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Exploring the Anti-Inflammatory and Apoptotic Potential of *Nigella Sativa* Compounds via Bioinformatics and Molecular Docking

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ABSTRACT

Nigella sativa is widely recognized for its anti-inflammatory, antioxidant, and immunomodulatory activities; yet, a systematic *in silico* assessment of its major phytocompounds against inflammation- and apoptosis-related targets remains limited. This study employed an integrated bioinformatics and molecular docking approach to evaluate the drug-likeness, toxicity, predicted bioactivity, and protein ligand interactions of four key *Nigella sativa* constituents: thymoquinone, thymohydroquinone, nigellidine, and nigellicine. Druglikeness and pharmacokinetic properties were analyzed using SwissADME, toxicity was predicted through ProTox 3.0, and bioactivity profiles were estimated using PASS Online. Molecular docking simulations were conducted using AutoDock Vina targeting TNF-α (PDB: 2AZ5) and caspase-3 (PDB: 3KJF), followed by interaction visualization in Discovery Studio 2024. All compounds satisfied Lipinski's Rule of Five, suggesting good oral drug-likeness, with nigellicine showing the highest predicted bioavailability score (0.85). Thymoquinone and thymohydroquinone demonstrated the safest toxicity profiles, whereas nigellidine and nigellicine indicated possible organ-specific risks. Docking results showed that nigellidine exhibited the strongest binding affinity to TNF-α (–8.2 kcal/mol) and caspase-3 (–6.7 kcal/mol), forming stable interactions with key active-site residues. Overall, these findings highlight nigellidine and nigellicine as promising dual-target inhibitors with potential therapeutic relevance for inflammation and apoptosis-associated conditions. This work supports the development of natural bioactive compounds and aligns with Sustainable Development Goal 3 (Good health and well-being).

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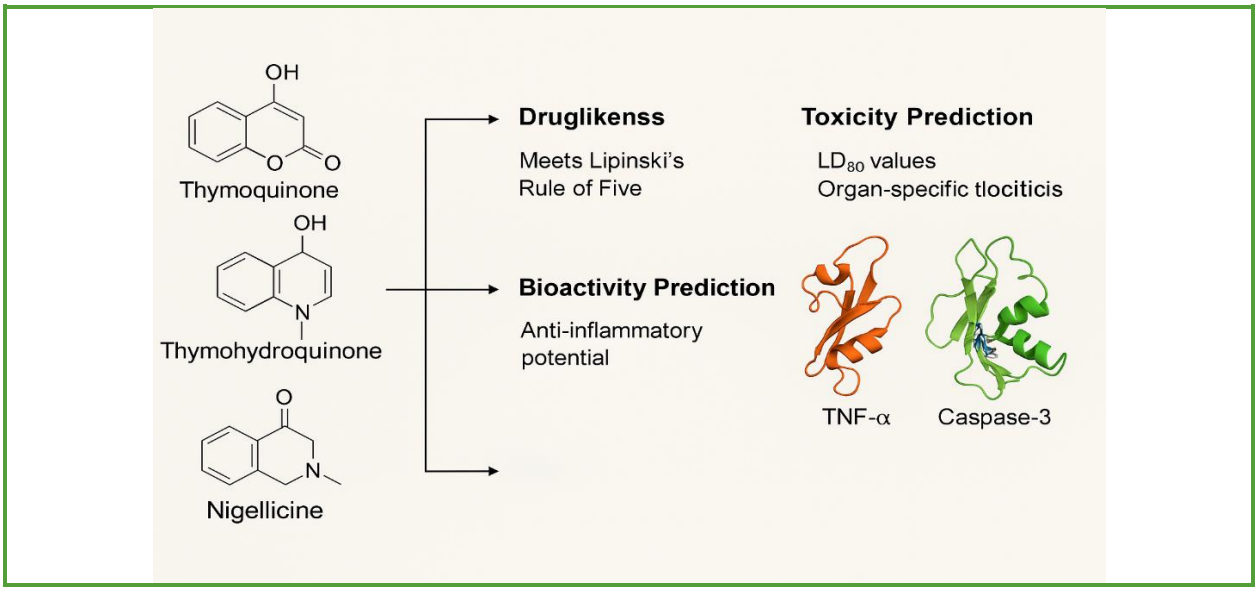
Graphical Abstract



Introduction

The dysregulation of inflammation and apoptosis plays a fundamental role in the development and progression of various chronic and degenerative conditions, particularly reproductive disorders such as cystic ovarian follicle formation. Inflammatory mediators like tumor necrosis factor-alpha (TNF-α) and apoptotic executors such as Caspase-3 are known to contribute to oxidative stress, cellular degeneration, and impaired ovarian folliculogenesis [1-3]. These molecular pathways are considered central targets for therapeutic modulation. *Nigella sativa* (black seed) has long been utilized in traditional medicinal systems due to its pharmacological versatility, which includes anti-inflammatory, antioxidant, antitumor, and immunomodulatory properties [4,5]. Its major phytoconstituents thymoquinone, thymohydroquinone, nigellidine, and nigellicine have shown potential biological activity in previous *in vitro* and *in vivo* studies. Nevertheless, detailed pharmacokinetic profiling, toxicity risk assessment, and molecular interaction analyses targeting inflammatory and apoptotic mechanisms remain limited. The advancement of cheminformatics and computational pharmacology tools now enables systematic *in silico* evaluation of natural products for therapeutic use. Web-based platforms such as SwissADME for pharmacokinetic analysis [6-9], ProTox 3.0 for toxicity prediction [10], and PASS Online for bioactivity prediction [11] provide rapid and efficient compound screening. Moreover, molecular docking has become a reliable method to explore ligand–protein binding interactions and to predict potential pharmacodynamic actions (4) [12-17]. Given the therapeutic relevance of TNF-α and Caspase-3 in inflammation and apoptosis driven disorders, especially within reproductive pathologies, further exploration of *Nigella sativa* compounds targeting these molecules is warranted. This study was therefore conducted to evaluate the drug-likeness, toxicity, predicted bioactivity, and molecular docking interactions of four major *Nigella sativa* compounds. The ultimate aim is to identify promising natural dual-target inhibitors of TNF-α and Caspase-3 that may support future therapeutic development aligned with

Sustainable Development Goal (SDG) 3: ensuring healthy lives and promoting well-being for all at all ages. This study aimed to identify promising *Nigella sativa* phytoconstituents with dual-target inhibitory activity against TNF-α and Caspase-3 through a comprehensive *in silico* pipeline. This is the first integrated computational study evaluating all four major *Nigella* sativa phytochemicals as dual-target inhibitors against both TNF-α and Caspase-3.

Experimental

Chemicals and data sources

All compounds evaluated in this study Thymoquinone, thymohydroquinone, nigellidine, and nigellicine were identified from *Nigella sativa* seed extract, which was obtained commercially in powdered form (Herb Farma, Surabaya, Indonesia). Extraction was performed using ethanol 96% (Merck, Darmstadt, Germany). LC-MS analysis was conducted using an LC-MS/MS system (Waters, Milford, USA) to verify the presence of major bioactive compounds. SMILES (Simplified Molecular Input Line Entry System) codes and structural information for each compound were retrieved from PubChem.

Preparation of chemical structures

Compounds were drawn and optimized using ChemDraw Ultra 12.0 (PerkinElmer, USA), and converted to 3D structures in PDBQT format using Open Babel software (version 3.1.1). Energy minimization was applied using MMFF94 force fields to ensure optimal conformation before docking.

Drug-likeness and pharmacokinetic prediction

Drug-likeness and pharmacokinetic parameters were predicted using the Swiss ADME web tool developed by the Swiss Institute of Bioinformatics (SIB). The evaluated parameters included molecular weight, LogP, number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), topological polar surface area (TPSA), and Lipinski's rule of five compliance [18-20].

Toxicity prediction

Toxicological assessments were carried out using the ProTox 3.0 online server, hosted by Charité–Universitätsmedizin Berlin, Germany. Parameters assessed included median lethal dose (LD_{50}), toxicity class, and organ-specific toxicity (hepatotoxicity, nephrotoxicity, respiratory, cardiotoxicity, and immunotoxicity). Predictions were expressed as probability scores ranging from 0 to 1.

Prediction of biological activity

Bioactivity profiling was conducted using the PASS online server developed by the Institute of Biomedical Chemistry, Russia. The probable activity (Pa) values were calculated for each compound, with values >0.5 indicating likely biological activity. Emphasis was placed on anti-inflammatory, antioxidant, and apoptotic regulatory properties.

Target identification and network construction

Target prediction was performed using Swiss Target Prediction, which employs 2D and 3D similarity to known ligands. Predicted protein targets were further analyzed through STRING v11.5 for protein–protein interaction (PPI) networks and pathway enrichment. Cytoscape 3.9.1 software (National Resource for Network Biology, USA) was used for network visualization.

Molecular docking simulation

TNF-α and Caspase-3 were selected due to their well-established roles as pivotal targets in inflammation and apoptosis pathways, especially in reproductive-related pathologies. Crystal structures of TNF-α (PDB ID: 2AZ5) and Caspase-3 (PDB ID: 3KJF) were downloaded from the RCSB Protein Data Bank. Protein structures were prepared using AutoDock Tools 1.5.6 (Scripps Research Institute, USA) by removing water molecules and co-crystallized ligands, adding polar hydrogens, and assigning Gasteiger charges. Grid boxes were defined to include the active sites based on co-crystallized ligand positions. Docking was performed using AutoDock Vina 1.1.2, and binding affinities were recorded in kcal/mol. Binding interactions were visualized using BIOVIA Discovery Studio Visualizer 2024 (Dassault Systèmes, France). All computational procedures were conducted with default parameters unless stated otherwise. Each docking simulation was performed in triplicate to ensure reproducibility and accuracy of results. No experimental uncertainties arose beyond acceptable computational deviation limits.

Results and Discussion

Drug-likeness and bioavailability

The four major compounds of *Nigella sativa* thymoquinone, thymohydroquinone, nigellidine, and nigellicine demonstrated favorable pharmacokinetic characteristics according to Lipinski's Rule of Five. All compounds had molecular weights under 500 g/mol, acceptable LogP values (1.28–2.39), and no rule violations, indicating good drug-likeness and oral absorption potential. Nigellicine presented the highest predicted oral bioavailability (score 0.85), suggesting superior intestinal absorption (Table 1) [3,4,17].

All four compounds exhibited profiles compatible with oral drug development, with nigellicine being the most favorable in terms of absorption prediction.

Toxicity prediction

Toxicity profiles varied among the compounds. Thymoquinone showed the highest LD_{50} (2500 mg/kg) and was classified in toxicity class 5, indicating a relatively safe profile. Thymohydroquinone, nigellidine, and nigellicine were placed in class 4. Organ-specific toxicity predictions suggested potential respiratory toxicity for nigellidine and both nephrotoxicity and respiratory toxicity for Nigellicine (Table 2) [7,9].

These results suggest that while all compounds fall within safe toxicity ranges, nigellidine and nigellicine require caution due to their potential organ-specific toxicities.

Prediction of biological activity

Thymoquinone, thymohydroquinone, and nigellidine were predicted to exhibit high anti-inflammatory activity (Pa > 0.5) via pathways involving TNF-α and JAK2. Nigellicine showed lower scores. Thymoquinone and thymohydroquinone also displayed antioxidant potential, likely via Nrf2–ARE signaling (Figure 1).

Molecular docking analysis

Docking simulations showed that Nigellidine had the strongest binding to TNF-α (−8.2 kcal/mol) and Caspase-3 (−6.7 kcal/mol), forming stable hydrogen bonds with key residues such as Tyr119, Gly121, and Arg207 (Table 3).

Table 1. Drug-likeness and bioavailability parameters of *Nigella sativa* compounds

Compound	MW (g/mol)	LogP	HBA	HBD	Violations	Bioavailability score
Thymoquinone	164.2	1.80	2	0	0	0.55
Thymohydroquinone	166.22	2.10	2	1	0	0.55
Nigellidine	294.35	2.39	2	1	0	0.55
Nigellicine	246.26	1.28	3	1	0	0.85

Table 2. Predicted toxicity of *Nigella sativa* compounds

Compound	LD_{50} (mg/kg)	Toxicity class	Hepatotoxicity	Nephrotoxicity	Respiratory toxicity	Cardiotoxicity	Immunotoxicity
Thymoquinone	2500	5	0.63	0.71	0.78	0.75	0.97
Thymohydroquinone	1000	4	0.77	0.66	0.55	0.89	0.89
Nigellidine	1000	4	0.64	0.62	−0.78	0.78	0.95
Nigellicine	1300	4	0.60	−0.61	−0.78	0.75	0.99

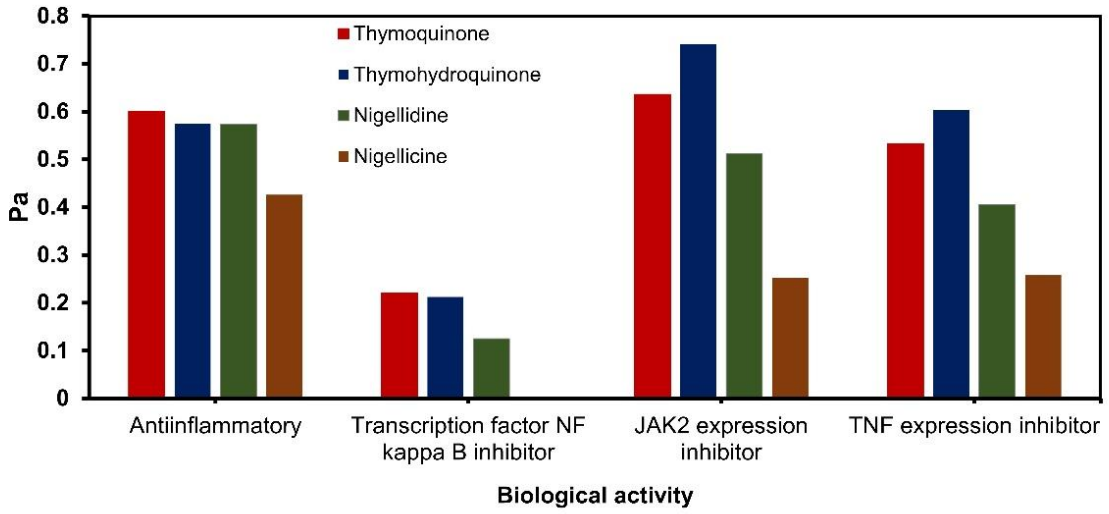


Figure 1. Biological activity prediction (Pa scores) of *Nigella sativa* compounds for selected pathways (Note: Pa > 0.5 indicates probable biological activity; plotted data include TNF-α inhibition, JAK2 modulation, and antioxidant response)

Table 3. Binding affinities from molecular docking simulations

Compound	TNF-α (kcal/mol)	Caspase-3 (kcal/mol)
Inhibitor (control)	−9.8	−9.1
Thymoquinone	−6.0	−5.2
Thymohydroquinone	−5.9	−5.2
Nigellidine	−8.2	−6.7
Nigellicine	−7.0	−6.5

Nigellicine showed slightly lower binding affinity. Thymoquinone and Thymohydroquinone had modest affinities ranging from −5.2 to −6.0 kcal/mol (Figures 2 and 3).

Collectively, these results support the dual-target potential of nigellidine and nigellicine in modulating TNF-α and Caspase-3 activity. While thymoquinone and thymohydroquinone exhibited lower docking scores, their superior safety and antioxidant profiles enhance their therapeutic value for chronic disease management. These insights highlight a complementary pharmacological profile, where potency and safety may be balanced through compound selection or formulation strategies such as nano-delivery systems [16].

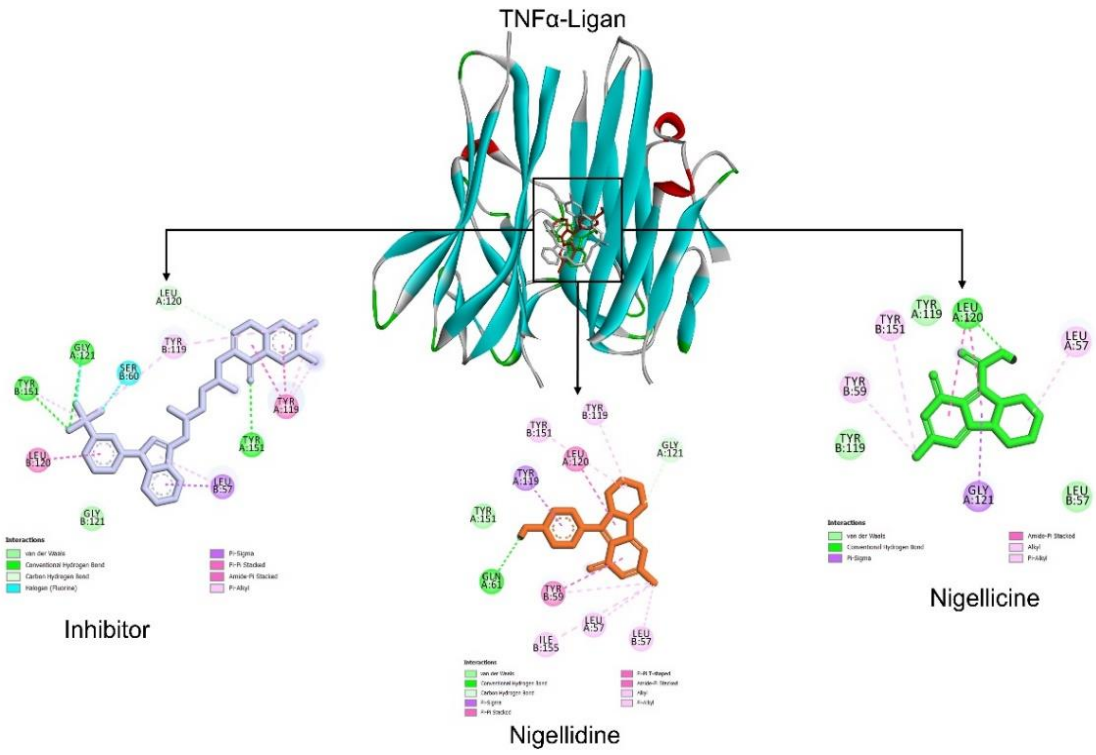


Figure 2. Docking visualization of TNF-α binding with control inhibitor, nigellidine, and nigellicine (Ligands shown interacting at the LEU120, TYR119, and GLY121 residues within TNF-α active site)

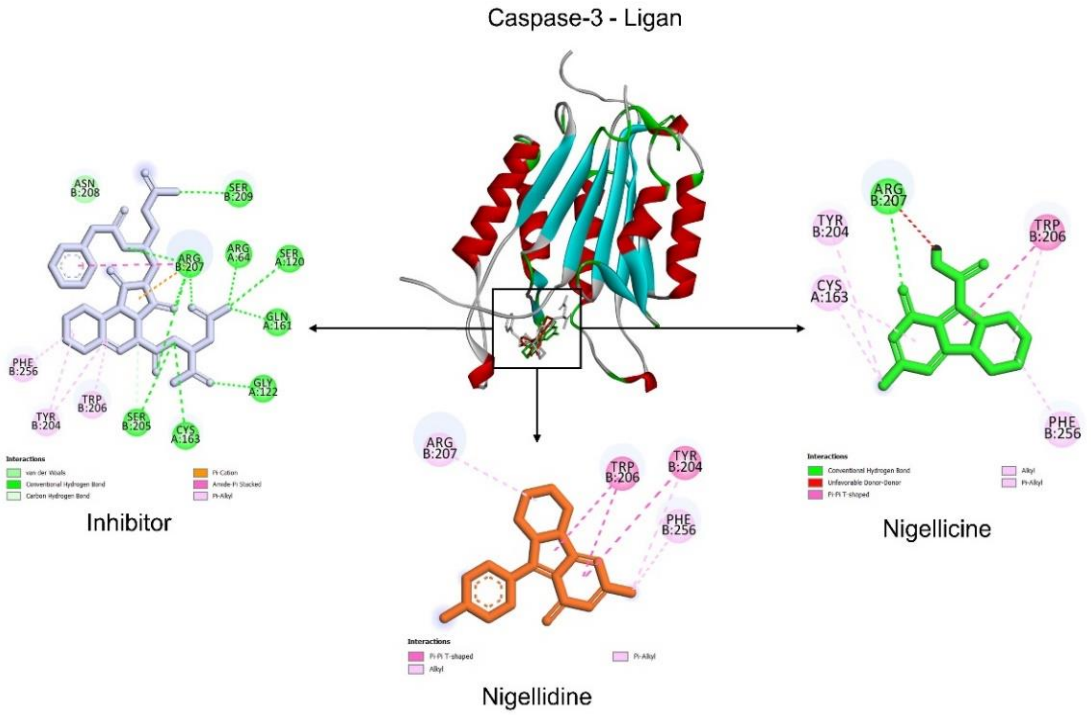


Figure 3. Docking visualization of Caspase-3 binding with control inhibitor, nigellidine, and nigellicine (Docked ligands interact with ARG207, PHE256, TRP206, and TYR204 via hydrogen and hydrophobic contacts)

Conclusion

This study provides a comprehensive *in silico* evaluation of four major *Nigella sativa* compounds thymoquinone, thymohydroquinone, nigellidine, and nigellicine highlighting their potential pharmacological applications in inflammation- and apoptosis-related disorders. All compounds fulfilled essential drug-likeness criteria, with nigellicine exhibiting the highest predicted oral bioavailability, while nigellidine demonstrated the strongest binding affinity toward both TNF-α and caspase-3. Toxicity profiling indicated that Thymoquinone and Thymohydroquinone are the safest for further development, whereas nigellidine and nigellicine possess potent activity but require structural refinement due to organ-specific risks. These findings support the dual-target therapeutic promise of *Nigella sativa* phytochemicals and justify further experimental validation through *in vitro* and *in vivo* studies. This work contributes toward the discovery of natural anti-inflammatory and anti-apoptotic agents and aligns with Sustainable Development Goal 3 (Good Health and Well-being).

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Disclosure Statement

The authors declare that no conflicts of interest exist.

Authors' Contributions

Viski Fitri Hendrawan: Conducted bioinformatics and molecular docking analysis, wrote the initial Draft. Epy Muhammad Luqman: Supervised the study design, guided data interpretation, and finalized the manuscript. Rimayanti and Widjiati Widjiati: Provided guidance in pharmacological interpretation and methodology validation. Iwan Sahrial Hamid and Moh. Anam Al Arif: Analyzed toxicity and PASS data. Hani Plumeriastuti and Tri Wahyu Suprayogi: Supported data visualization and editing. All authors read and approved the final manuscript.

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