

Molecular Basis for the Adjuvant Potential of *Nigella sativa*-Derived Bioactive Constituents in Overcoming Resistance to Cancer Therapy

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Abstract

Resistance to cancer therapy remains a major cause of treatment failure, tumor recurrence, and disease progression because malignant cells can adapt through interconnected mechanisms involving prosurvival signaling, apoptosis suppression, drug efflux, epithelial–mesenchymal plasticity, cancer stemness, and the tumor microenvironment. This narrative review evaluates the molecular basis and adjuvant potential of *Nigella sativa*-derived bioactive constituents in overcoming resistance to conventional cancer therapies. Relevant literature was identified from Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar, with emphasis on resistance-related mechanisms, treatment sensitization, and combination-therapy studies. Among the identified constituents, thymoquinone has the strongest preclinical evidence. It modulates PI3K/Akt/mTOR, JAK/STAT, NF-κB, and Wnt/β-catenin signaling; enhances mitochondrial apoptosis; regulates ABC drug transporters; and influences autophagy, epithelial–mesenchymal transition, and cancer stem-cell properties. Thymoquinone has also enhanced the effects of chemotherapy, radiotherapy, and selected targeted agents in several experimental models, although treatment responses vary according to cancer type, dose, formulation, therapeutic combination, and microenvironmental conditions. Evidence for α-hederin, thymohydroquinone, dithymoquinone, thymol, and carvacrol remains comparatively limited, particularly in validated models of established treatment resistance. Clinical translation is further constrained by poor thymoquinone solubility and bioavailability, chemical instability, phytochemical variability, limited pharmacokinetic data, and insufficient evaluation of long-term safety and drug interactions. Overall, *N. sativa*-derived constituents, particularly thymoquinone, represent promising multi-target adjuvant candidates rather than alternatives to established cancer treatments. Future research should prioritize chemically standardized preparations, resistant cancer models, quantitative combination analyses, clinically relevant dosing, and well-designed pharmacokinetic, toxicological, and early-phase clinical studies.

Keywords : *Nigella sativa*; Thymoquinone; Cancer therapy resistance; Adjuvant therapy; Chemosensitization.

INTRODUCTION

Resistance to cancer therapy remains a major barrier to achieving durable treatment responses. Despite substantial advances in chemotherapy, radiotherapy, targeted therapy, hormonal therapy, and immunotherapy, many tumors show an inadequate initial response or gradually lose sensitivity during treatment. The persistence of resistant cancer-cell populations can promote disease progression, tumor recurrence, and reduced progression-free and overall survival. Resistance may also limit subsequent treatment options by producing cross-resistance to agents with different structures or mechanisms of action. Consequently, therapeutic resistance is recognized as one of the principal causes of treatment failure and poor clinical outcomes among patients with cancer. Identifying strategies capable of preventing

or reversing resistance is therefore essential for improving the effectiveness and durability of current cancer treatments (Ashrafi et al., 2022; Lei et al., 2023).

Cancer-treatment resistance can be broadly classified as intrinsic or acquired. Intrinsic resistance exists before therapeutic exposure and may arise from pre-existing genetic alterations, epigenetic states, cellular heterogeneity, or resistant subpopulations within the tumor. In contrast, acquired resistance develops after an initial therapeutic response through clonal selection, adaptive signaling, phenotypic plasticity, or treatment-induced molecular changes. Both forms are supported by multiple and frequently overlapping mechanisms, including increased drug efflux, altered drug targets, enhanced DNA-damage repair, suppression of apoptosis, and activation of prosurvival signaling pathways. Cancer stem cells and interactions within the tumor microenvironment can further maintain resistant phenotypes and facilitate tumor repopulation following treatment. The coexistence of these mechanisms explains why inhibition of a single molecular target often produces only temporary or incomplete responses (Bou Antoun & Chioni, 2023; Li et al., 2021; Safa, 2022).

The molecular complexity of therapeutic resistance supports the development of multi-target adjuvant strategies. Cancer cells can compensate for the inhibition of one pathway by activating alternative survival networks, metabolic adaptations, or microenvironment-mediated signals. Agents capable of simultaneously modulating several resistance-related processes may therefore improve tumor sensitivity to established therapies. Natural bioactive compounds have attracted increasing attention as potential adjuvants because they can affect cell proliferation, apoptosis, oxidative stress, inflammation, angiogenesis, and drug-response pathways. Several phytochemicals have shown chemosensitizing or radiosensitizing activity in preclinical models, indicating their potential to enhance therapeutic responses or reduce the effective doses of conventional agents. Nevertheless, natural products should be investigated as complementary interventions rather than replacements for evidence-based cancer treatment, and their development must be supported by rigorous assessments of efficacy, selectivity, pharmacokinetics, safety, and drug interactions (Chimento et al., 2023; Devarajan et al., 2021; Kilmister et al., 2022; Yesilkanal et al., 2021).

Among medicinal plants with potential application in cancer treatment, *Nigella sativa* L., commonly known as black seed or black cumin, has received considerable scientific attention. Its seeds, oils, extracts, and isolated constituents have been investigated for antioxidant, anti-inflammatory, immunomodulatory, and anticancer properties. Preclinical studies suggest that *N. sativa* preparations can regulate cancer-cell proliferation, cell-cycle progression, apoptosis, angiogenesis, invasion, and metastasis. These effects are associated with the modulation of multiple molecular targets rather than a single signaling pathway, making *N. sativa* particularly relevant to the multifactorial nature of therapeutic resistance. However, the pharmacological activity of whole extracts and seed oils may differ from that of isolated constituents because their effects depend on phytochemical composition, extraction method, and active-compound concentration (Ansary et al., 2021; Dalli et al., 2022).

Thymoquinone is the most extensively investigated bioactive constituent of *N. sativa* in cancer-related research. Its reported effects include the inhibition of prosurvival signaling, induction of cell-cycle arrest and apoptosis, regulation of oxidative stress, and enhancement of cancer-cell sensitivity to anticancer agents. Other constituents, including α -hederin, thymohydroquinone, dithymoquinone, thymol, and carvacrol, may also contribute to the overall pharmacological activity of *N. sativa*, although their individual roles in overcoming therapeutic resistance remain less clearly established. Interpretation of the existing evidence is further complicated by variations in plant genotype, geographical origin, cultivation conditions, extraction procedures, storage, and phytochemical standardization. Differences in thymoquinone content and chemical composition can result in nonequivalent biological effects among preparations labeled as *N. sativa* extracts or oils, highlighting the importance of chemical characterization and standardized dosing in future studies (Alberts et al., 2024; Ansary et al., 2021; Mani et al., 2023).

Previous reviews have primarily addressed the general pharmacological properties, chemopreventive activity, or broad anticancer mechanisms of *N. sativa* and thymoquinone. In

contrast, evidence concerning their ability to sensitize resistant cancer cells remains dispersed across different cancer types, resistance mechanisms, therapeutic modalities, and experimental preparations. Limited attention has also been given to distinguishing direct cytotoxic effects from genuine resistance-reversing or treatment-sensitizing activity. A focused synthesis is therefore required to clarify how *N. sativa*-derived constituents interact with the molecular networks responsible for therapeutic resistance and how these interactions may support their use as cancer-treatment adjuvants. Accordingly, this narrative review evaluates the molecular basis and adjuvant potential of *N. sativa*-derived bioactive constituents in overcoming resistance to cancer therapy, with emphasis on resistance-associated signaling, apoptosis, drug transport, autophagy, epithelial-mesenchymal transition, cancer stemness, combination-treatment evidence, and the major challenges affecting clinical translation.

RESEARCH METHODS

This article employed a narrative literature review approach to examine the molecular basis and adjuvant potential of *Nigella sativa*-derived bioactive constituents in overcoming resistance to cancer therapy. Relevant literature was identified through Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar using combinations of the keywords “*Nigella sativa*,” “black seed,” “thymoquinone,” “ α -hederin,” “cancer resistance,” “chemoresistance,” “radioresistance,” “treatment sensitization,” “adjuvant therapy,” and “combination therapy.” Priority was given to peer-reviewed in vitro, in vivo, and clinical studies addressing resistance-related mechanisms, treatment sensitization, or combinations of *N. sativa* constituents with conventional cancer therapies. Review articles were used to support theoretical concepts and identify additional relevant studies. Duplicate publications, non-cancer studies, and articles without a clear relationship to cancer-treatment resistance were excluded. The selected evidence was synthesized descriptively to identify molecular mechanisms, adjuvant effects, translational limitations, and future research directions.

RESULTS AND DISCUSSION

The available evidence indicates that *Nigella sativa* and its bioactive constituents may overcome cancer therapy resistance by modulating multiple molecular pathways. The following sections summarize the current evidence on resistance mechanisms, bioactive constituents, molecular targets, adjuvant effects, and future research directions.

Resistance to Cancer Therapy and the Rationale for Multi-Target Adjuvants

Resistance to cancer therapy is sustained by interconnected signaling networks that enable malignant cells to maintain proliferation, survival, and adaptive capacity during therapeutic exposure. The PI3K/Akt/mTOR pathway is among the most frequently activated signaling systems in human cancers and regulates cell growth, metabolism, cell-cycle progression, and resistance to apoptosis. Its dysregulation can reduce responsiveness to chemotherapy and targeted treatments while facilitating disease progression. PI3K/Akt/mTOR signaling also interacts with the RAS/MAPK and Wnt/ β -catenin pathways, forming compensatory networks that preserve survival signals when an individual molecular target is inhibited. In parallel, NF- κ B-mediated inflammatory and antiapoptotic responses may further protect cancer cells from treatment-induced injury. These interactions indicate that resistance is not determined by an isolated pathway but by dynamic crosstalk, feedback regulation, and pathway reactivation that limit the durability of single-target therapies (Glaviano et al., 2023; Lei et al., 2023).

Cancer cells can also acquire resistance by reducing intracellular drug activity and preventing treatment-induced cell death. Increased expression of ATP-binding cassette transporters lowers intracellular drug concentrations by actively removing anticancer agents from malignant cells. Enhanced DNA-repair capacity can diminish the cytotoxic effects of DNA-damaging treatments, whereas alterations in apoptotic regulation allow damaged cells to survive despite sustained therapeutic stress. These alterations may include loss of proapoptotic activity, increased expression of antiapoptotic proteins, and disruption of

mitochondrial cell-death pathways. Such mechanisms can exist before treatment as intrinsic resistance or emerge during repeated exposure through clonal selection and adaptive reprogramming. Moreover, drug efflux, DNA repair, and apoptosis suppression frequently occur together, allowing cancer cells to compensate when only one resistance mechanism is therapeutically inhibited (Lei et al., 2023; Safa, 2022; Su et al., 2021).

Epithelial-mesenchymal transition, cancer stem-cell plasticity, tumor hypoxia, and the tumor microenvironment further reinforce resistant phenotypes. During epithelial-mesenchymal transition, cancer cells lose epithelial characteristics and acquire mesenchymal properties associated with increased motility, invasiveness, apoptosis resistance, and metastatic potential. This process is closely connected to the acquisition of stem-like traits, which enable cancer stem cells to survive therapy, self-renew, and repopulate tumors after an initial response. Hypoxic conditions can strengthen these phenotypes through hypoxia-inducible signaling and promote metabolic adaptation, drug resistance, and cellular plasticity. The surrounding tumor microenvironment-including stromal and immune cells, extracellular-matrix components, cytokines, and metabolic conditions-can provide additional protective signals that reduce sensitivity to chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Together, these mechanisms link therapeutic resistance with tumor recurrence and metastatic progression (Chen et al., 2025; Jiang et al., 2025; Xu et al., 2022; Zhou et al., 2021).

The coexistence of signaling, genetic, epigenetic, cellular, and microenvironmental mechanisms provides a compelling rationale for multi-target adjuvant strategies. Therapeutic pressure may eliminate sensitive tumor cells while selecting pre-existing resistant subclones or inducing adaptive cellular states. Inhibition of a single pathway may therefore be followed by the activation of alternative survival networks, changes in cell phenotype, or increased dependence on microenvironmental support. Multi-target adjuvants may complement conventional treatments by simultaneously modulating prosurvival signaling, apoptosis, intracellular drug accumulation, cancer-cell plasticity, and tumor-microenvironment interactions. Their use should nevertheless be guided by mechanistic compatibility, resistance biomarkers, quantitative combination analyses, pharmacokinetic considerations, and safety evaluation rather than by the assumption that broader biological activity necessarily produces greater therapeutic benefit. This systems-level rationale supports the investigation of *Nigella sativa*-derived bioactive constituents as potential adjuvants capable of influencing several resistance-associated processes, although their specific molecular and therapeutic effects require critical evaluation in the subsequent sections (Glaviano et al., 2023; Lei et al., 2023).

Bioactive Constituents of *Nigella sativa* Relevant to Resistance to Cancer Therapy

Thymoquinone is the principal and most extensively investigated bioactive constituent of *Nigella sativa* in cancer research. Its relevance to resistance to cancer therapy is supported by its capacity to regulate multiple processes involved in cancer-cell survival and treatment response. Thymoquinone has been reported to inhibit proliferation, induce cell-cycle arrest and apoptosis, modulate reactive oxygen species, and interfere with molecular regulators such as NF- κ B, PPAR γ , c-Myc, p53, and PTEN. These pleiotropic effects provide a mechanistic basis for its potential to sensitize malignant cells to conventional anticancer treatments. Thymoquinone has also been investigated against cancer stem cells, which contribute to therapeutic resistance, tumor recurrence, and disease progression. Nevertheless, its effects appear to be context-dependent. In a hypoxic oral cancer model, thymoquinone enhanced carboplatin-induced apoptosis but simultaneously increased the enrichment of CD133-positive cells, indicating that improved short-term cytotoxicity may coexist with the persistence of adaptive stem-like populations. Therefore, reductions in cancer-cell viability should not automatically be interpreted as complete reversal of resistance without evaluating residual stemness, long-term clonogenic survival, and tumor-repopulating capacity (Almajali et al., 2021; Fattat et al., 2021; Rahman et al., 2025).

Other constituents may contribute to the overall anticancer activity of *N. sativa*, although their resistance-related evidence is considerably less developed than that of thymoquinone. α -Hederin, a pentacyclic triterpenoid saponin, has received particular attention

because of its antiproliferative and proapoptotic properties and has been identified alongside thymoquinone as one of the principal constituents relevant to cancer treatment. Thymol has also demonstrated direct antiproliferative activity in MCF-7 breast cancer cells through the downregulation of Cyclin D1 and proliferating cell nuclear antigen, supporting its potential contribution to the suppression of cancer-cell proliferation. Thymohydroquinone, dithymoquinone, and carvacrol are recognized pharmacologically active constituents, but current evidence primarily establishes their general biological or anticancer activities rather than their ability to reverse established resistance to anticancer therapy. These compounds should therefore be regarded as potentially contributory constituents rather than assigned a level of evidence equivalent to that of thymoquinone. Direct comparisons using resistant cancer models are required to determine whether they independently sensitize cancer cells, enhance the effects of thymoquinone, or merely exert parallel cytotoxic effects (Ansary et al., 2021; Pandey et al., 2025; Vahitha et al., 2024).

Whole-seed extracts and black seed oils may produce biological effects that differ from those of isolated thymoquinone because they contain complex mixtures of quinones, terpenes, phenolic compounds, phytosterols, tocopherols, and fatty acids. Experimental and compositional evidence indicates that the antioxidant properties of black seed oil are not determined exclusively by thymoquinone concentration, suggesting that interactions among multiple constituents contribute to its aggregate activity. Black seed oil and thymoquinone have also been associated with the regulation of epigenetic mediators, including the UHRF1/DNMT1/HDAC1 complex, which participates in cancer-cell proliferation and survival. However, evidence of multicomponent activity in antioxidant, anti-inflammatory, or epigenetic models cannot be directly interpreted as synergistic reversal of resistance to cancer therapy. Demonstrating such synergy requires direct comparison of whole preparations with their isolated constituents, quantitative combination analyses, and functional outcomes in validated resistant models. Thus, whole preparations may provide broader molecular activity, but their compositional complexity also limits mechanistic attribution, dose reproducibility, and comparison across studies (Abbas et al., 2024; Alsanosi et al., 2022; Neunert et al., 2025).

The pharmacological activity of *N. sativa* preparations is influenced by geographical origin, genotype, extraction method, processing, storage, and formulation. Variations in thymoquinone content and other phytochemicals among seeds from different regions and extraction procedures result in differences in biological activity. Similar variability has also been observed in commercial products, indicating that preparations with the same botanical name are not necessarily chemically equivalent. Therefore, standardized preparations with comprehensive phytochemical characterization and detailed reporting of botanical source, extraction conditions, thymoquinone content, storage, and batch consistency are essential to ensure reproducible and reliable findings in cancer resistance research (Abdel-Razek et al., 2024; Chung et al., 2023; Kaushik & Barmanray, 2025; Khaikin et al., 2022).

To provide a concise summary of the major bioactive constituents of *Nigella sativa* and their reported anticancer activities, Table 1 presents the principal molecular targets, biological effects, and therapeutic implications associated with each compound. This overview serves as a bridge to the subsequent discussion on the molecular mechanisms underlying cancer-treatment resistance.

Table 1. Major bioactive constituents of *Nigella sativa* and their reported molecular targets, biological effects, and therapeutic implications in overcoming cancer-treatment resistance.

Bioactive constituent	Major molecular targets/pathways	Biological effects	Therapeutic implications
Thymoquinone	PI3K/Akt/mTOR, NF- κ B, JAK/STAT, Wnt/ β -catenin, ABC transporters	Induces apoptosis, inhibits proliferation, suppresses EMT and metastasis, reduces	Improves sensitivity to chemotherapy, radiotherapy, and targeted therapy; helps

	(ABCB1/P-gp), Nrf2, EMT, CSCs	drug efflux, decreases cancer stemness, enhances oxidative stress	overcome therapeutic resistance and reduce tumor recurrence
α -Hederin	PI3K/Akt, Bcl- 2/Bax, caspase signaling	Promotes apoptosis and inhibits tumor growth	Enhances anticancer efficacy and chemosensitivity
Thymol	NF- κ B, ROS- related pathways	Induces apoptosis, suppresses inflammatory signaling, inhibits proliferation	Potentiates the anticancer effects of conventional chemotherapy
Carvacrol	MAPK, PI3K/Akt, NF- κ B	Inhibits proliferation, migration, and invasion	Acts as a potential adjuvant to conventional cancer therapy
Thymohydroquinone	Oxidative stress- associated pathways	Promotes apoptosis and suppresses cell survival	Supports the anticancer activity of conventional therapies
Dithymoquinone	Multiple signaling pathways (limited evidence)	Exhibits antiproliferative and pro-apoptotic activities	Potential adjunctive agent requiring further investigation

As summarized in Table 1, the anticancer activity of *Nigella sativa* is attributed to the complementary actions of multiple bioactive constituents, each targeting distinct yet interconnected molecular pathways involved in cancer progression and treatment resistance. Among these compounds, thymoquinone has been the most extensively investigated and demonstrates the broadest spectrum of molecular targets, including PI3K/Akt/mTOR, NF- κ B, JAK/STAT, Wnt/ β -catenin, ABC transporters, Nrf2, EMT, and cancer stem cells (CSCs). In contrast, α -hederin, thymol, carvacrol, thymohydroquinone, and dithymoquinone exhibit more specific but complementary activities, primarily through the modulation of apoptosis, oxidative stress, inflammatory signaling, and cell-survival pathways. Collectively, these findings suggest that the therapeutic potential of *Nigella sativa* arises from the synergistic effects of its bioactive constituents, which collectively enhance the sensitivity of cancer cells to conventional therapies and provide a mechanistic basis for overcoming cancer-treatment resistance. The molecular pathways summarized in Table 1 are discussed in greater detail in the following section.

Molecular Modulation of Resistance to Cancer Therapy by *Nigella sativa*-Derived Constituents

The molecular mechanisms underlying the adjuvant potential of *Nigella sativa*-derived bioactive constituents are summarized in Figure 1. As illustrated, these constituents regulate multiple resistance-associated signaling pathways, leading to biological changes that enhance cancer-cell sensitivity to conventional therapies.

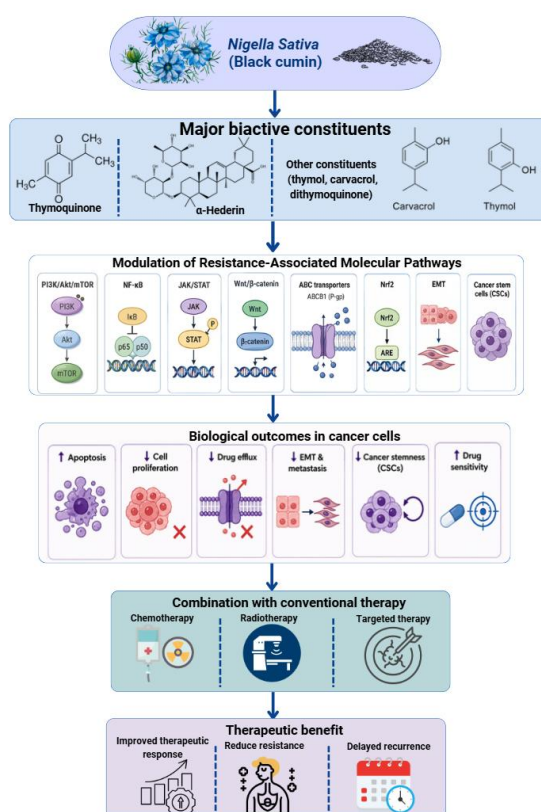


Figure 1. Conceptual overview of the molecular mechanisms through which *Nigella sativa*-derived bioactive constituents enhance cancer-cell sensitivity to conventional therapies by modulating multiple resistance-associated signaling pathways.

As illustrated in Figure 1, *Nigella sativa* contains several major bioactive constituents, including thymoquinone, α -hederin, thymol, carvacrol, thymohydroquinone, and dithymoquinone, with thymoquinone being the most extensively investigated compound. These bioactive constituents exert multitarget effects by modulating multiple molecular pathways involved in cancer-treatment resistance, including PI3K/Akt/mTOR, NF- κ B, JAK/STAT, Wnt/ β -catenin, ABC transporters (ABCB1/P-glycoprotein), Nrf2 signaling, epithelial–mesenchymal transition (EMT), and cancer stem cells (CSCs). Regulation of these interconnected pathways contributes to enhanced apoptosis, suppression of cancer-cell proliferation, reduced drug efflux, inhibition of EMT and metastatic progression, attenuation of cancer stemness, and increased sensitivity of cancer cells to anticancer therapies. Collectively, these molecular effects provide a mechanistic basis for the ability of *Nigella sativa*-derived bioactive constituents to enhance the therapeutic efficacy of chemotherapy, radiotherapy, and targeted therapy, thereby improving treatment response, overcoming therapeutic resistance, and potentially reducing the risk of tumor recurrence.

Current mechanistic evidence concerning resistance to cancer therapy is dominated by thymoquinone, whereas the resistance-modulating effects of other *Nigella sativa*-derived constituents remain insufficiently characterized. Thymoquinone acts on several interconnected signaling networks that regulate cancer-cell proliferation, survival, and adaptation to therapeutic stress. In MV4-11 and K562 myeloid leukemia cells, thymoquinone reduced the expression and phosphorylation of PI3K, Akt, and mTOR while increasing PTEN expression and suppressing JAK2/STAT3 and STAT5 signaling. These alterations were accompanied by reduced proliferation and increased apoptotic activity, indicating that thymoquinone can simultaneously interfere with complementary survival pathways. Evidence from pancreatic and colorectal cancer models further implicates NF- κ B and Wnt/ β -catenin signaling in its anticancer effects. In pancreatic cancer, thymoquinone has been associated with reduced NF- κ B activation and improved sensitivity to gemcitabine, whereas modulation of Wnt/ β -catenin

signaling in colorectal cancer has been linked to decreased viability, migration, and survival-related activity. These findings support thymoquinone as a multi-pathway modulator; however, pathway inhibition observed in individual tumor models should not be interpreted as universal reversal of resistance across cancer types or treatment modalities (Al-Rawashde et al., 2022; Majdalawieh et al., 2024; Moulana et al., 2024; Zhao et al., 2023).

The inhibition of prosurvival signaling by thymoquinone may enhance apoptotic competence and facilitate treatment-induced cancer-cell death. PTEN upregulation and Akt inhibition can attenuate signals that normally protect malignant cells from therapeutic injury, while modulation of p53 and p21 may promote cell-cycle arrest and the removal of damaged cells. Across preclinical cancer models, thymoquinone has been associated with increased Bax expression, reduced Bcl-2 activity, mitochondrial cytochrome c release, and activation of caspase-9 and caspase-3. These molecular changes shift the Bax/Bcl-2 balance toward mitochondrial membrane permeabilization and intrinsic apoptosis. Thymoquinone-containing combinations have likewise been reported to increase caspase activation and poly(ADP-ribose) polymerase cleavage while suppressing antiapoptotic signaling. Nevertheless, apoptosis induction in treatment-naïve cells does not necessarily demonstrate reversal of established resistance. Resistant subpopulations may persist through quiescence, protective autophagy, alternative survival pathways, or stem-like plasticity. Resistance-focused investigations should therefore complement short-term viability and apoptosis assays with clonogenic survival, tumor-repopulation, and recurrence-related assessments to determine whether treatment sensitization is durable (Almajali et al., 2021; Al-Rawashde et al., 2022; Majdalawieh et al., 2024).

Regulation of membrane transport provides another mechanism through which thymoquinone may improve the intracellular availability of anticancer agents. Overexpression of ATP-binding cassette transporters, particularly P-glycoprotein/ABCB1, promotes multidrug resistance by exporting structurally diverse anticancer drugs and preventing the attainment of effective intracellular concentrations. In HCT116 colorectal cancer cells, thymoquinone significantly enhanced imatinib uptake in a time- and concentration-dependent manner. The combination also decreased *ABCB1* and *ABCG2* expression and substantially increased the cellular uptake-to-efflux ratio compared with imatinib alone. Quantitative combination analysis indicated synergistic activity, providing direct evidence that thymoquinone can potentiate a conventional anticancer agent through modulation of cellular drug disposition rather than through independent cytotoxicity alone. However, this evidence remains confined to a specific drug-cell model, and the findings do not establish that thymoquinone universally inhibits ABC transporters. Further studies are required to determine whether it directly affects transporter function, indirectly regulates transporter expression through upstream signaling, or produces both effects. Confirmation in transporter-overexpressing resistant models, animal pharmacokinetic studies, and clinically relevant combinations is essential before this mechanism can be generalized to multidrug-resistant cancers (Dong et al., 2023; Thabet et al., 2021).

Thymoquinone also modulates autophagy, epithelial-mesenchymal plasticity, and cancer stem-cell properties, although the direction and therapeutic consequences of these effects are highly context-dependent. In HT-29 colorectal cancer cells, thymoquinone increased Beclin-1 and the LC3-II/LC3-I ratio while reducing p62, consistent with activation of autophagic flux alongside reduced viability and migration. Conversely, in bladder carcinoma cells, thymoquinone impaired autophagosome-lysosome fusion, leading to autophagosome accumulation and apoptosis. The same study demonstrated activation of the miR-877-5p/PD-L1 axis, suppression of epithelial-mesenchymal transition, and reduced migration and invasion. In breast cancer stem cells, thymoquinone inhibited vasculogenic capacity, reduced drug-efflux activity, interfered with Wnt/PI3K signaling, and promoted mesenchymal-to-epithelial transition. Nevertheless, evidence from hypoxic oral cancer cells demonstrates that its effects are not uniformly advantageous: thymoquinone enhanced carboplatin-induced apoptosis but simultaneously increased enrichment of CD133-positive stem-like cells. Immediate cytotoxic sensitization may therefore coexist with the survival or selection of

subpopulations capable of supporting subsequent adaptation and recurrence. Long-term clonogenic assays, sphere-formation studies, lineage tracing, and in vivo recurrence models are needed to establish whether thymoquinone eliminates resistant cancer-cell populations or merely alters their transient phenotypic state (Haiaty et al., 2021; Moulana et al., 2024; Rahman et al., 2025; Zhou et al., 2021).

Adjuvant Effects of *Nigella sativa*-Derived Constituents with Conventional Cancer Therapies

Thymoquinone has demonstrated adjuvant activity with several conventional anticancer agents, although the strength of evidence varies according to the drug, cancer type, experimental model, and formulation. Across the preclinical literature, thymoquinone has been combined with antimetabolites, platinum compounds, taxanes, anthracyclines, and kinase inhibitors, with many studies reporting greater anticancer activity than either treatment alone. Among the most clearly characterized examples, thymoquinone-loaded calcium carbonate nanoparticles combined with 5-fluorouracil inhibited CT26 colorectal cancer-cell proliferation, migration, and spheroid growth while inducing apoptosis and G0/G1 cell-cycle arrest. Quantitative interaction analysis supported a synergistic effect at the evaluated concentrations. In a separate study using LoVo colorectal cancer cells and a xenograft model, combined thymoquinone and 5-fluorouracil treatment produced greater cytotoxic, genotoxic, proapoptotic, and tumor-suppressive effects than either monotherapy. These findings support the potential of thymoquinone to enhance chemotherapy responses, but their preclinical nature precludes conclusions regarding efficacy in patients (Al-Damook et al., 2025; Deng et al., 2024; Guler et al., 2025).

The potentiating effects of thymoquinone appear to involve complementary regulation of apoptosis, oxidative stress, prosurvival signaling, and intracellular drug disposition. In colorectal cancer models, its combination with 5-fluorouracil increased reactive oxygen species, DNA damage, and apoptosis while reducing mitochondrial membrane potential and glutathione levels. Thymoquinone also sensitized HCT116 colorectal cancer cells to imatinib, as demonstrated by formal combination-index analysis and a substantial reduction in the effective concentration of imatinib. This effect was accompanied by increased intracellular imatinib uptake, downregulation of the efflux-associated genes *ABCB1* and *ABCG2*, and an increased uptake-to-efflux ratio. These observations indicate that thymoquinone can enhance anticancer activity not only through independent cytotoxicity but also by modifying cellular drug transport and weakening resistance-associated defenses. Nevertheless, the term *synergistic* should be reserved for studies using validated quantitative interaction models. Similarly, reductions in effective concentrations in preclinical systems should be interpreted as potential dose-sparing effects rather than evidence that clinical chemotherapy doses can be safely reduced (Deng et al., 2024; Guler et al., 2025; Thabet et al., 2021).

Thymoquinone has also shown radiosensitizing potential in colorectal cancer models. In two-dimensional cultures, three-dimensional spheroids, and patient-derived organoids, combining thymoquinone with ionizing radiation reduced cancer-cell survival and clonogenic recovery more effectively than either intervention alone. The combination also inhibited sphere-forming capacity and altered molecular processes associated with cancer stemness and DNA-damage repair, suggesting that thymoquinone may impair the ability of residual cancer cells to survive and repopulate tumors after irradiation. These endpoints are particularly relevant because clonogenic and organoid-forming assays provide a more durable measure of treatment response than short-term metabolic viability alone. However, the therapeutic value of a radiosensitizer depends on selective enhancement of tumor-cell injury without increasing radiation damage in normal tissues. Although the study reported limited toxicity toward non-tumorigenic intestinal cells under the tested conditions, comprehensive evaluation in normal-tissue models and in vivo radiotherapy settings remains necessary before clinical translation (Al Bitar et al., 2022).

Evidence involving targeted therapy is less extensive than that involving cytotoxic chemotherapy and remains largely restricted to cell-based studies. Thymoquinone enhanced

imatinib activity in HCT116 cells through modulation of drug uptake and efflux, providing an example of its potential interaction with a tyrosine kinase inhibitor. Thymoquinone also enhanced the antiproliferative and cytotoxic effects of sorafenib in HepG2 liver cancer and MDA-MB-231 triple-negative breast cancer cells. The sorafenib study associated the combination with G2/M arrest, apoptosis, reduced DNMT3B and HDAC3 expression, and increased miR-29c expression. However, several treatment groups also included piperine, making the specific contribution of thymoquinone difficult to isolate. Other target-directed investigations have identified interactions between *N. sativa* constituents and neuropilins in breast cancer models, but such findings do not yet demonstrate sensitization to an approved targeted therapy. Direct evidence for combinations with hormonal therapy remains particularly scarce. Future research should therefore employ molecularly characterized resistant models, clinically relevant treatment sequences, quantitative synergy analyses, pharmacokinetic evaluation, and normal-tissue controls before *N. sativa*-derived constituents can be positioned as reliable adjuvants to targeted or endocrine cancer therapies (El-Shehawy et al., 2023; Thabet et al., 2021; Zafar et al., 2023).

Translational Challenges and Future Research Directions

The clinical development of thymoquinone remains limited by its unfavorable physicochemical and pharmacokinetic properties. Its hydrophobicity and poor aqueous solubility can restrict oral dissolution and absorption, while sensitivity to light, temperature, and pH may reduce chemical stability during formulation and storage. First-pass metabolism and variable systemic exposure may further limit the attainment of therapeutically relevant concentrations in tumor tissues. Nanoemulsions, liposomes, polymeric nanoparticles, and other delivery systems have therefore been investigated to improve thymoquinone stability, bioavailability, and tumor delivery. However, enhanced cytotoxicity in experimental models should not be interpreted as improved clinical performance without supporting pharmacokinetic, biodistribution, and safety data (Pal et al., 2021).

The reproducibility of *Nigella sativa* preparations is also challenged by substantial phytochemical variability. Extraction solvent, temperature, duration, and processing technique can alter the recovery of thymoquinone, phenolic compounds, and other bioactive constituents, thereby producing preparations with different pharmacological activities. Plant genotype, geographical origin, cultivation conditions, oxidation, and storage may introduce further variation. Consequently, seed oils and extracts marketed under the same botanical name cannot be assumed to be chemically equivalent. Future studies should report botanical authentication, geographical source, extraction procedures, phytochemical profiles, thymoquinone concentration, storage conditions, and batch consistency. Standardization based solely on thymoquinone may nevertheless be insufficient for whole extracts because other constituents can contribute to their overall biological effects (Alberts et al., 2024; Gueffai et al., 2022).

Safety, selectivity, and potential interactions with conventional anticancer agents require equally careful evaluation. Thymoquinone-containing combinations may enhance apoptosis and reduce the effective concentrations of some anticancer drugs in preclinical models, but these findings do not establish safe dose reduction in patients. The same mechanisms that increase intracellular drug exposure or suppress resistance pathways may also enhance toxicity in normal tissues. Therefore, future investigations should assess hepatic, renal, hematological, cardiovascular, and immunological toxicity, together with effects on drug-metabolizing enzymes and membrane transporters. Comparisons between malignant and nonmalignant cells are also necessary to determine whether treatment enhancement is selective for cancer cells. Although *N. sativa* has a history of traditional and dietary use, this does not automatically establish the safety of concentrated extracts or purified constituents administered with multidrug cancer regimens (Akalin et al., 2025; Alberts et al., 2024).

Future research should prioritize reproducible and clinically relevant evidence rather than further demonstrations of general cytotoxicity. Chemically standardized preparations should be evaluated in paired treatment-sensitive and treatment-resistant models with

validated molecular resistance characteristics. Cancer stem-cell systems, patient-derived organoids, resistant xenografts, and models incorporating hypoxia and the tumor microenvironment may provide greater translational relevance. Combination studies should use clinically achievable concentrations and quantitative interaction methods, such as combination-index or isobolographic analysis, to distinguish synergy from additive effects. Promising formulations should subsequently undergo pharmacokinetic, biodistribution, toxicity, and drug-interaction testing before progression to early-phase clinical trials. Computational approaches may support target identification and compound optimization, but their predictions must be confirmed experimentally. Ultimately, controlled clinical studies using standardized preparations, resistance-related biomarkers, and systematic safety monitoring are required to determine whether *N. sativa*-derived constituents can provide reproducible benefits as adjuvants to conventional cancer therapy (Arif et al., 2024; Fatfat et al., 2021).

CONCLUSION

Nigella sativa-derived bioactive constituents exhibit promising multi-target adjuvant potential for overcoming cancer therapy resistance. Thymoquinone has the strongest preclinical evidence, modulating prosurvival signaling, apoptosis, drug efflux, autophagy, epithelial-mesenchymal transition, and cancer stem-cell properties while enhancing the efficacy of chemotherapy, radiotherapy, and targeted therapies. However, its effects vary depending on cancer type, treatment regimen, formulation, and experimental conditions. Evidence for other constituents remains limited. Current research is largely preclinical and constrained by phytochemical variability, poor bioavailability, limited pharmacokinetic data, and insufficient safety evaluation. Future studies should emphasize standardized formulations, resistant cancer models, pharmacokinetics, toxicity, and clinical validation.

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