THYMOQUINONE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS: A REVIEW

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ABSTRACT

Thymoquinone (TQ) is a versatile bioactive moiety initially found in the seeds of *Nigella sativa* L., along with other medicinal plants such as *Thymus vulgaris* L., *Satureja montana* L., and *Monarda fistulosa* L. Due to its diverse pharmacological properties and potency to treat many diseases like cancer, convulsions, inflammation, etc., it has gained interest from chemists. TQ acts as an anticancer by modulating the key pathways in cellular mechanisms by inhibiting the proliferation of cells, inducing apoptosis, and disrupting the cell cycle. Its clinical applications are still limited due to poor bioavailability, fast metabolism, and low blood retention. To overcome these limitations, advancements in drug delivery systems and QSAR studies are being explored. To develop optimized TQ-based therapies, the understanding of molecular mechanisms is important. The present review provides a better understanding and knowledge of the phytochemical profile of *Nigella sativa*, the biological activities of thymoquinone, its therapeutic potential in cancer treatment, and highlights ongoing efforts in formulation strategies and drug design to translate its promising pharmacological effects into clinical applications.

Keywords: Thymoquinones, *Nigella sativa*, anticancer agents, medicinal plants, QSAR studies.

1. INTRODUCTION

Black seed (Synonym: *Nigellia sativa* or Black cumin) is a flowering plant which grows annually. Thymoquinone (TQ), the main bioactive compound of *Nigella sativa,* is increasingly recognized as an effective anti-cancer compound because of its ability to modulate specific oncogenic signaling pathways pertinent to tumor growth, survival and metastasis. TQ modulation of PI3K/Akt/mTOR, NF-κB and Wnt/β-catenin pathway signaling arises in cancer by preventing tumor cell proliferation, inducing apoptosis and preventing the acquisition of metastatic ability [1]. Its unique redox-active chemistry enables TQ to specifically target malignant cells while leaving normal cells without injury. There is ample in vitro and in vivo evidence of the efficacy of TQ, demonstrating its significant potential in the field of integrative oncology, as a natural and low-toxicity anti-cancer therapy [2,3].

For centuries, the seeds of *Nigella sativa* have been recognized for their incredible healing capabilities, written into history and tradition for use in the important religious and traditional medicine of Arab and Islamic countries, and are often referenced as a "miraculous cure." These seeds can be used as both a therapeutic and feed additive for a wide variety of medical dysfunctions [4-6]. The seeds of the *N. sativa* plant have a variety of the essential nutrients including carbohydrates, amino acids, proteins, essential fatty acids, and minerals such as iron, magnesium, calcium, sodium, zinc, and dietary fibers [7-10]. Among many bioactive compounds, thymoquinone seems to be the most important active ingredient that gives it such powerful medicinal benefits. Thymoquinone has been studied extensively in both in vivo and in vitro studies and it demonstrated very significant biological activities, thereby suggesting its capacity for therapeutic effects. Thymoquinone and other bioactive compounds are derived from natural plant products and provide potent efficacy with minimal toxicity and cost-effectiveness when compared to some of their man-made counterparts. As such, the impact of natural products with healing properties continues to be fundamental to traditional medicine, and, more recently, modern medicine [11-13].

Originally isolated by El-Dakhakhany in 1963, thymoquinone is a monoterpene that has been the subject of numerous preclinical studies focused on its anticancer properties. *N. sativa* has traditionally been employed in the treatment of hypertension, diabetes, inflammation, respiratory issues, and gastrointestinal disturbances. Over time, the range of illnesses *N. sativa* has proposed therapeutic benefits for has expanded, thanks to the research efforts of many scientists [14,15]. Thymoquinone is lipophilic which facilitates its ability to penetrate the blood-brain barrier and exert pharmacological effects on cellular molecules across many tissues. Upon reaching the liver, thymoquinone undergoes reduction to dihydrothymoquinone, which is considered an active metabolite and certainly is a contributor to its many pharmacological benefits in the literature. These metabolic conversions only improve thymoquinone's biological effect, making it one of the most promising candidates in natural medicine [16].

Thymoquinone (TQ), the main bioactive compound of *Nigella sativa*, is increasingly recognized as an effective anti-cancer compound because of its ability to modulate specific oncogenic signaling pathways pertinent to tumor growth, survival and metastasis. Thymoquinone has anticancer effects in addition to apoptosis-inducing effects, mediated through additional mechanisms such as inhibition of angiogenesis, modulation of epigenetic regulators (e.g., histone deacetylases), induction of autophagic cell death, downregulation of multidrug resistance proteins [17]. TQ modulation of PI3K/Akt/mTOR, NF-κB and Wnt/β-catenin pathway signaling arises in cancer by preventing tumor cell proliferation, inducing apoptosis and preventing the acquisition of metastatic ability [18]. Recent research emphasizes that thymoquinone can increase the effectiveness of standard chemotherapeutic agents, such as cisplatin and doxorubicin, by sensitizing tumor cells to standard agents and reducing drug resistance. The possibility of synergistic interactions creates a viable option for TQ to be included in combination therapy modalities [19]. Its unique redox-active chemistry enables TQ to specifically target malignant cells while leaving normal cells without injury. There is ample in vitro and in vivo evidence of the efficacy of TQ, demonstrating its significant potential in the field of integrative oncology, as a natural and low-toxicity anti-cancer therapy [20]. Thymoquinone has displayed significant efficacy for preclinical cancer models, however, clinical translation is still being investigated, with early-phase trials being developed to look at pharmacokinetics, safety, and efficacy in humans [21]. Thymoquinone, when compared to other phytochemicals, namely curcumin and resveratrol, has a unique balance of redox activity, lipophilicity, and tumor-selective cytotoxicity, allowing för it to remain a competitor in natural cancer therapeutics [22].

Emerging nano-formulations and targeted drug delivery approaches associated with thymoquinone (TQ) in cancer therapeutics captures recent advances in the field. In particular, previously identified issues for TQ, such as low bioavailability, rapid metabolism and poor water solubility, are being addressed [23]. Researchers are working on enhancing the effectiveness of TQ through lipid-based nanocarriers, which includes nano-emulsions and liposomes, which would have controlled drug release and ultimately, increased absorption. Researchers have also used polymeric nanoparticles as drug delivery systems offering improved stability and better targeting cancer cells. Additional novel strategies involve biomolecule conjugation, where peptides or antibodies provide enhanced specific targeting of tumors to decrease systemic toxicity. Emerging approaches like these offer clinically impactful directions that will expand TQ as a natural, low-toxicity option for clinical practice and therapeutic integration as an alternative to traditional chemotherapy, reaffirming thymoquinone's role as a contemporary agent for future oncology therapies. In recent years, a marked increase in scientific publications and patents results has been seen concerning thymoquinone, reflecting a growing world-wide interest in its pharmacological use and therapeutic commercialization [24].

Some features of *N. sativa* are given in Table 1

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Table 1 *Features of Nigella sativa.*

Features	Details
Biological Name	*Nigella sativa*
Regular Names	Black seed, Black cumin
Biological Family	Ranunculaceae
Plant Type	Annual flowering herb
Geographical Origin	Originating in the Southwest Asian and Mediterranean regions
Cultivation Regions	Proliferated over Asia, North Africa, and the Mediterranean coast
Physical Characteristics	Black, tiny seeds with a fragrant scent and a somewhat bitter flavor
Historical Use	Used for a long time in religious and traditional healing rituals
Key Bioactive Component	Thymoquinone—the active ingredient that gives it its therapeutic effects
Traditional Applications	Reduces inflammation, high blood pressure, diabetes, lung problems, and gastrointestinal issues.
Modern Research	Researched for potential neuroprotective, antioxidant, antibacterial, and anticancer effects.

1.1. Chemistry of Thymoquinone

Thymoquinone ($C_{10}H_{12}O_2$) or 2-isopropyl-5-methyl-1,4-benzoquinone, is the active biological component in *Nigella sativa* (black seed) oil. Thymoquinone belongs to the quinone group of compounds, which are redox active compounds, and are bioactive and can be therapeutically active [25-26]. Thymoquinone was first isolated in 1963 by El-Dakhakhany, and more recently has been studied pharmacologically, due to its antioxidant, anti-inflammatory and anticancer activities. Thymoquinone has bicyclic aromatic quinone ring system, and has two carbonyl groups (C=O), which are responsible for the redox activity of the molecule. The bicyclic aromatic quinone structure allows thymoquinone to undergo electron transfer reactions [27-29]. The simple chemical structure is shown below: **Figure 1**

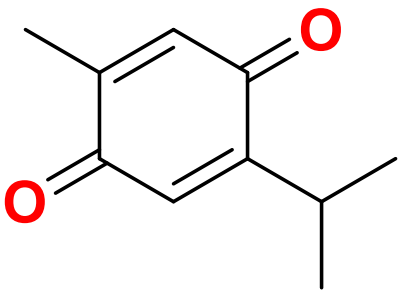

Figure 1: Chemical structure of thymoquinone

.Thymoquinone is lipophilic and can easily penetrate the blood-brain barrier, giving the possibility for contact with different tissues and cellular components. When metabolized hepatically, thymoquinone undergoes reduction that results in dihyrothymoquinone that leads to greater biological efficacy [30-32]. Chemical properties of TQ are given in Table 2

Table 2 Physical and chemical properties of thymoquinone (TQ).

Property	Details
Molecular Weight	~164.2 g/mol
Solubility	Soluble in ethanol and lipids, low water solubility
Appearance	Yellow crystalline substance
Odor & Taste	Distinctive aromatic odor with a pungent taste
Polarity	Moderately lipophilic
Redox Activity	Easily undergoes oxidation-reduction reactions

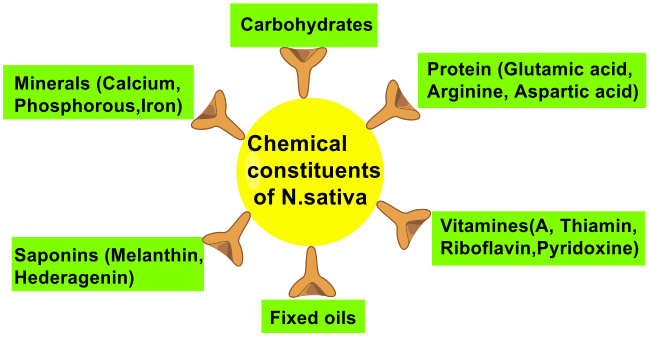

Figure 2: Various constituents of Nigella sativa [33]

1.2. Antineoplastic Effect of Thymoquinone

TQ has strong anti-cancer effects because it modulates multiple biologicals signaling pathways such as, PI3K/Akt/mTOR, NF-κB, and Wnt/β-catenin. It has been shown to induce apoptosis, inhibit cell proliferation and inhibit angiogenesis and metastasis in numerous cancer cell lines including breast, colon, lung, and prostate cancers [34,35]. the induction of Apoptosis is shown in Figure 3

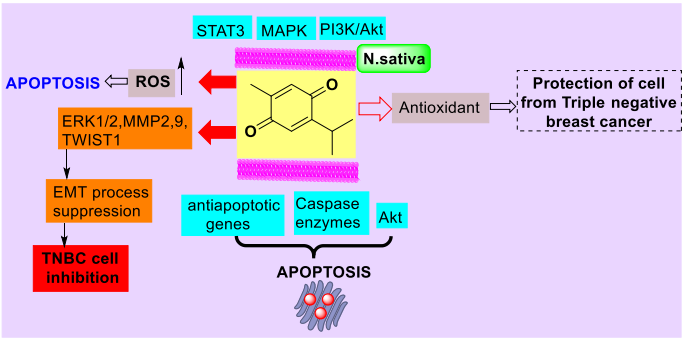

Figure 3: Antineoplastic effect of Thymoquinone (TQ)

1.3. Thymoquinone as Anticancer

TQ is the key constituent found in the black seed of *Nigella sativa*. It has promising anticancer properties. TQ is also found to have several important properties like arrest of cell cycle, initiation of apoptosis and ROS generation. The anticancer therapies available in the market have wide range of side effects. This drug boosts the immune system thereby lessening the side effects arises from established anticancer therapy. Cancer metastasis is also controlled by the drug Thymoquinone. Cancer is a perishable disease worldwide. In cancer the normal healthy cells of body convert into cancerous cells which ultimately leads to malignant form. Many different cancer treatments are already available in the market like chemotherapy, radiotherapy, surgery, targeted therapy, immunotherapy. To deal with this deadly disease many natural plants with its bio-active components are used now a days. Currently, the use of bio-active compounds as anticancer is helping the chemist [36,37].

TQ are used to treat various types of cancers like breast cancer, prostate cancer, bladder, lung, colon cancer, duodenal cancer. TQ acts as anticancer agents by influencing different pathways which were involved in cell proliferation, regulation of cell cycle, apoptosis, angiogenesis and metastatic cancer. Some patents on TQ are given below Table 3 [38-41].

Table 3 Patents on thymoquinone derivatives as anticancer

Patent	Title	Key Focus
US10501428B2	Derivatives of thymoquinone as anticancer.	Discovery of new thymoquinone derivatives with better efficacy.
US9446034B2	Methods of Using Thymoquinone for Cancer Therapy	Discusses the mechanisms of thymoquinone to inhibit cancer cell proliferation.
WO2019163055A1	Combination Therapy Including Thymoquinone	Highlights the synergistic effects of thymoquinone with other anticancer agents.
EP3215478A1	Formulations of thymoquinone for drug delivery	Advanced drug delivery systems which enhance bioavailability of thymoquinone.

As per many research, TQ reduces the side effects associated with chemotherapy drugs. TQ have cytoprotective properties against various chemicals used to treat cancer. They have the property to inhibit various stages of cancer like progression, migration, and invasion. [42] Thymoquinone act as anticancer by causing apoptosis by different pathways such as caspases activation and preventing antiapoptotic proteins. [43]

Abdelazeem et al. and others created hydrazino thymohydraquinone by mixing thymoquinone with hydrazine hydrate, which then reacted with isocyanates, isothiocyanates, and acyl halides to produce new versions of semicarbazone, semithiocarbazone, and acyl hydrazone. Derivatives with IC_{50} values of **1** (9.6 μM) and **2** (10 μM) showed much better effectiveness against breast cancer by causing cell death through the activation of caspase 3/7 enzymes, as confirmed by the MTT test, compared to thymoquinone. [44]

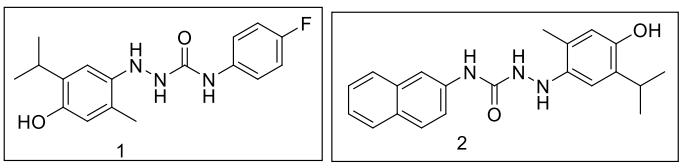

Figure 4: Structures of potent compound 1,2

Eltayeb et al. and others studied how thymoquinone (TQ) works with sulfobutylether-β-cyclodextrin (SBE-β-CD) at different temperatures between 293 and 318 K. TQ showed excellent anticancer activity but has limited solubility and poor delivery. To overcome these limitations, researchers combine TQ with SBE--CD. This study tested the effects of TQ alone and TQ combined with SBE-β-CD on six types of cancer cells—HCT-116, HT-29, MDA-MB-231, MCF-7, SK-BR-3, and HepG2—using the MTT assay. The results revealed that the solubility of TQ was improved and it actively penetrates into the SBE-β-CD cavity. The IC_{50} values for TQ combined with SBE-β-CD, compound **3**, were 0.1 ± 0.01 μg/mL for SK-BR-3 human breast cancer cells and 1.2 ± 0.16 μg/mL for HCT-116 human colorectal cancer cells. By enhancing its solubility and bioavailability, TQ's anticancer activity can be enhanced. The IC_{50} values of TQ alone were found to be 0.2 ± 0.01 μg/mL to 4.7 ± 0.21 μg/mL [45].

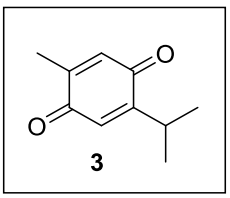

Figure 5: Structure of potent compound 3

From thymoquinone, *Ulfa et al.* synthesized bromoalkylquinones with seven and ten carbon atoms as derivatives. The synthesis involved one and a half hours of reflux oxidation and alkylation of 2,3-dimethylhydroquinone. The compounds were analyzed using 13C-NMR, 1H-NMR, and FT-IR techniques. The yields of the alkylation products, 5-(7-bromoheptyl)-2,3-dimethyl-1,4-benzoquinone (**4**) and 5-(10-bromodecyl)-2,3-dimethyl-1,4-benzoquinone (**5**), were 31.93% and 16.89%, respectively. C7 and C10 demonstrated significant potential as anticancer drugs, with PTEN IC_{50} values of 23.13 and 18.31 ppm, respectively [46].

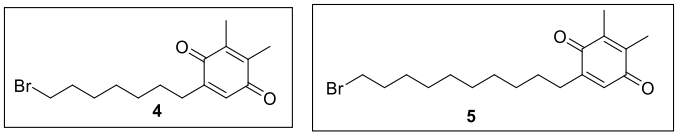

Figure 6: Structures of potent compounds 4,5

Kale et al. investigated possible effects of TQ-Ox on ovarian cancer cells. Finding if it may induce cell death or DNA damage was the goal. NMR studies defined TQ-Ox's structure. Measuring calcium levels helped one to evaluate changes in glutathione, reactive oxygen species, and mitochondrial membrane potential. Stains for acridine orange and ethidium bromide were used to evaluate cell viability. DNA fragmentation was measured using the comet test. With a statistically significant outcome ($p < 0.001$), the more TQ-Ox is used shows improved action against cancer cells. Lowering of GSH (glutathione) and MMP (mitochondrial membrane potential) followed from higher TQ-Ox concentrations. The results show that by improving its cytotoxic effectiveness, TQ-Ox (compound **6**) could be efficient in treating ovarian cancer [47].

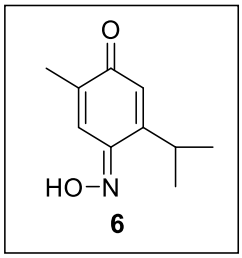

Figure 7: Structure of compound 6

Frohlich et al. prepared seven new thymoquinone-artemisinin hybrids through different linkers and tested their anticancer activity against a range of tumor cell lines. HCT116 and HT29, human colorectal cancer cell lines, have been employed for this. The literature supports the concept of hybridization by proving that the type and size of linkers control the biological activity of hybrid drugs. The thymoquinone-artesunic acid hybrid **7** was twenty times more effective than its original compounds, thymoquinone and artesunic acid, and it did not harm healthy colon cells ($IC_{50} > 100$ μM). The **7** has cytotoxic effects on colorectal cancer cells at concentrations of 2.4 μM and 2.8 μM against both specified cell lines [48].

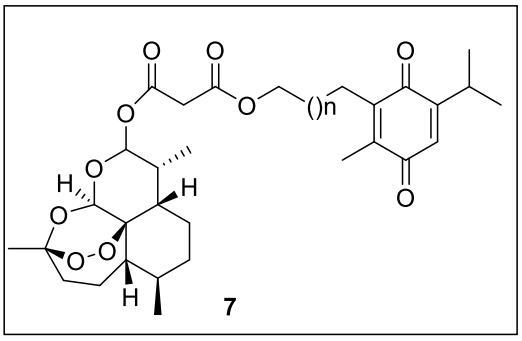

Figure 8: Structure of potent compound 7

Glamoclija et al. found that the 3-aminothymoquinone (ATQ), a natural compound with antibacterial and anticancer properties, is the precursor for ten new benzoxazole derivatives. Elements, infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance (1H,13C) in solution were employed to synthesise and study the derivatives. The study revealed that three benzoxazole compounds exhibited the highest activity among all the compounds.

The strongest compound was 2-(4-fluorophenyl)-4-methyl-7-isopropyl-1,3-benzoxazole-5-ol (Compound **8**) was one of the most effective compounds because it reduces the activity of protein kinase (Akt) and insulin-like growth factor-1 receptor (IGF1R β) in HeLa and HepG2 cells during tests. Docking scores showed that compounds 8, possess tumour survival receptors [49].

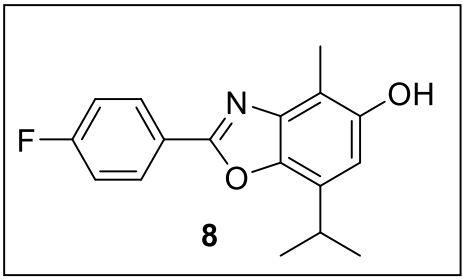

Figure 9: Structure of potent moiety 8

Czajkowska et al. looked into how natural compounds might help prevent and treat various diseases, including cancer. Research in pharmacology has shown that Nigella sativa has a wide range of biological effects. Instead of relying solely on monotherapy, combining natural and pharmaceutical agents could boost anticancer effectiveness while reducing side effects. This study specifically explored the cytotoxic and pro-apoptotic effects of a new octahydropyrazino[2,1-a:5,4-a′] diisoquinoline derivative, Compound **9** both on its own and alongside Nigella sativa oil or extract, in human gastric cancer cells (AGS). They also referenced etoposide in their findings. The results suggest that using the new octahydropyrazino[2,1-a:5,4-a′] diisoquinoline derivative (OM-90) together with Nigella sativa oil or extract is more effective than using monotherapy or etoposide alone in human gastric cancer cells (AGS). This combination activates the mitochondrial pathway, which is essential for these drugs to successfully trigger apoptosis [50].

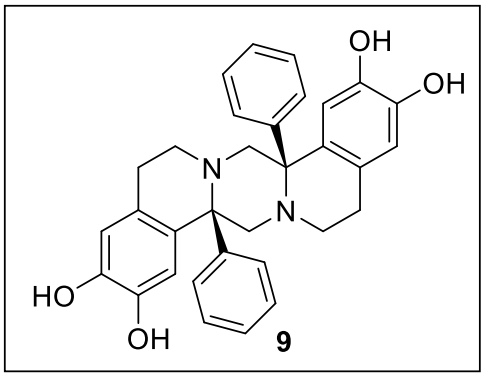

Figure 10: Structure of potent moiety 9

According to *Ismail et al.* hepatocellular carcinoma is one of the most common cancers worldwide, and nutraceuticals are popular cancer therapies. Resveratrol makes *Polygonum cuspidatum* chemo preventive. Thymoquinone is a potent component of *Nigella sativa* that has many therapeutic applications. This study will examine the cancer response of the HepG2 cancer cell line to thymoquinone and also resveratrol. We will examine the responses of HepG2 to thymoquinone, to resveratrol and to their combination treatments. We will evaluate cell viability, caspase-3, glutathione and malondialdehyde. Thymoquinone and resveratrol inhibited cells and elevated caspase-3, suggesting apoptosis. Both drugs raised reactive oxygen species, lowered glutathione, and had minimal impact on lipid peroxidation. Thymoquinone and resveratrol showed significant anti-tumor activity against HepG2 cells with IC_{50} values of 46μM and 64.5μM, respectively. The cell viability was 47.2% and 49.9%. Thymoquinone and resveratrol, compound **10**, boosted caspase-3 enzyme levels by 77% and 98.5%, respectively, whereas glutathione dropped by 22.8% and 35.6%. Malondialdehyde fell 18% and 29.6%. Thymoquinone decreased cell viability 9.9% and resveratrol 12.6%. Caspase-3 rose 89% and 67.5%, whereas glutathione fell 25.6% and 12.8%. Malondialdehyde dropped 32.3% and 20.7% compared to thymoquinone and resveratrol. This proposes an innovative, effective anticancer combo [51].

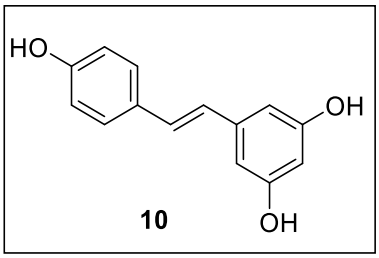

Figure 11: Structure of potent compound 10

Dirican et al. identified thymoquinone in *Nigella sativa* seeds. Breast, prostate, bladder, and kidney cells benefit from its antioxidant, anti-inflammatory, and cancer-fighting properties. We wanted to know how nivolumab and thymoquinone destroy kidney cell carcinoma cells. Using mononuclear cells, Caki-1 and Renca kidney cancer cell lines were established in vitro. Thymoquinone and nivolumab were tested using MTT and Annexin V + PI. In vivo, four groups of eight-week-old male C57BL/6 mice received RENCA cells beneath their skin to simulate renal cell carcinoma. Thymoquinone (20 mg/kg IP every 1, 4, 7, and 14 days) and nivolumab (10 mg/kg IP every 1, 6, and 13 days) comprised the control group. Seven animals were randomly selected for each test group. We used quantitative real-time PCR to check the levels of certain proteins related to cell death and blood vessel growth in the tumor tissue. The heart, lungs, brain, liver, and kidney cells were checked for side effects. Plasma biochemistry was studied. Mann-Whitney U tests were performed for statistical analysis, and p-values were below 0.05. Results are: Thymoquinone killed cancers in lab experiments and live creatures. Nivolumab also killed more immune cells. Tumor tissue samples from the combination group revealed tumor death or complete therapeutic response. Our study reveals that thymoquinone, **11** boosts nivolumab's immune action and eliminates tumors from kidney cell carcinoma. It does not harm other organs whether taken alone or with additional medications [52].

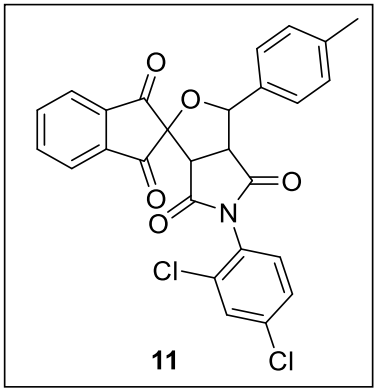

Figure 12: Structure of potent derivative 11

Farghaly et al. found that reducing oxidative stress and improving endoplasmic reticulum proteins could help thymoquinone (TQ) and cisplatin treat HCC. The 50-adult male Wistar albino rats were divided into five equal groups of ten: a control group, a group with HCC caused by dendrimers amine/carbon tetrachloride, a group receiving cisplatin (2 mg/kg ip), a group receiving thymoquinone (20 mg/kg orally), and a group receiving both drugs from Groups 3 and 4. After confirming the model, treatment began for four weeks. The 50-adult male Wistar albino rats were split into five equal groups of ten: control, dendrimers amine/carbon tetrachloride-induced HCC model, cisplatin (2 mg/kg ip), thymoquinone (20 mg.kg−1oral), and both drugs from Groups 3 and 4. 4-week treatment regimens were initiated after the model was validated. The HCC model demonstrated higher ER chaperone, glucose-regulated protein-78 (GRP78), and lower CHOP-mediated apoptosis. Alpha-fetoprotein (AFP) levels rose and liver function was decreased. The preliminary results showed that thymoquinone modified the GRP78/CHOP pathway to enhance cisplatin cytotoxicity. The cisplatin/TQ group, **12** induced CHOP-mediated apoptosis in treatment-related liver cells and exhibited substantially lower GRP78 levels compared with both the HCC group and cisplatin group.

However, the cancer cell treatments had significantly higher AFP and lower liver function when compared to the cisplatin/TQ group. In summary, thymoquinone modified the GRP78/CHOP/caspase-3 pathway to enhance the effect of cisplatin therapy in HCC. Overall, thymoquinone may enhance HCC treatment and mitigate the liver damage caused by cisplatin [53].

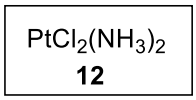

Figure 13: Structure of potent derivative 12

Suriyah et al. found that Cisplatin (CDDP) is an additional chemotherapy dose for oral cancer and it could trigger a major side effect. Researchers in cancer field discovered the natural ingredients to combat the disease cancer. Chemist found out that the TQ, primary active constituent of *N. sativa* may kill many cancer cels without affecting normal healthy ones. In the present investigation, oral cancer cells HSC-4 cells are explored that how much TQ increases the CDDP's cytotoxicity. It was found out that the CDDP(1.66μM) and TQ(1.52μM) were the deadliest to HSC-4 cells, with CI values of 0.362 and 0.538. The current study found that low doses of CDDP decreased HSC-4 cell viability to the same extent as the IC_{50} dose (16.9 μM and 1.97 μM at 24h and 48h, respectively). The low CDDP dose did not reduce the survival of human mouth fibroblasts in the cytotoxicity test. TQ may enhance the chemotherapeutic effect of CDDP in the treatment of oral cancer, while also reducing harm to healthy cells [54].

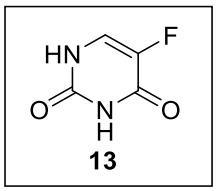

Figure 14: Structure of compound 16

Aumeeruddy et al. state that breast cancer is the most common illness in women and a worldwide danger, despite better screening technologies. Effective treatment is still needed. Single-agent treatments such as surgery, radiation, endocrine therapy, and chemotherapy have high tumour return and disease development rates; hence, combination therapy is the standard breast cancer treatment. In recent decades, studying how medicinal plant phytochemicals interact with cancer medicines has risen in interest. This therapy combines drugs with different effects. This lowers cancer cell resistance and single-treatment side effects. Three substances have been studied because they may fight cancer: piperine **(14),** found in black pepper (Piper nigrum L.) and long pepper (Piper longum L.); sulforaphane **(15)**, found in green vegetables; and thymoquinone **(16)**, from black seed. To find medications that work better together, this study examined how these three substances affect regular cancer treatment. This study underlines the need for nano formulations of these beneficial phytochemicals to make them more accessible by targeting delivery mechanisms to minimize body intake [55].

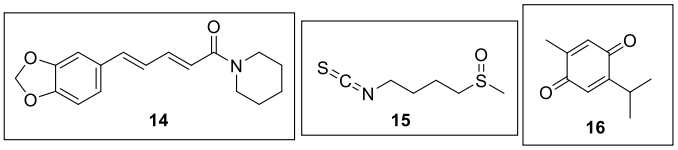

Figure 15: Structures of potent compounds 14,15,16

Eloraby et al. stated that tongue cancer is the most frequent oral cancer. Drugs for cancer treatment increasingly need natural product extracts. Good cancer fighter thymoquinone (TQ). 5-Flourouracil (5-FU) is being developed into a cancer therapy drug series. Cancer combination treatment has grown in popularity. 5-Fluorouracil (5-FU), Thymoquinone (TQ), and their combinations were tested on HNO-97 tongue squamous cell carcinoma cells in vitro. HNO-97, a tongue cancer cell line, was separated into four groups: untreated, 0.5-3.0 μM/ml, 25-50 μM/ml, 7.25-15.65–23.05μM/ml. Methyl thiazole tetrazolium, nuclear morphometric analysis, microscopy, and annexin-v/propidium iodide staining investigated sampling agents' cytotoxicity on HNO-97 cells. Five-FU, TQ, or combination treatment may affect HNO-97 or tongue squamous cell carcinoma cell lines dose-dependently. Cells in 5-FU carriers, TQ with 5-FU **(17)**, and dead cells were examined under the microscope. Significant at 0.05. 5-Fluorouracil and TQ found this HNO-97 sample very cytotoxic [56].

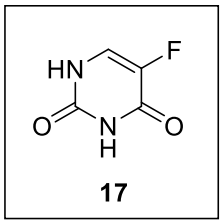

Figure16: Structure of compound 17

Fath et al. claimed that natural polyphenolic thymoquinone has strong anticancer and anti-proliferative effects, claims. The efficacy of thymoquinone to promote cisplatin-induced death in oral squamous cell carcinoma cells was investigated. SCC-25 cancer cells were cultured in varying thymoquinone and cisplatin doses. Following the MTT experiment—which gauges cell viability—Ros and antioxidant activity kits were used. By qRT-PCR and Western blotting, Bax, Bcl-2, caspase-3 mRNA and protein, DNA damage, lipid peroxidation, and protein oxidation were assessed. Together, cisplatin and thymoquinone killed SCC-25 cancer cells. Increased protein, lipid, and DNA peroxidation thyroid quinone and cisplatin. Cysteine and thymoquinone lowered Bcl-2 (**18**), Bax mRNA and protein as well as caspase-3. Thymoquinone reduced cellular oxidative stress and increased the anticancer action of cisplatin in OSCC [57].

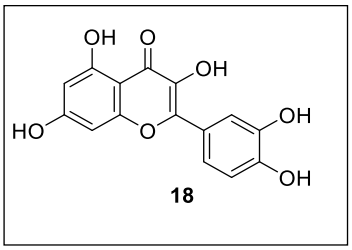

Figure17: Structure of compound 18

Thymoquinone (TQ) and piperine from *Nigella sativa* (cumin) and Piper longum (black pepper) were anticancer, according to *Talib et al.* This study investigates TQ-piperine against chemically induced breast cancer in mice. In the MTT experiment, TQ, piperine, and a combination will be investigated for cytotoxicity on the mouse epithelial breast cancer cell line EMT6/P. CI was calculated using isobolographic analysis. To assess angiogenesis' major suppressor, all therapies would inhibit VEGF or endogenous subjects. EMT6/P cells were injected into Balb/C mice and treated with TQ, piperine, or both to measure tumor suppression. Treatment growth was assessed by measuring tumor size and comparing final tumor histology with various hematoxylin/eosin methods. TUNEL chromogen and caspase-3 activity assays demonstrated apoptosis. Serum levels of IFN-γ, IL-4, IL-2, and IL-10 were evaluated using ELISA. AST, ALT, and creatinine were used to evaluate treatment toxicity. Chemical synergy has been shown, with a CI of 0.788. With 60% cure rate, and 0% mortality, the combination treatment suppressed tumor progression. The combination treatment was able to cause tumor apoptosis and regional necrosis. VEGF decreased substantially with combo treatment [58].

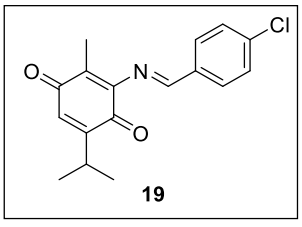

Figure 18: Structure of potent compound 19

Pazhouhi et al. demonstrated that chemotherapy may induce apoptosis in tumour cells. Temozolomide and thymoquinone were studied to see how they affect the process that leads to cell death in the U87MG human glioblastoma multiforme cell line. After temozolomide and thymoquinone treatment, U87MG cells were tested for proliferation. We used a variety of approaches to study apoptosis and cell death. Combining temozolomide and thymoquinone enhanced cell viability. Thymoquinone boosted temozolomide-induced apoptosis. Natural anticancer drugs boosted TMZ's cytotoxic efficacy in vitro. Clinical trials found that using carmustine with TMZ before surgery for GBM was both effective and safe, suggesting that combining medications could be beneficial. Cell antioxidant GSH reduces ROS accumulation. Decreased GSH and increased ROS may activate caspase 3 and destroy cells. ROS signify cell transduction, while excessive levels induce apoptosis. Thus, ROS may biochemically induce apoptosis. The research revealed that TMZ and TQ boosted U87MG cell ROS. Combination treatment enhanced it more than TMZ or TQ alone. ROS are detoxified by reduced glutathione, a brain antioxidant. Nitric oxide reduces neuronal caspase activation and apoptosis. Nitric oxide's significance in glial cell death is unknown. In U87MG cells, TMZ and TQ reduced NO production and may negate NO's anti-apoptotic action [59].

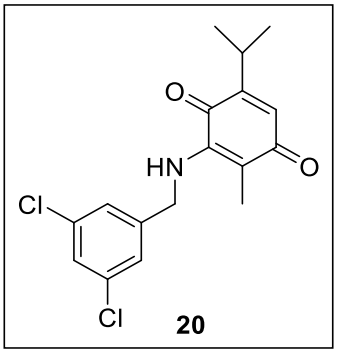

Figure19: Structure of compound 20

Bai P et al. discovered that temozolomide is the only oral first-line glioblastoma treatment. MGMT repairs TMZ's lethal O6-MeG lesion, making it TMZ-resistant. Small-molecule covalent MGMT inhibitors cause haematological damage, limiting their therapeutic utility. New methods are required now to overcome MGMT-mediated resistance. We explored modulating Wnt/β-catenin signaling in glioblastoma to reduce MGMT expression and overcome TMZ resistance. Within 24 hours, thymoquinone (TQ) completely stopped MGMT expression in glioblastoma SF763 and SF767 cell lines, among eight natural or approved Wnt/β-catenin pathway inhibitors. As expected, TQ and TMZ killed SF763 and SF767 cells synergistically, although MGMT-negative SF126 cells only displayed additive effects. TQ also greatly boosted TMZ's inhibition of cell proliferation, clone creation, invasion, migration, and death. In a biosafety-resistant SF763 mouse tumour xenograft model, TQ significantly increased TMZ tumour growth inhibition. Western blotting revealed that TQ greatly reduced the movement of β-catenin into the nucleus and the levels of related proteins like Cyclin D1 and MGMT. Adding the Wnt activator LiCl lowered the effects of TQ on β-catenin moving into the nucleus and the production of MGMT and Cyclin D1. Research suggests that TQ might make glioma more sensitive to TMZ by changing the Wnt/β-catenin pathway and reducing MGMT levels [60].

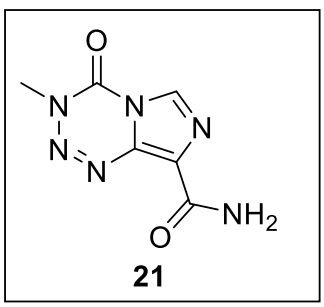

Figure 20: Structure of compound 21

Alam et al. discovered several side effects and large expenses in treatments for non-small cell lung cancer. Development of anticancer treatment depends on alternative medicinal systems, especially those using natural substances. Natural Bax and Bcl2 apoptotic compounds were investigated in silico and in vitro using respective approaches. Examining their interactions and stability in explicit solvent conditions, Tq and Qu were docked and MD-simulated with Bax and Bcl2. Western blot, MTT, and apoptotic tests were used in vitro to examine these molecules. Significant Bax (−6.2 and −7.1 kcal/mol) and Bcl2 (−5.6 and −6.4 kcal/mol) binding affinities for Tq and Qu suggested structural compatibility. Tq and Qu, respectively, had IC_{50} values of 45.78 and 35.69 μM in A549 cells. With a total IC_{50} value lowered to 22.49 μM, there is a notable efficiency increase. Comparatively to 40.6% and 33.3%, respectively, Tq and Qu (**22**) cause 50.9% early death. Both drugs lower Bcl2 expression and raise Bax to sensitize NSCLC cells toward death [61].

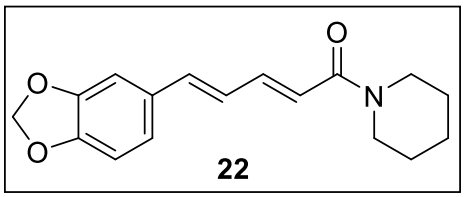

Figure 21: Structure of potent compound 22

Wei et al. studied many types of anti-tumour drug, thymoquinone (TQ). The mechanism and effects are investigated. TQ has weak anti-tumour activity in several cancer cell lines and animal models, according to our lab and others. Higher TQ is needed to design cancer drugs with low-toxicity, high-efficiency ingredients. Anhydrous NaN_3 dissolved in EtOH and acetic acid generated various TQ changes. The reaction mixture cooled to room temperature after 6 h of 80°C heating and stirring. TLC verified a full TQ response. We studied TQFL12 versus TQ in vitro and in vivo on TNBC cells in a mouse model. More than TQ, TQFL12 suppresses TNBC cell growth. We found that TQFL12 (**19**) affects the proliferation of breast cancer cells, migration, invasion, and apoptosis. In cancer cell–derived xenograft mice, TQFL12 decreased tumour growth and metastasis with less toxicity than TQ. The study showed that TQFL12 helped keep AMPKα stable and boosted AMPK/ACC activity, with molecular docking proving they are directly connected. A novel chemical, TQFL12, suppresses TNBC cells better and safer in vitro and in vivo. Thus, TQFL12 may treat breast cancer [62].

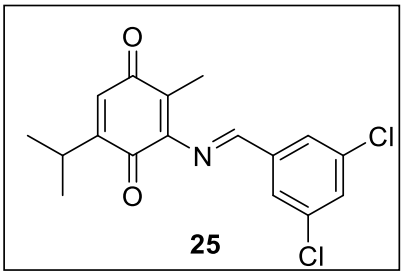

Figure 22: Structure of compound 23

Alaa et al. discovered that thymoquinone (TQ) prevents and treats cancer. Human telomere DNA controls gene transcription. In malignant cells, G-quadruplexes inhibit telomerase overexpression. Selectively stabilizing G-quadruplexes may combat cancer. Methods were used TQ was tested for interactions with telomeric G-quadruplex (5′-AGGG(TTAGGG)3-3′) and duplex DNAs by UV–vis, fluorescence, circular dichroism, liquid and solid NMR (1H and 13C), melting temperature, and docking Changing UV–vis, CD, fluorescence, ^{1}H and ^{13}C NMR spectra, melting temperatures, and docking simulations indicated TQ's interactions with G-quadruplex. TQ binds to G-quadruplex at two sites in the TTA loop, with binding values of 1.80 × 105 and 1.12 × 107 M− 1. TQ stabilized G-quadruplex at 5.6 °C and destabilized ct-DNA at 5.1. Tests indicate that TQ binds more to G-quadruplex than duplex, with selectivity factors of 2.80–3.33 × 10− 3. The findings suggest a π-π stacking-based intercalation binding mechanism. TQ may stabilize G-quadruplex DNA and suppress telomerase and cancer progression, according to our research [63].

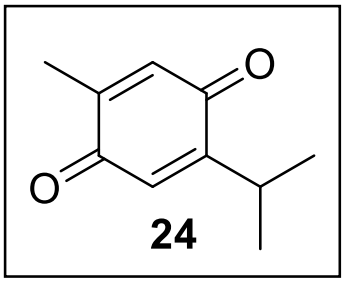

Figure 23: Structure of compound 24

Far et al. synthesized TQFL19, a novel thymoquinone (TQ), which regulates the progression of TNBC in this study. TQFL19 was synthesized from TQ, yielding 65% 3-amino-thymoquinone (NTQ). Red solid NTQ. NTQ was constantly agitated in 100% ethanol with strong hydrochloric acid as a catalyst to synthesize TQFL19. TQFL19, which more efficiently stimulates the AMPK/ACC pathway than TQ, reduces metastasis and invasion in TNBC. The 4T1, BT-549, and MDA-MB-231 cancer cell lines were efficiently used. TQFL19 exhibited an IC_{50} of 26.169 μM against 4T1 cancer cells after 24 hours, whereas TQ had an IC_{50} of 72.05 μM. The IC_{50} of TQFL19 (**25**) against BT-549 cells is 44.271 μM after 24 hours, whereas TQ exhibits an IC_{50} over 80 μM at both 24-hour intervals.The IC_{50} values for TQFL19 against MDA-MB-231 cells after 24 hours are 28.15 μM, whereas TQ has an IC50 of 48.76 μM. The findings indicate that TQFL19 may have prevented the proliferation, metastasis, and invasion of TNBC [64].

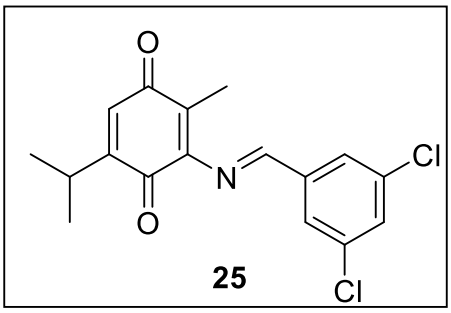

Figure 24: Structure of compound 25

Shadyro et al. developed and studies combination of doxorubicin (Dox) and linseed oil (LO). This combination was evaluated for xenograft tumor models, which includes Pliss lymphosarcoma (PLS) in rats and lewis lung adenocarcinoma (LLC) in mice. The results indicates that the monotherapy of TQ suppressed the tumor growth, combination therapy increases the antitumor effects of doxorubicin thereby reducing the growth of tumor. TQ was administered orally in animals for 12 days are different doses. The final outcome was TQ and its combination have the clinical tendency as an adjuvant in cancer chemotherapy [65].

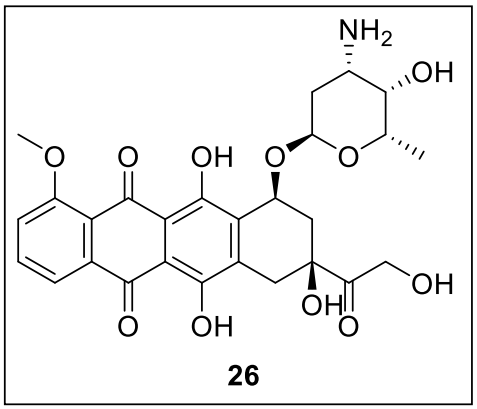

Figure 25: Structure of compound 26

CONCLUSION

In conclusion, thymoquinone (TQ) shows better potential as an anticancer agent; it has the tendency to inhibit cancer cell proliferation, induce apoptosis, regulate the cell cycle, and suppress angiogenesis. The clinical utility of thymoquinone derivatives is still limited in inspite of its promising anticancer activity. Some limitations are poor availability, rapid metabolism, and less systematic retention. The aim is to address these limitations and thereby improve thymoquinone therapeutic efficacy through advancements in drug delivery technologies and structural modifications. Thorough understanding of its molecular mechanism supports the development of optimized drugs.

1.4. **Abbreviations:** TQ: Thymoquinone; QSAR: Quantitative Structure-Activity Relationship; PI3K: Phosphoinositide 3-kinase; Akt: Protein Kinase B; m TOR: mechanistic target of rapamycin; N. sativa: Nigella sativa; Wnt: Wingless-related integration site.

AUTHOR CONTRIBUTION

The authors confirm their contribution to the paper as follows:

Study conception and design: NS

Draft manuscript: NS, BS, AH

All authors reviewed the results and approved the final version of the manuscript.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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