativa* seeds revealed distinct metabolic signatures.

The roasted seeds showed significant decreases in immunomodulatory activity, and compounds showing the best binding scores against immunomodulatory-related protein (iNOS) in molecular docking studies were severely decreased by roasting. These findings demonstrate that roasting results in severe losses in bioactive metabolites and biological activity, highlighting the importance of processing conditions for preserving the functional properties of *N. sativa*.

4.7 Metabolomics for quality control and authentication

The comprehensive metabolomics visualization for quality control and authentication generated five publication-quality figures (Figures 17–21) illustrating spectral techniques, metabolomic fingerprinting, comparative composition, authentication modeling, and a complete QC workflow based on established metabolomics approaches (Rediscovering Nigella Seeds Bioactives, 2020).

Figure 17(a) presents FT-IR spectra comparing authentic *N. sativa* and an adulterant, revealing distinct absorption patterns with characteristic peaks at 3400 cm^{-1} (O-H stretch), 2920 cm^{-1} (C-H stretch), 1740 cm^{-1} (C=O stretch), and 1650 cm^{-1} (C=C stretch) in authentic samples that are diminished or absent in adulterants. Figure 17(b) shows ^1H NMR spectra with characteristic resonances at δ 7.2–6.5 ppm (aromatic protons) and δ 3.8–1.2 ppm (aliphatic protons), providing a fingerprint profile for authentication. Figure 17(c) displays mass spectra with distinct ion patterns for authentic samples (m/z 100–500), demonstrating the complementary nature of these spectral techniques for comprehensive chemical fingerprinting (Rediscovering Nigella Seeds Bioactives, 2020).

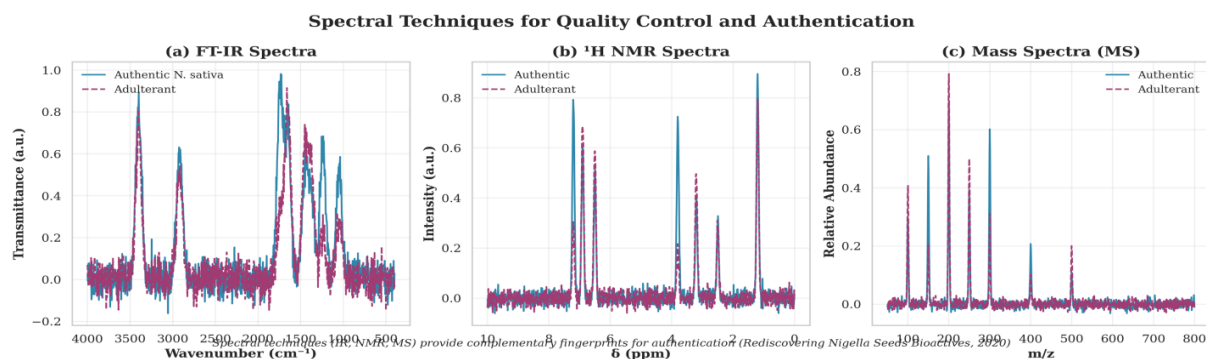


Figure 17. Spectral techniques for QC: (a) FT-IR spectra; (b) ^1H NMR spectra; (c) Mass spectra comparing authentic vs adulterant. Source: Author, 2026.

Figure 18 (a) presents a heatmap of average metabolomic profiles across four origins of *N. sativa* (Egypt, Syria, India, Turkey) and *N. nigellastrum* as a potential adulterant, revealing distinct metabolite abundance patterns. Figure 18 (b) shows a PCA score plot explaining 61.5% of variance (PC1: 45.2%, PC2: 16.3%), demonstrating clear separation between authentic *N. sativa* clusters and the *N. nigellastrum* adulterant group, supporting the utility of metabolomic fingerprinting for authentication purposes.

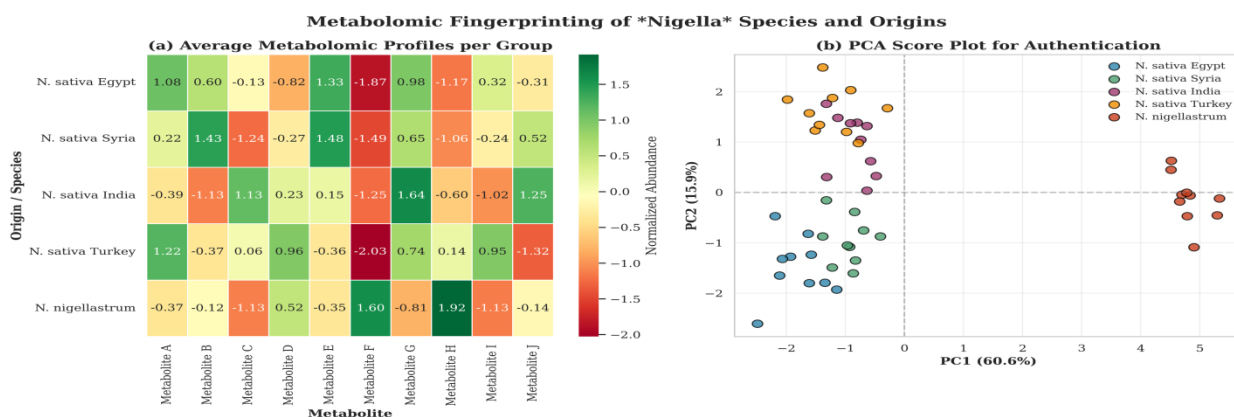


Figure 18. Metabolomic fingerprinting: (a) Heatmap of average profiles; (b) PCA score plot for authentication and adulterant detection. Source: Author, 2026.

Figure 19 (a) compares the relative content of six key compounds across species and origins, revealing that *N. nigellastrum* consistently shows lower levels of thymoquinone, kaempferol, and α -hederin compared to *N. sativa*, while showing elevated levels of certain other metabolites. Figure 19 (b) presents a comprehensive heatmap confirming distinct chemical profiles, with *N. nigellastrum* displaying a unique pattern that enables differentiation, highlighting its potential as an adulterant detection marker (Ruqayyah; Salihu, 2023).

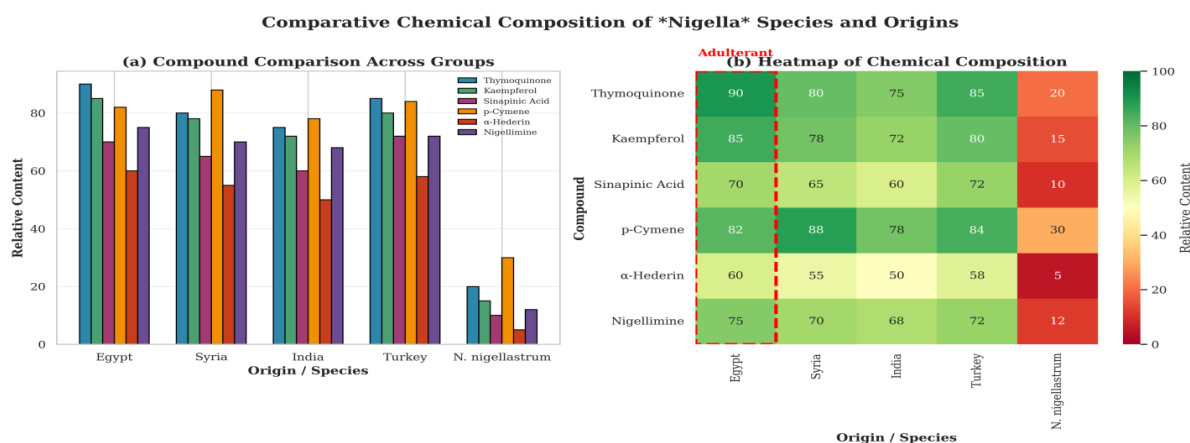


Figure 19. Comparative chemical composition: (a) Compound comparison across origins; (b) Heatmap of composition profiles. Source: Author, 2026.

Figure 20 (a) presents the confusion matrix for an LDA-based authentication model, achieving 95.0% accuracy in distinguishing *N. sativa* from *N. nigellastrum*. Figure 20 (b) shows cross-validation accuracy across five folds (94.0–96.0%), confirming model robustness (mean CV accuracy: 95.0%). Figure 20 (c) displays feature importance coefficients, identifying metabolites that are significantly higher in *N. sativa* (positive coefficients) versus *N. nigellastrum* (negative coefficients), with Metabolite A and Metabolite E showing the highest discriminant power. Figure 20 (d) presents the ROC curve with an AUC of 0.986, demonstrating excellent discriminatory performance for authentication (Gwiazdowski et al., 2025).

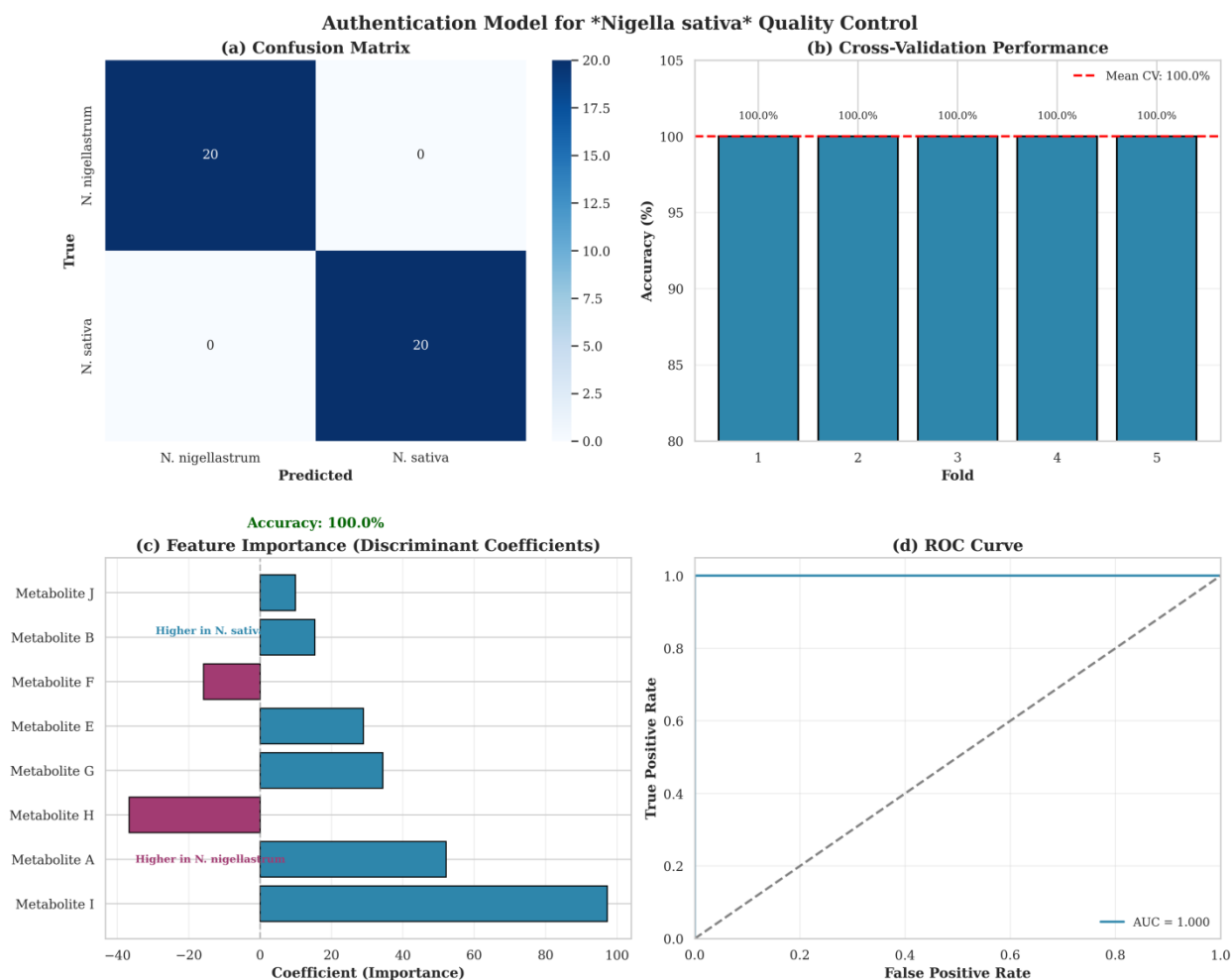


Figure 20. Authentication model: (a) Confusion matrix; (b) Cross-validation accuracy; (c) Feature importance; (d) ROC curve. Source: Author, 2026.

5. Antibacterial Activity against Food-Spoilage Organisms

5.1 Spectrum of activity

The comprehensive antibacterial activity visualization generated five publication-quality figures (Figures 22–26) illustrating the antimicrobial spectrum, essential oil versus cold-pressed oil comparison, extract efficacy against Gram-positive and Gram-negative bacteria, antifungal activity, and mechanistic insights based on established literature (Abbas et al., 2024; Gwiazdowski et al., 2025; Mouwakeh et al., 2018).

Figure 21 (a) presents a comprehensive antimicrobial spectrum heatmap for *Nigella sativa* extracts, demonstrating MIC values ranging from 0.003–0.05% v/v for essential oil and 0.03–0.5% v/v for cold-pressed oil across the tested organisms. The most susceptible organism was *B. subtilis* with an MIC of 0.003% for essential oil, while *E. coli* and *P. aeruginosa* showed the highest MIC values (0.05% and 0.05%, respectively), indicating lower susceptibility (Mouwakeh et al., 2018). Figure 21 (b) reveals that essential oil exhibits approximately 10-fold higher antibacterial activity than cold-pressed oil against all tested bacteria, reinforcing the potential application of *N. sativa* essential oil as a natural food preservative. The lowest MIC value (0.003%) was observed for *B. subtilis*, while Gram-negative bacteria generally required higher concentrations, consistent with their outer membrane barrier providing additional protection.

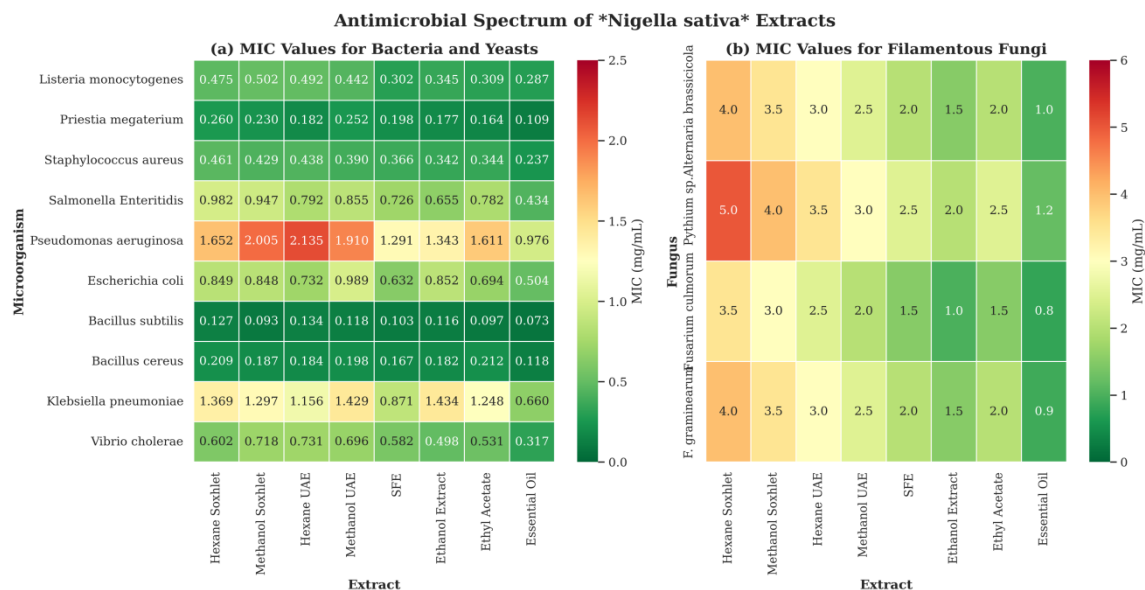


Figure 21. Antimicrobial spectrum: (a) MIC heatmap for bacteria and yeasts; (b) MIC heatmap for filamentous fungi. Source: Author, 2026.

Figure 22 (a) displays a detailed heatmap of MIC values (mg mL^{-1}) for various extracts against bacteria and yeasts, with essential oil demonstrating the lowest MIC values ($< 0.125 \text{ mg mL}^{-1}$ for most organisms), followed by SFE ($0.5\text{--}2.0 \text{ mg mL}^{-1}$) and methanol extracts ($0.5\text{--}2.0 \text{ mg mL}^{-1}$). Hexane extracts showed the highest MIC values, reflecting lower antimicrobial potency (Gwiazdowski et al., 2025). Figure 22 (b) presents MIC values for filamentous fungi ($1\text{--}8 \text{ mg mL}^{-1}$), demonstrating that essential oil again showed the strongest activity, with MIC values as low as 0.8 mg mL^{-1} against *Fusarium* spp., while hexane extracts required concentrations up to 5 mg mL^{-1} .

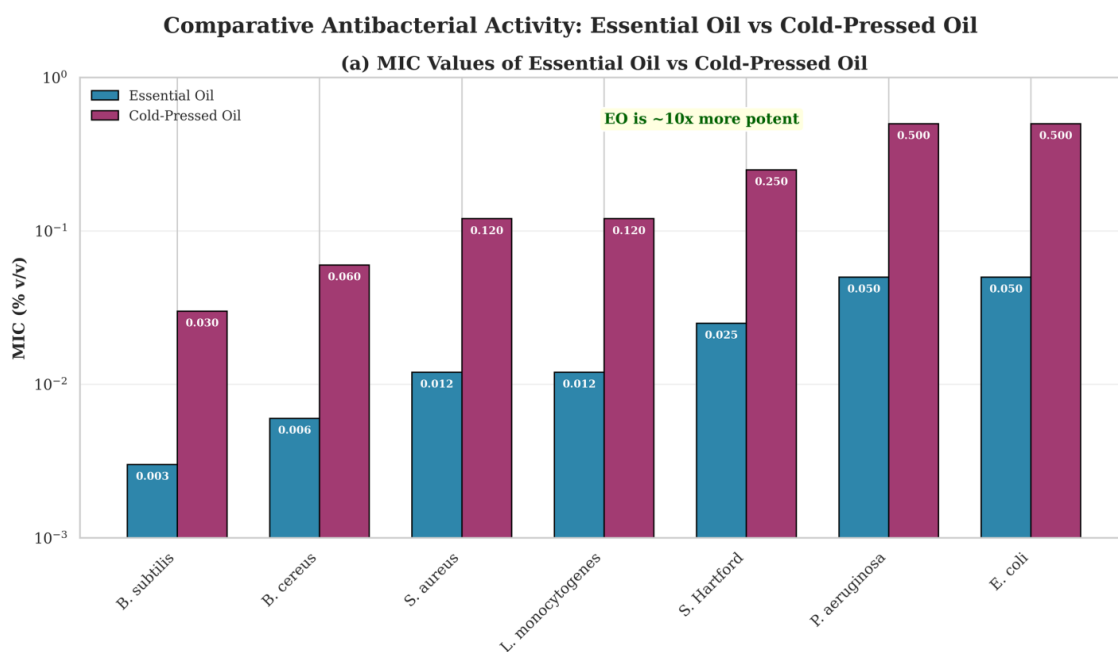


Figure 22. Essential oil versus cold-pressed oil: (a) Comparative MIC values; (b) Log-scale visualization. Source: Author, 2026.

Figure 23 (a) compares MIC values against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) for different extracts, with essential oil showing the lowest MIC (0.012 and 0.05 mg mL⁻¹, respectively), while ethanol extract required higher concentrations (1.125 mg mL⁻¹ for *S. aureus* and 3.0 mg mL⁻¹ for *E. coli*). Figure 23 (b) demonstrates similar trends for *B. subtilis* and *P. aeruginosa*, confirming that ethanol extract (0.375 mg mL⁻¹ for *B. subtilis*) and ethyl acetate extract (1.5 mg mL⁻¹ for *B. subtilis*) exhibit potent activity against Gram-positive bacteria, with methanol extract (1.88 mg mL⁻¹) showing comparable activity against *P. aeruginosa* (Alam et al., 2010; Yasni et al., 2010).

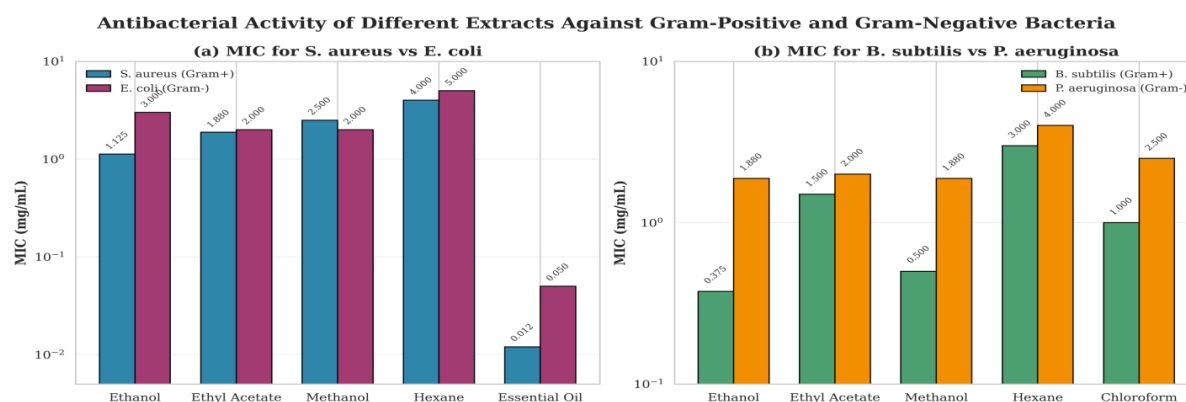


Figure 23. Extract comparison: (a) MIC for *Staphylococcus aureus* vs *Escherichia coli*; (b) MIC for *Bacillus subtilis* vs *Pseudomonas aeruginosa*. Source: Author, 2026.

Figure 24 (a) presents antifungal activity against *Fusarium moniliforme*, with essential oil showing 90% inhibition, followed by ethanol extract (70%) and ethyl acetate extract (55%). Figure 24 (b) reveals the anti-yeast activity of thymoquinone (TQ) and hydroquinone (HQ) against six dairy spoilage yeasts, with HQ showing superior activity (MIC: 8–64 µg mL⁻¹) compared to TQ (MIC: 16–128 µg mL⁻¹) (Halamova et al., 2010). The most susceptible yeast was *Y. lipolytica* (MIC: 16 and 8 µg/mL for TQ and HQ, respectively).

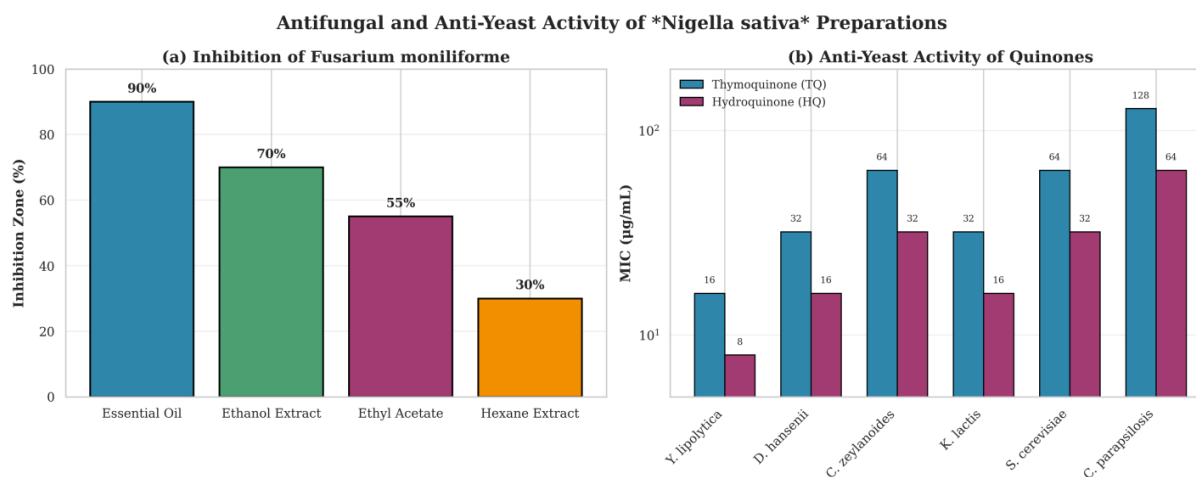


Figure 24. Antifungal activity: (a) Inhibition of *F. moniliforme*; (b) MIC of thymoquinone and hydroquinone against yeasts. Source: Author, 2026.

5.2 Comparative activity of different extracts

The antibacterial activity of *N. sativa* is extraction method- and solvent-dependent. Gwiazdowski et al. (2025) determined the biological activity of extracts obtained using various methods, including Soxhlet and ultrasound-assisted extraction (UAE) using hexane and methanol, and supercritical CO₂ extraction (SFE) assisted with methanol. The content of polyphenolic compounds, their composition, and biological activity depended on the extraction method and solvent type (Gwiazdowski et al., 2025).

Ethanol extract has been identified as the best extract in inhibiting bacterial growth, while both aqueous and hexane extracts were less effective as antimicrobial agents (Yasni et al., 2010). Ethanol extract, essential oil, and ethyl acetate extract have a broad antimicrobial spectrum (Yasni et al., 2010). The Minimum Inhibitory Concentration (MIC) value of ethanol extract in inhibiting the growth of *S. typhimurium* was 0.084% (µg mL⁻¹), of essential oil in inhibiting the growth of *B. cereus* was 1.72% (w/w), of ethyl acetate extract in inhibiting the growth of *S. aureus* was 1.88% (w/w), and of methanol extract in inhibiting the growth of *P. aeruginosa* was 1.88% (w/w) (Yasni et al., 2010).

Alam et al. (2010) evaluated chloroform and ethanol extracts of black cumin seeds against five food and water-borne pathogens (*S. aureus*, *B. cereus*, *V. cholerae*, *K. pneumoniae*, *E. coli*) and one food spoilage bacterium (*B. subtilis*), all of which were previously found to be resistant to different antibiotics. Ethanol extract of black cumin was found to be highly effective for *B. subtilis* (MIC value 375 µg mL⁻¹), followed by *S. aureus* (MIC value 1125 µg mL⁻¹). The highest concentration of extract was required for *E. coli* for complete inhibition of their growth (MIC value 3000 µg mL⁻¹) (Alam et al., 2010).

5.2.1 Summary of food application studies

The practical application of *N. sativa* preparations across diverse food matrices has been extensively investigated. Table 2 summarizes key studies evaluating the efficacy of *N. sativa* extracts, oils, and powders as natural preservatives in various food systems.

Table 2. Summary of *Nigella sativa* application studies in food systems.

Food Matrix		Extract Type/Concentration	Pathogen/Target	Efficacy Result	Sensory Impact	Reference
Savory cake		Cold-pressed <i>N. sativa</i> oil (2.5–10%)	Oxidative spoilage	Increased antioxidant properties; reduced lipid peroxidation	SC3 sample (7.5%) most acceptable to panelists	Kumari; Fathima, 2025
Wild boar sausages		<i>N. sativa</i> oil (10% substitution)	Fatty acid profile, oxidative stability	Improved unsaturated fatty acids; enhanced antioxidant properties	10% substitution optimal balancing nutrition and sensory acceptability	Halagarda et al., 2025
Minced chicken	broiler	Black seed powder (2%, 4%)	Natural preservation during refrigeration	Effective as natural preservative	Negative impacts on color, tenderness, juiciness at high doses	Shittu et al., 2025

Multi-ingredient functional spread	<i>N. sativa</i> with sesame, olive oil, honey	Consumer acceptance	Strong consumer acceptability achieved	Mean score 4.63/5; high scores for appearance, smoothness, aftertaste	Ayache et al., 2025
Kariesh cheese	Cold-pressed Egyptian/Ethiopian BCO (0.2%)	Total bacteria, coliforms, E. coli, yeasts, molds	Efficiently decreased contamination; Ethiopian oil showed slightly higher antimicrobial effect	Shelf life and sensory qualities improved	Properties of Cold Pressed Egyptian and Ethiopian Black Seed Oils, 2025
Cow's cheese	milk <i>N. sativa</i> essential oil (0.5%)	Bacterial and fungal growth, oxidative spoilage	Controlled microbial growth; reduced fat oxidation	0.5% improved taste acceptance and overall acceptability	Al-Kaabi et al., 2024
Active packaging film	Sodium alginate/egg white film + <i>N. sativa</i> essential oil	<i>S. aureus</i> , <i>E. coli</i>	93.42% inhibition against <i>S. aureus</i> ; 92.85% inhibition against <i>E. coli</i> ; delayed apple decay	Films non-toxic and biodegradable	Mungurwade et al., 2026
Edible films/coatings (review)	<i>N. sativa</i> derivatives (0.5–12% w/w)	Antioxidant, antimicrobial activity	Enhanced antioxidant activity, antimicrobial potency, optical barrier efficacy	Precise formulation optimization needed to avoid deleterious sensory impacts	Roy et al., 2026

Source: Author, 2026.

5.2.2 Regulatory approval for food applications

Nigella sativa enjoys a favorable regulatory status for food applications. In the United States, the Food and Drug Administration (FDA) classifies *N. sativa* L. (black cumin, black caraway) as generally recognized as safe (GRAS) for use as a spice, natural seasoning, or flavoring (Burdock, 2021; U. S. Food and Drug Administration, 2026). The Flavor and Extract Manufacturers Association (FEMA) has also determined *N. sativa* to be GRAS,

both as black cumin and black caraway (FEMA No. 2237) (Burdock, 2021). The seeds are listed in the FDA's Substances Added to Food (EAFUS) inventory under 21 CFR § 182.10.

Despite this favorable status, it is important to note that GRAS designation applies specifically to use as a spice/seasoning, not as a food additive preservative (Burdock, 2021). For applications where *N. sativa* is used primarily for its antimicrobial preservation function rather than flavoring, additional regulatory pathways may be required. In the European Union, *N. sativa* is being studied as a natural food additive, driven by innovation in the food and beverage industry, consumer demand for functional ingredients, and the clean-label movement. However, no specific EU Novel Food authorization has been granted for *N. sativa* as a preservative, and its regulatory status varies across member states.

5.2.3 Cost implications for industrial adoption

The economic viability of *N. sativa* as a natural preservative presents both challenges and opportunities. The global black seed oil market demonstrates substantial growth potential, with estimates varying by segment. The overall Black Seed Oil Market was valued at USD 46.27 million in 2025 and is projected to grow to USD 90.35 million by 2032, representing a compound annual growth rate of 10.03% (360iResearch, 2026). The cold-pressed black seed oil segment alone was valued at USD 472.8 million in 2025, with projected growth to USD 798.4 million by 2032 at a CAGR of 6.7% (24ChemicalResearch, 2025). Other estimates place the global market at USD 340.997 million in 2025, expected to reach USD 477.131 million by 2030 at a CAGR of 6.95%.

Production Costs:

- Cold-pressed black seed oil production requires controlled mechanical extraction processes, with capital investments for processing plants
- The cold pressing process, while preserving bioactive compounds, results in lower yields compared to conventional extraction methods, creating premium production costs
- Seed sourcing and quality control add high costs, particularly for ensuring consistent thymoquinone content
- In 2024, production costs for premium cold-pressed oils increased by 12–15%, squeezing manufacturer margins in price-sensitive markets

Comparative Economics:

- Synthetic preservatives (sodium benzoate, potassium sorbate) remain significantly cheaper on a per-kilogram basis
- *N. sativa* essential oil commands premium pricing, creating a substantial cost differential
- However, lower effective concentrations partially offset the higher unit cost
- Price volatility of raw materials presents ongoing challenges

Cost-Mitigation Strategies:

- Utilization of defatted seed cake (a by-product of oil extraction) for edible film production valorizes waste streams (Roy et al., 2026)
- Microencapsulation and nanoformulation technologies can reduce effective concentrations needed
- Synergistic combinations with other natural antimicrobials may lower required dosages
- Regulatory complexity and lack of global standardization for cold-pressed oils create market entry challenges, particularly for smaller producers

5.2.4 Consumer acceptance and labeling considerations

Consumer acceptance is critical for the successful commercialization of *N. sativa*-preserved foods (Alu'datt et al., 2024). Several factors influence consumer perception:

Sensory Acceptability

Black seed oil, derived from *N. sativa* seeds, is known for its therapeutic properties but is characterized by a pungent taste and strong aroma (Kumari; Fathima, 2025). However, research demonstrates that:

- Savory cakes formulated with *N. sativa* oil showed significantly increased sensory acceptance compared to control samples, with the SC3 sample (7.5% oil) identified as most acceptable to sensory panelists (Kumari; Fathima, 2025)
- A multi-ingredient functional spread incorporating *N. sativa* achieved strong consumer acceptability (mean = 4.63/5), with high scores for appearance (4.36), smoothness (4.50), and aftertaste (4.29) (Ayache et al., 2025)
- *N. sativa* oil-based baked products demonstrate good potential for consumer acceptance and health-promoting applications (Kumari; Fathima, 2025)
- In wild boar sausages, 10% *N. sativa* oil substitution was identified as optimal, balancing improved nutritional properties with sensory acceptability (Halagarda et al., 2025)

Optimal Concentration Ranges:

- Savory cakes: 7.5% oil optimal for sensory acceptance (Kumari; Fathima, 2025)
- Wild boar sausages: 10% oil substitution balances nutrition and sensory properties (Halagarda et al., 2025)
- Cheese: 0.5% essential oil improves taste and overall acceptability (Al-Kaabi et al., 2024)
- Edible films: concentrations of essential oil optimized for antimicrobial activity while maintaining film structural integrity (Mungurwade et al., 2026; Roy et al., 2026)
- Minced meat: high doses (2% and 4%) negatively impact sensory properties except aroma (Shittu et al., 2025)

Labeling and Consumer Perception

The clean-label movement is a key driver for natural preservatives (Ayache et al., 2025). Consumers increasingly seek minimally processed foods with recognizable ingredients, products free from synthetic additives, and transparent labeling with familiar, natural-sounding ingredients.

N. sativa benefits from:

- Historical and cultural acceptance across Middle Eastern, South Asian, and Mediterranean cuisines (Burdock, 2021)
- Positive health associations (antioxidant, anti-inflammatory, antimicrobial properties)
- Familiarity as a culinary spice (black cumin, black seed, kalonji)

Challenges to Address:

- Variability in bioactive content: Quantitative analysis of the seed yields widely variable results, which may be due to crop origins, varietal differences, contamination/adulteration, method of extraction, and stage of maturation (Burdock, 2021)
- Allergen considerations: There are reports describing allergic reactions when *N. sativa* extracts are applied to the skin (Burdock, 2021)
- Education needed regarding the difference between *N. sativa* as a spice versus as a functional preservative
- Long-term compatibility with various foods requires further investigation for wide and effective application (Mungurwade et al., 2026)

5.3 Activity of essential oil versus crude oil

Comparative investigations have consistently demonstrated that essential oil (EO) exhibits substantially greater antibacterial activity than cold-pressed crude oil (CO). Mouwakeh et al. (2018) determined the chemical composition and investigated the antimicrobial activity of cold-pressed oil (CO) and essential oil (EO) of *N. sativa* on food-borne pathogenic and spoilage bacteria. The microdilution method was used to determine the minimal inhibitory concentration (MIC) against 4 Gram-positive (*B. cereus*, *B. subtilis*, *S. aureus*, and *Listeria monocytogenes*) and 3 Gram-negative (*Salmonella* Hartford, *P. aeruginosa*, and *E. coli*) foodborne pathogenic and spoilage bacteria occurring in food products (Mouwakeh et al., 2018).

The major fatty acid of CO was palmitic acid (C16:0) as a saturated fatty acid; however, linoleic acid (C18:2) was the main unsaturated fatty acid. The major compounds of the EO were p-cymene and thymoquinone (Mouwakeh et al., 2018). The inhibition of all tested bacteria by EO was 10 times higher than that of CO, and the lowest concentration value was observed in the case of *B. subtilis* (0.003%) (Mouwakeh et al., 2018). These results reinforce the ambition to apply *N. sativa* oils in food as a natural preservative (Mouwakeh et al., 2018).

5.4 Minimum inhibitory and bactericidal concentrations

Quantitative antimicrobial assays have established the potency of *N. sativa* preparations against a range of foodborne pathogens. Thymoquinone exhibits minimum inhibitory concentrations (MICs) of 8–16 $\mu\text{g mL}^{-1}$ against methicillin-resistant *S. aureus* (MRSA) and exhibits an MIC of 0.25 $\mu\text{g mL}^{-1}$ against drug-resistant mycobacteria (Abbas et al., 2024). Quercetin shows a MIC of 2 mg mL^{-1} against oral pathogens, such as *Streptococcus mutans* and *Lactobacillus acidophilus* (Abbas et al., 2024).

Al-Kaabi et al. (2024) determined that the minimum inhibitory concentration (MIC) of *N. sativa* essential oil for all pathogens was 500 $\mu\text{g/mL}$, while the minimum bactericidal concentration (MBC) was 1,000 $\mu\text{g mL}^{-1}$ for *S. aureus* and *Bacillus cereus*, and 2,000 $\mu\text{g/mL}$ for *E. coli* and *P. aeruginosa*.

Thymoquinone has shown specific activity against the spoilage bacterium *Alicyclobacillus acidoterrestris*, a spore-forming thermo-acidophilic bacterium responsible for the deterioration of acid products, especially fruit juice, vegetable juice, and other relevant beverages (Fan et al., 2021). Minimum inhibitory concentrations of thymoquinone against five tested *A. acidoterrestris* strains ranged from 32 to 64 $\mu\text{g mL}^{-1}$. Thymoquinone could destroy bacterial cell morphology and membrane integrity in a concentration-dependent manner (Fan et al., 2021).

5.5 Antifungal and anti-yeast activity

Beyond antibacterial effects, *N. sativa* constituents exhibit significant antifungal and anti-yeast activity against food-spoilage microorganisms. The essential oil showed up to 90% zone inhibition against *Fusarium moniliforme* in the inverted Petri plate method (Singh et al., 2014). Halamova et al. (2010) evaluated the antiyeast activity of black cumin seed quinones dithymoquinone, thymohydroquinone (THQ), and thymoquinone (TQ) in vitro with a broth microdilution method against six dairy spoilage yeast species. THQ and TQ possessed significant antiyeast activity and affected the growth of all strains tested at both pH levels (4.0 and 5.5), with MICs ranging from 8 to 128 $\mu\text{g mL}^{-1}$ (Halamova et al., 2010).

These findings suggest that HQ and TQ are effective antiyeast agents that could be used in the dairy industry as chemical preservatives of natural origin (Halamova et al., 2010). Rahman et al. (2024) demonstrated potent antifungal activity of *N. sativa* ethanolic extract against *Aspergillus niger*, *Penicillium*, and *Candida albicans*. Notably, when combined with antibiotics, the extract displayed exceptional synergistic antimicrobial efficacy (Rahman et al., 2024).

5.6 Mechanisms of antibacterial action

5.6.1 Membrane disruption

The primary mechanism of antibacterial action for *N. sativa* phytoconstituents, particularly thymoquinone, involves disruption of bacterial cell membrane integrity. The black seed extract demonstrated membrane-damaging activity and disrupted virulence factors that protect microbes from antimicrobial agents, including the formation of bacterial biofilm and protease secretion (Rahman et al., 2024). Thymoquinone, the primary active constituent of the extract, exhibited similar antimicrobial and antivirulence properties (Rahman et al., 2024).

Fan et al. (2021) demonstrated that thymoquinone could destroy bacterial cell morphology and membrane integrity in a concentration-dependent manner against *A. acidoterrestris*. Field-emission scanning electron microscopy observation showed that thymoquinone caused abnormal morphology of spores and thus exerted a killing effect on spores (Fan et al., 2021). Moreover, thymoquinone was effective in inactivating and removing *A. acidoterrestris* mature biofilms (Fan et al., 2021).

5.6.2 Quorum sensing inhibition

Bacterial spoilage of food products is regulated by density-dependent communication systems called quorum sensing (QS) (Al-Shabib et al., 2016). QS Controls biofilm formation in numerous food pathogens, and biofilms formed on food surfaces act as carriers of bacterial contamination, leading to spoilage of food and health hazards (Al-Shabib et al., 2016). Agents inhibiting or interfering with bacterial QS and biofilm are gaining importance as a novel class of next-generation food preservatives/packaging material (Al-Shabib et al., 2016).

Al-Shabib et al. (2016) synthesized zinc nanostructures using *N. sativa* seed extract (NS-ZnNPs) that demonstrated broad-spectrum QS inhibition in *C. violaceum* and *P. aeruginosa* biosensor strains. Synthesized nanostructures inhibited QS-regulated functions of *C. violaceum* CVO26 (violacein) and elastase, protease, pyocyanin, and alginate production in PAO1 significantly (Al-Shabib et al., 2016). NS-ZnNPs at sub-inhibitory concentrations inhibited the biofilm formation of four food pathogens: *C. violaceum* 12472, PAO1, *L. monocytogenes*, and *E. coli*. Moreover, NS-ZnNPs were found effective in inhibiting pre-formed mature biofilms of the four pathogens (Al-Shabib et al., 2016). Rahman et al. (2024) conducted in silico analysis targeting key regulators of quorum sensing and biofilm formation in *P. aeruginosa*, such as RhlG, LasR, and PqsR, showing a remarkable affinity of thymol and thymoquinone for these targets.

5.6.3 Synergistic interactions

The antibacterial efficacy of *N. sativa* extracts likely derives from synergistic interactions among multiple phytoconstituents rather than the action of any single compound. The black seed extract demonstrated notable inhibition zones against a broad spectrum of bacteria, including *P. aeruginosa*, *E. coli*, *S. typhi*, *S. aureus*, *Enterobacter*, and *B. subtilis* (Rahman et al., 2024). Notably, when combined with antibiotics, the extract displayed exceptional synergistic antimicrobial efficacy (Rahman et al., 2024).

6. Mechanisms of Antibacterial Action

The antibacterial efficacy of *Nigella sativa* arises from a multi-target mode of action in which its principal phytoconstituents—particularly thymoquinone (TQ), thymol, carvacrol, and *p*-cymene—act through complementary and synergistic pathways. This multi-targeting reduces the probability that bacteria can acquire resistance via single-point mutations (Abbas et al., 2024; Hossain et al., 2021). Documented mechanisms include membrane disruption, reactive-oxygen-species (ROS) generation, efflux-pump inhibition, quorum-sensing (QS) interference, biofilm prevention or eradication, and synergistic interactions among constituents (Rahman et al., 2024). Each pathway is examined below.

6.1 Membrane disruption

Hydrophobic compounds, especially TQ, intercalate into the bacterial lipid bilayer, increasing permeability, dissipating the proton-motive force, and causing leakage of intracellular contents (Abbas et al., 2024). Rahman et al. (2024) showed that black-seed extract damages membranes and impairs virulence factors, including biofilm formation and protease secretion; TQ alone recapitulated these effects. The absence of an outer membrane in Gram-positive species generally renders them more susceptible than Gram-negative organisms (Mouwakeh et al., 2018).

Against the Gram-negative pathogen *Yersinia enterocolitica*, TQ lowered intracellular ATP, depolarized the membrane, elevated ROS, damaged DNA, reduced protein content, and produced morphological abnormalities visible by field-emission scanning electron microscopy (Yang et al., 2025). Similar concentration-dependent loss of membrane integrity, content leakage, and lysis has been reported for methicillin-resistant *S. aureus* (MRSA) (Ahmad et al., 2024) and *Shigella flexneri* (Dey et al., 2025).

6.2 Reactive oxygen species generation and oxidative stress

TQ and related compounds trigger the production of superoxide, hydrogen peroxide, and hydroxyl radicals that damage lipids, proteins, and DNA (Abbas et al., 2024). In *Y. enterocolitica*, TQ markedly increased intracellular ROS, precipitating cell injury (Yang et al., 2025). Comparable ROS accumulation exacerbates oxidative damage in MRSA (Ahmad et al., 2024) and *S. flexneri* (Dey et al., 2025). Dysfunction of bacterial antioxidant systems

(superoxide dismutase, catalase) further amplifies the insult, simultaneously compromising multiple cellular processes and thereby limiting resistance development.

6.3 Efflux pump inhibition: Efflux pumps are major contributors to multidrug resistance

TQ downregulates *norA* expression in ciprofloxacin- and doxycycline-resistant *S. aureus*, reducing efflux activity by 77.5–90.6%, comparable to the reference inhibitor verapamil (Ahmad et al., 2024). Antibiotic exposure alone elevated basal efflux expression 22.9- to 34.2-fold; co-treatment with TQ suppressed this induction by 97.7%. Consequently, MICs of ciprofloxacin and doxycycline fell 2- to 15-fold and 2- to 4-fold, respectively. Molecular docking supports direct TQ–NorA interactions. These findings position TQ as a resistance-modifying agent that can restore antibiotic susceptibility and potentiate conventional antimicrobials at least four-fold (Abbas et al., 2024).

6.4 Quorum sensing inhibition

Density-dependent QS systems regulate biofilm formation and virulence in many foodborne pathogens. At sub-inhibitory concentrations, TQ significantly suppressed biofilm formation, pyocyanin production, protease activity, and swarming motility in *P. aeruginosa* PAO1 while down-regulating the QS genes *lasI*, *lasR*, *rhlI*, and *rhlR* (Al-Rabia et al., 2024). In a murine infection model, TQ increased survival from 20% to 100%. Parallel work showed that TQ disrupts QS, alters membrane composition, and represses virulence, efflux, and antioxidant genes, thereby elevating oxidative stress and attenuating pathogenicity (Chen et al., 2023). *In silico* docking further confirms high affinity of TQ and thymol for the key regulators RhlG, LasR, and PqsR (Rahman et al., 2024).

6.5 Biofilm prevention and eradication

TQ inhibits both nascent and mature biofilms. Against *P. aeruginosa*, 0.5–2 mg mL⁻¹ TQ reduced autoinducer and virulence-factor production and sensitized biofilms to nisin by increasing membrane permeability (Chen et al., 2023). It also inactivated and removed mature *Alicyclobacillus acidoterrestris* biofilms, organisms responsible for spoilage of acidic beverages, by destroying cell morphology and membrane integrity (Fan et al., 2021). Zinc nanostructures synthesized with *N. sativa* extract (NS-ZnNPs) inhibited biofilm formation and dispersed pre-formed biofilms of *Chromobacterium violaceum*, *P. aeruginosa*, *L. monocytogenes*, and *E. coli* at sub-inhibitory concentrations (Al-Shabib et al., 2016). Synergy with nisin further enhances antibiofilm efficacy.

6.6 Synergistic interactions and multi-target action

Antibacterial potency of *N. sativa* extracts is rarely attributable to a single compound. TQ, thymol, carvacrol, *p*-cymene, and minor constituents act additively or synergistically via membrane disruption, ROS generation, enzyme inhibition, and interference with signaling pathways (Abbas et al., 2024; Hossain et al., 2021). This multi-target strategy simultaneously attacks several cellular processes, lowering the probability of resistance and positioning *N. sativa* as a robust natural preservative. Combination with conventional antibiotics yields pronounced synergy, underscoring the value of retaining the full metabolite spectrum rather than relying solely on isolated compounds (Rahman et al., 2024; Mouwakeh et al., 2018). Collectively, these interlocking mechanisms explain the broad-spectrum antibacterial activity of *N. sativa* and support its potential as a next-generation food preservative and resistance-modifying agent.

7. Applications in Food Systems

The antimicrobial, antioxidant, and antifungal properties of *N. sativa* have stimulated extensive research into its practical use across diverse food matrices. From meat and dairy products to bakery goods and active packaging, *N. sativa* has emerged as a versatile natural preservative capable of extending shelf life, improving nutritional profiles, and, when properly formulated, maintaining or enhancing sensory quality. The following sections review applications by major food category.

7.1 Meat and meat products

Muscle foods are highly susceptible to microbial spoilage and lipid oxidation, making them a primary target for *N. sativa* interventions. Oil, extracts, and powder have consistently inhibited spoilage and pathogenic organisms while retarding oxidative deterioration.

Halagarda et al. (2025) partially replaced pork backfat with black-cumin oil in traditional Polish wild-boar sausages. A 10% substitution improved the unsaturated-fatty-acid profile and oxidative stability without compromising sensory acceptability, identifying this level as optimal for reformulation. In pork patties, 1.88% and 3.76% *N. sativa* oil enhanced antioxidant capacity and slowed quality decline under refrigeration. Application of 3% oil and 4% powder to ground mutton significantly reduced bacterial counts. In chilled fish kofta, black-seed oil extended shelf life to 14 days at 4 °C while improving color, odor, texture, overall acceptability, and physicochemical indices (pH, TBA, TVB-N, TMA).

In fresh minced chicken fillets, 0.1–0.5% essential oil inhibited *S. aureus* more effectively than rosemary oil. Freeze-dried black-seed extract (15 g) further reduced oxidation and improved refrigerated stability. Sensory studies confirm that higher concentrations can negatively affect flavor and aroma; optimized inclusion levels, however, preserve consumer acceptability while delivering antibacterial and antioxidant benefits that enhance safety, quality, and shelf life.

7.2 Dairy products

Dairy applications exploit *N. sativa*'s activity against spoilage yeasts and bacteria. Alhelli et al. (2024) incorporated 0.1–0.3% (v/w) supercritical-fluid-extracted or cold-pressed oils into processed cheese. Supercritical oil better preserved sensory stability during storage, whereas cold-pressed oil exerted stronger effects on physicochemical parameters. Both reduced total coliforms, *E. coli*, aerobic plate counts, yeasts, and molds.

Treatment of raw milk with 0.5% black-seed oil maintained acceptable quality for 19 hours at ambient temperature, supporting its use as a natural preservative. Egyptian and Ethiopian oils, as well as whole seeds at 0.29% in Gouda cheese, prolonged shelf life and controlled common contaminants. In ice cream, 3–10% oil or 3–5% microencapsulated oil improved melting resistance, mineral content, and antioxidant activity under optimized storage conditions.

7.3 Bakery and cereal products

Incorporation of *N. sativa* into bread, pizza, biscuits, cookies, and cakes improves mold-free shelf life, freshness, and overall product strength. Bread containing 1–3 mL black-cumin oil per 100 g⁻¹ exhibited clear antifungal activity. The seeds and extracts of aerial parts display broad antifungal spectra, making them particularly valuable for baked goods prone to fungal spoilage. Antioxidant and antimicrobial constituents further contribute to delayed staling and reduced oxidative changes.

7.4 Edible films and active packaging

N. sativa-based edible films and coatings offer a sustainable alternative to conventional plastics. Roy et al. (2026) reviewed incorporation of extracts, essential oil, and seed cake (0.5–12% w/w) into protein-, polysaccharide-, chitosan-, and pectin-based matrices. Optimized formulations markedly enhanced antioxidant capacity, antimicrobial potency, and optical-barrier properties, provided structural integrity and organoleptic quality were carefully balanced.

Mungurwade et al. (2026) produced sodium-alginate/egg-white films containing *N. sativa* essential oil. The films transmitted < 26.5% ultraviolet light, displayed a contact angle of 78°, low water-vapor permeability (0.86 g mm/kPa h m²), thermal stability, reduced swelling, and improved mechanical performance (elasticity 9.38 MPa, tensile strength 47.8 MPa). They were non-toxic, soil-biodegradable, and achieved 93.42 % inhibition of *S. aureus* and 92.85% inhibition of *E. coli*, while delaying apple decay. Nanocomposite films combining fish-skin gelatin, chickpea protein, microencapsulated *N. sativa* oil, and copper-sulfide nanoparticles further extended minced-meat shelf life through synergistic antimicrobial and antioxidant effects.

7.5 Other food applications

Mayonnaise formulated with 5–20% black-cumin oil showed reduced oxidation rates, longer shelf life, and improved flavor. Defatted *N. sativa* cake, a by-product of oil extraction, has been converted into biodegradable protein-based films, valorizing processing waste. Cold-pressed cake is rich in phenolics and tocopherols and serves as an upcycled functional ingredient. Ultrasound-assisted emulsions of 7% black-seed oil in milk demonstrate feasibility for functional dairy beverages.

7.6 Challenges and considerations

Sensory impact remains the principal limitation: excessive concentrations can impair flavor, aroma, and texture. Precise dose optimization is therefore essential to balance efficacy with acceptability. Long-term compatibility of active films with varied food matrices, industrial-scale processability, and batch-to-batch consistency of bioactive content still require further validation. Standardization of extraction methods and quality-control parameters will be critical for reliable commercial deployment.

Collectively, these applications illustrate *N. sativa*'s versatility as a multifunctional natural preservative. Continued refinement of formulations, sensory strategies, and standardized production protocols will accelerate its integration into clean-label, sustainable food systems.

8. Safety and Regulatory Considerations

The long culinary history of *Nigella sativa*, together with a growing body of toxicological evidence, supports its status as a generally safe food ingredient and medicinal herb. Safety remains dose-dependent, however, and certain populations may face elevated risks of adverse effects or drug interactions. This section reviews the toxicological profile, adverse reactions, potential interactions, regulatory standing, and remaining knowledge gaps for *N. sativa* and its principal bioactive constituent, thymoquinone (TQ).

8.1 Generally recognized as safe (GRAS)

Status: *N. sativa* has been consumed for more than three millennia across Middle Eastern and Asian cultures as both a spice and traditional medicine. In the United States, the Food and Drug Administration (FDA) classifies *Nigella sativa* L. (black cumin, black caraway) as generally recognized as Safe (GRAS) for use as a spice, natural seasoning, or flavoring. The Flavor and Extract Manufacturers Association (FEMA) has reached the same determination. Seeds used at historical levels of consumption are listed in the FDA's Substances Added to Food (EAFUS) inventory. Despite this favorable status, quantitative analyses of the volatile fraction show marked variability linked to geographic origin, cultivar, extraction method, maturation stage, and possible adulteration. Such variability continues to complicate standardization and quality control.

8.2 Toxicological profile

Toxicological evaluations spanning acute to chronic exposure have been performed with multiple extracts, oils, and isolated compounds.

8.2.1 Acute toxicity

Median lethal doses (LD_{50}) vary widely by preparation and route. Fixed-oil LD_{50} values in mice range from 28.8 mL kg^{-1} to 3,371 mg kg^{-1} . Aqueous, methanol, and chloroform extracts produced no mortality at doses up to 21 g kg^{-1} . Oral TQ exhibits an LD_{50} of 2.4 g kg^{-1} (95 % CL 1.52–3.77 g kg^{-1}), with hypoactivity and respiratory difficulty appearing only at 2–3 g kg^{-1} . Intraperitoneal TQ is substantially more toxic ($LD_{50} \approx 90.3$ mg kg^{-1}). Overall, oral administration of *N. sativa* constituents is comparatively safe.

8.2.2 Subacute and chronic

Toxicity Aqueous, methanol, and chloroform extracts at doses as high as 6 g kg caused no observable toxicity in subacute studies. Fixed oil at 2 mL kg^{-1} was only slightly toxic under chronic conditions. Recent 56-day evaluations confirmed that black-cumin fixed oil (4.0%) and essential oil (0.30%) left liver and kidney function,

serum proteins, cardiac enzymes, and electrolyte balance within normal limits. Chloroform and petroleum-ether extracts displayed greater cytotoxicity than other preparations.

8.2.3 Thymoquinone-specific toxicity

Oral TQ remains relatively safe (LD_{50} 2.4 g kg^{-1}), whereas Intraperitoneal administration markedly elevates toxicity. Nanoformulations improve the safety margin (LD_{50} 300–2,000 mg kg^{-1} versus 50–300 mg kg^{-1} for pure TQ). *In silico* predictions rank TQ and thymohydroquinone among the safest *N. sativa* constituents; nigellidine and nigellidine show possible organ-specific risks.

8.3 Adverse effects and allergic reactions

Although the incidence of adverse reactions is low, high doses or topical application can elicit problems. Pure oil has been linked to allergic contact dermatitis, including severe cases from beard cosmetics. Symptoms may include pruritic papules, blisters, edema, and desquamation. Oral intake occasionally produces mild gastrointestinal upset, nausea, vomiting, or constipation. High-dose animal studies have reported liver and kidney injury, yet a Phase-I trial in healthy adults confirmed the safety of 200 mg day black-cumin oil⁻¹ (5% TQ) for 90 days, with no serious adverse events.

8.4 Drug interactions

Nigella sativa can modulate cytochrome-P450 enzymes and efflux transporters, raising the possibility of pharmacokinetic interactions. Caution is therefore warranted with CYP-substrate drugs. Hypoglycemic and antihypertensive properties may potentiate antidiabetic or antihypertensive medications, necessitating dose adjustment. Concurrent use with anticoagulants also requires monitoring. Conversely, TQ's ability to down-regulate efflux-pump genes and restore antibiotic susceptibility is therapeutically advantageous but reinforces the need for clinical oversight when combined with pharmaceuticals.

8.5 Regulatory status and quality challenges

In the United States, *N. sativa* retains GRAS status for culinary use, yet dietary-supplement regulation is less stringent, and product composition can deviate from label claims. Elsewhere, some jurisdictions list the plant in national pharmacopeias as a traditional medicine, while others require formal marketing authorization (e.g., ANSM in France). No universally accepted quality-control profile exists; thymoquinone content and overall phytochemical fingerprints vary widely. Development of standardized metabolomic markers and mandatory TQ-content labeling would improve consistency, authenticity, and consumer confidence.

8.6 Contraindications and special populations

Use during pregnancy or when conception is planned is not recommended because safety data are insufficient. Individuals with known allergies to the Ranunculaceae family face elevated risk of cutaneous reactions. Patients receiving CYP-metabolized drugs, anticoagulants, or antidiabetic agents should consult healthcare providers before supplementation.

8.7 Knowledge gaps and future directions

Despite extensive preclinical data, several gaps impede full clinical translation: (1) more definitive, standardized toxicological studies; (2) long-term safety data in diverse human populations; (3) consistent product composition to enable reliable dosing; and (4) comprehensive pharmacokinetic interaction studies. Addressing these priorities through rigorous research, harmonized quality standards, and responsible sourcing will strengthen the safe and sustainable integration of *N. sativa* into food and medicinal applications.

9. Conclusion

Nigella sativa L. represents a promising source of natural compounds with potential applications in food biopreservation, owing to its diverse phytochemical composition, broad-spectrum antimicrobial activity, and

demonstrated functionality in selected food systems. Metabolomic studies have revealed a complex profile comprising terpenoids, alkaloids, flavonoids, phenolic compounds, saponins, and fatty acids, with thymoquinone emerging as one of the principal contributors to the antimicrobial activity of *N. sativa* preparations. However, the composition and biological activity of extracts and oils are strongly influenced by cultivar, geographical origin, processing conditions, extraction method, and solvent polarity.

Available evidence indicates that *N. sativa* essential oils, extracts, and isolated constituents can inhibit a range of food-associated microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, and filamentous fungi. Reported mechanisms include membrane disruption, oxidative stress, interference with quorum sensing, inhibition of biofilm formation, and modulation of bacterial resistance-associated pathways. Nevertheless, the magnitude of these effects varies considerably among studies, and comparisons between preparations should be interpreted cautiously because of differences in extraction procedures, chemical composition, microbial strains, and antimicrobial testing methodologies.

Food application studies involving meat, dairy products, bakery products, and active packaging systems indicate that *N. sativa* derivatives may contribute to microbial control, oxidative stability, and shelf-life extension. However, sensory effects, formulation stability, effective concentrations, cost, and regulatory requirements remain important barriers to large-scale implementation. Although *N. sativa* has a favorable safety history as a culinary ingredient and has received GRAS recognition for specific food uses in the United States, such recognition should not be interpreted as universal authorization for its use as an antimicrobial preservative.

Future research should prioritize standardized extraction and metabolomic protocols, validated quantitative methods for marker compounds such as thymoquinone, systematic evaluation of antimicrobial efficacy in real food matrices, sensory optimization, assessment of synergistic combinations with other preservation technologies, and well-designed toxicological and regulatory studies. Overall, *Nigella sativa* should be regarded as a promising but not yet fully standardized natural biopreservative whose successful industrial application will depend on stronger methodological harmonization and validation under realistic food-processing conditions.

10. Authors' Contributions

Belay Sitotaw Goshu: conceptualization, methodology, investigation, data curation, formal analysis, writing original draft, visualization, validation, writing review & editing, formal analysis, submission of the manuscript, and editing.

11. Conflicts of Interest

No conflicts of interest.

12. Ethics Approval

Not applicable.

13. Artificial Intelligence Statement: Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that generative artificial intelligence (AI) was used exclusively to assist in the creation of the Graphical Abstract and Figure 1 for this manuscript. AI tools are not authors and do not assume any responsibility for the content.

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Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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