Indonesian Journal of Pharmacy and Natural Product
http:/jurnal.unw.ac.id/index.php/ijpnp
Volume 08 Nomor 02 , September 2025
p-ISSN : 2656-3215
e-ISSN : 2615-6903

In Silico Evaluation of Black Cumin Oil Constituents for Selective Anti-Inflammatory Inhibitor of COX-2 And 5-LOX

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ABSTRACT

Black cumin oil (Nigella sativa) is widely known for its anti-inflammatory properties, attributed to various bioactive compounds. This study aimed to evaluate the binding affinity and selectivity of five major compounds from black cumin oil: dithymoquinone, thymoquinone, thymohydroquinone, p-cymene, and thymol, against key inflammatory enzymes: COX-1, COX-2, and 5-LOX, using an in silico approach. All compounds were assessed for drug-likeness using Lipinski's Rule of Five before molecular docking with AutoDock Vina. Celecoxib, a selective COX-2 inhibitor, was used as a reference compound. Dithymoquinone demonstrated strong binding affinity to COX-2 (-9.3 kcal/mol) and 5-LOX (-8.4 kcal/mol), comparable to celecoxib (-9.9 and -8.3 kcal/mol, respectively), while showing lower affinity for COX-1 (-7.5 kcal/mol). The interaction analysis revealed hydrogen bonds and van der Waals forces with several active site residues, suggesting a stable and selective interaction. Other compounds showed moderate to high affinity but lacked the same degree of selectivity due to their interaction with COX-1. These findings indicate that dithymoquinone has the potential to act as a selective anti-inflammatory agent with a reduced risk of gastrointestinal side effects commonly associated with non-selective NSAIDs. The results support further investigation of dithymoquinone in preclinical and clinical settings to validate its efficacy and safety.

***Keywords**: Black Cumin Oil, COX-2, 5-LOX, Anti-Inflammatory, Docking.*

INTRODUCTION

Inflammation is an adaptive response of the body to tissue damage, infection, or exposure to harmful agents. However, when the acute phase progresses into chronic inflammation, this protective mechanism may instead become a trigger for degenerative diseases, including arthritis, cardiovascular disorders, and metabolic abnormalities, which contribute significantly to public health concerns and economic burden (Gusev and Zhuravleva, 2022; Linschoten et al., 2021). Conventional therapeutic approaches to inflammation remain largely dependent on nonsteroidal anti-inflammatory drugs (NSAIDs), which act by inhibiting cyclooxygenase (COX) enzymes. Due to their non-selective inhibition of both COX-1 and COX-2, compounds such as aspirin and ibuprofen are often associated with gastrointestinal toxicity, including ulceration and bleeding. Consequently, the development of more selective anti-inflammatory agents has become a key focus in healthcare (Ahmadi et al., 2022).

The development of selective COX-2 inhibitors, such as celecoxib, aims to minimize these adverse effects (El-Malah et al., 2022). However, selective inhibition of COX-2 may shift arachidonic acid metabolism toward the 5-lipoxygenase (5-LOX) pathway, leading to increased production of pro-inflammatory leukotrienes and potentially elevating cardiovascular risks. Therefore, a dual inhibition strategy targeting both COX-2 and 5-LOX is considered a more effective approach to controlling inflammation with minimal side effects (Mukhopadhyay et al., 2023) effects.

Submitted: 2025-01-15 Revised : 2025-08-28 Accepted : 2025-08-20

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Bioactive compounds from medicinal plants have been extensively investigated as alternative anti-inflammatory therapies with improved safety profiles. One such example is black cumin (*Nigella sativa*), traditionally used for its anti-inflammatory, antioxidant, and immunomodulatory properties. *Nigella sativa* has emerged as a promising herbal candidate, supported by both historical and religious significance (Dabeer et al., 2022). Extracts and oils of *Nigella sativa* have been reported to exhibit diverse biological activities, including antimicrobial (Shafodino et al., 2022), analgesic and anti-inflammatory (Shaheen et al., 2022), antioxidant (S et al., 2021), antidiabetic (Shaukat et al., 2023), anticancer (Mir et al., 2022), and gastroprotective effects (Sanpinit et al., 2023).

Black cumin oil contains several active constituents, including thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, and thymol, which are thought to contribute to its pharmacological activities (K et al., 2021) While thymoquinone has been extensively studied (Chatterjee et al., 2025), research on other compounds, particularly dithymoquinone which formed by the dimerization of thymoquinone during extraction, remains limited. The unique chemical structure of dithymoquinone may enable specific interactions with inflammatory enzymes, suggesting its potential as a selective anti-inflammatory agent.

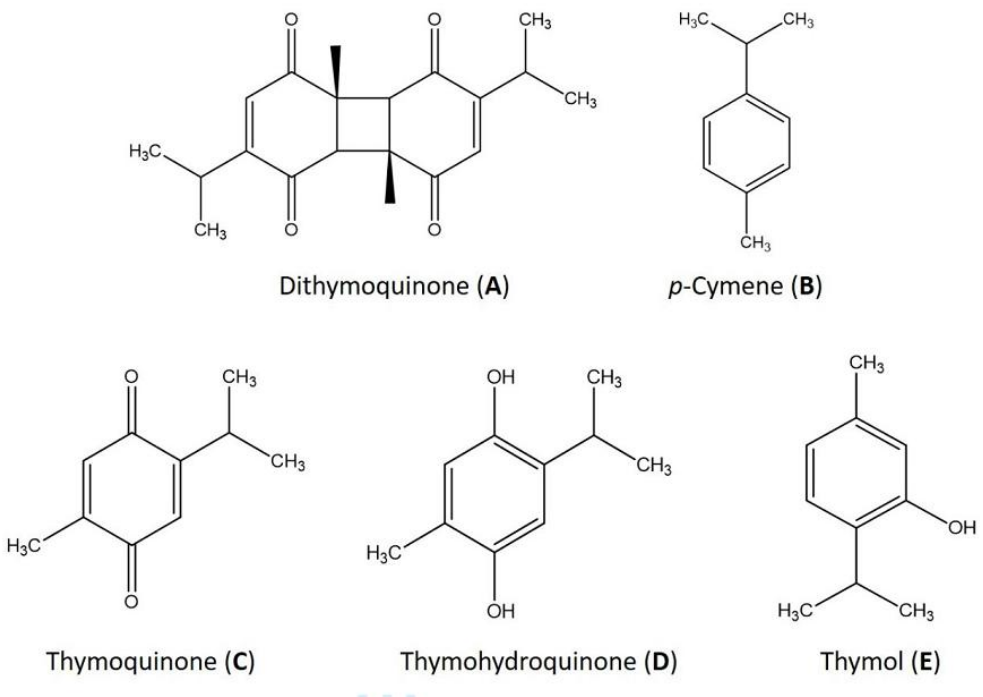

Figure 1. Chemical Structures of Bioactive Compounds in Black Cumin Oil

This study aims to evaluate the binding affinity and selectivity of five major black cumin oil constituents: dithymoquinone, p-cymene, thymoquinone, thymohydroquinone, and thymol, towards COX-1, COX-2, and 5-LOX through an in silico approach. Molecular docking analysis was performed using AutoDock Vina (Eberhardt et al., 2021) to predict the interactions of these compounds with the target enzymes, with celecoxib, a selective NSAID, serving as the reference drug. In addition, the pharmacokinetic feasibility of the compounds was assessed based on Lipinski's Rule of Five.

The findings of this study are expected to identify compounds with selective inhibitory activity against COX-2 and 5-LOX, thereby offering potential as anti-inflammatory agents with

Submitted: 2025-01-15 Revised : 2025-08-28 Accepted : 2025-08-20

minimal gastrointestinal and cardiovascular side effects. Moreover, these results may provide a foundation for further *in vitro* and *in vivo* investigations.

MATERIALS AND METHODS

Equipment and Materials

This study employed the following software and data sources: AutoDock Vina 1.1.2 for molecular docking (Trott and Olson, 2010); AutoDock Tools 1.5.6 for protein and ligand preparation; Open Babel for molecular file format conversion; PyMOL for 3D visualization of protein–ligand structures; Discovery Studio Visualizer 2017 R2 for 2D and 3D protein–ligand interaction analysis; the Protein Data Bank (PDB) as the source of target protein structures; and PubChem as the source of ligand structures. Docking simulations were performed on a Windows system equipped with an Intel(R) Core(TM) i7-3537U CPU @ 2.00 GHz and 4 GB RAM.

Methods

Ligand Preparation

Five major bioactive compounds of black cumin oil: dithymoquinone, p-cymene, thymoquinone, thymohydroquinone, and thymol, were retrieved in Structure Data File (SDF) format were obtained from the PubChem database. Their chemical structures were converted into Protein Data Bank (PDB) format using Open Babel 3.1.1. Ligand preparation was carried out in AutoDock Tools 1.5.7 by adding polar hydrogen atoms, calculating Gasteiger partial charges, and saving the files in PDBQT format. Celecoxib was used as the positive control and processed using the same procedure.

Protein Preparation

Three inflammatory enzymes, COX-1 (PDB ID: 2OYE), COX-2 (PDB ID: 1CX2), and 5-LOX (PDB ID: 3O8Y), were obtained from the Protein Data Bank. Protein structures were modified by removing water molecules, ions, and co-crystallized ligands, followed by the addition of polar hydrogen atoms and calculation of Kollman charges using AutoDock Tools. The final protein files were saved in PDBQT format after verifying the absence of missing residues or atoms.

Docking Procedure

Molecular docking was performed using AutoDock Vina 1.1.2 with specific parameters for each enzyme. For COX-1, the grid box was centered on residues Tyr385 and Ser530 with dimensions of 20 × 20 × 20 Å. For COX-2, the grid box was defined around Val523 and Ser353 with dimensions of 25 × 25 × 25 Å. For 5-LOX, the grid box encompassed the Fe^{2+} binding site at residues His372 and His550 with dimensions of 22 × 22 × 22 Å. Docking parameters included an exhaustiveness value of 8 and a maximum of 10 conformations. Binding affinity was expressed as Gibbs free energy (ΔG) in kcal/mol.

Protein–Ligand Interaction Analysis

Docking results were analyzed to determine binding affinities and interaction types. 2D and 3D visualizations were generated using Discovery Studio Visualizer 2021 and PyMOL 2.5 to identify hydrogen bonds, hydrophobic interactions, van der Waals forces, and electrostatic interactions with catalytic residues such as Arg120 in COX-2. Validation of the docking protocol was conducted by redocking the native ligand into the active site of the target protein, with a root-mean-square deviation (RMSD) < 2.0 Å considered as the validation threshold.

Submitted: 2025-01-15 Revised : 2025-08-28 Accepted : 2025-08-20

RESULTS AND DISCUSSION

This study performed a computational analysis of five active compounds from black cumin oil as potential anti-inflammatory candidates using molecular docking. Preliminary results showed that all compounds complied with Lipinski's Rule of Five (Table 1), with molecular weights ranging from 134.22 to 328.39 g/mol and logP values between 2.47 and 3.82, indicating favorable oral bioavailability (Ischak et al., 2023; Lipinski, 2004).

Table 1. Physicochemical Parameters of the screened compounds based on Lipinski's Rule of Five.

Compounds	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Molar Refractivity	Rules Satisfied
Dithymoquinone (**A**)	328	0	4	2,71	89,06	5/5
p-cymene (**B**)	134	0	0	3,12	45,27	5/5
Thymoquinone (**C**)	164	0	2	1,67	46,69	5/5
*Thymohydroquinon*e (**D**)	166	2	2	2,53	48,59	5/5
Thymol (**E**)	150	1	1	2,82	46,93	5/5

Collectively, these findings suggest that all five compounds possess promising drug-like characteristics. Dithymoquinone, despite having a relatively higher molecular weight as a dimer of thymoquinone, remains within acceptable limits and exhibits balanced lipophilicity and polarity that may support passive membrane diffusion. p-Cymene, a small and lipophilic monoterpene hydrocarbon, readily fulfills Lipinski criteria, with its low molecular weight and moderate logP contributing to good membrane permeability despite the lack of hydrogen bond donors and acceptors. Thymoquinone displays an optimal balance of molecular weight, lipophilicity, and hydrogen bond acceptors, suggesting favorable solubility permeability characteristics for oral administration (Mir et al., 2022). Thymohydroquinone, containing additional hydroxyl groups, shows increased hydrogen bonding capacity and improved aqueous solubility while remaining within Lipinski thresholds, indicating preserved oral bioavailability. Likewise, thymol demonstrates low molecular weight, moderate lipophilicity, and minimal hydrogen bonding functionality, which are consistent with efficient gastrointestinal absorption and further support its classification as a drug-like natural compound. These predictive pharmacokinetic parameters provide an essential foundation for the development of natural compound-based therapeutics.

Molecular docking analysis was conducted against three key inflammatory enzymes; COX-1 (PDB ID: 2OYE), COX-2 (PDB ID: 1CX2), and 5-LOX (PDB ID: 3O8Y) to represent the major inflammatory pathways involving prostaglandin and leukotriene biosynthesis. COX-1 was included to assess potential gastrointestinal side effects related to constitutive prostaglandin inhibition, whereas COX-2 and 5-LOX were selected to evaluate anti-inflammatory efficacy through suppression of inducible and pro-inflammatory mediators (Ahmadi et al., 2022; Mukhopadhyay et al., 2023). Binding affinity analysis revealed an intriguing pattern, in which dithymoquinone exhibited the most promising performance with Gibbs free energy (ΔG) values of –9.3 kcal/mol against COX-2 and –8.4 kcal/mol against 5-LOX. These results were not only comparable to celecoxib, the positive control (–9.9 and –8.3

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kcal/mol, respectively), but also indicated superior selectivity by displaying weaker binding affinity toward COX-1 (–7.5 kcal/mol compared to celecoxib at –8.3 kcal/mol). This suggests that dithymoquinone has the potential to act as an anti-inflammatory agent with a reduced risk of gastrointestinal side effects.

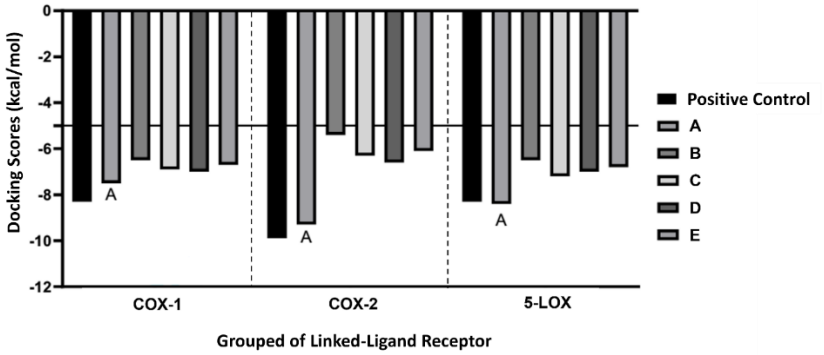

Figure 1. Docking Scores Against Target Receptors: COX-1, COX-2, and 5-LOX. (Positive Control: celecoxib; A: dithymoquinone; B: p-cymene; C: thymoquinone, D: thymohydroquinone, and E: thymol)

Molecular interaction mapping revealed a complex binding mechanism that can be explained in the context of enzyme active-site function and substrate recognition. In COX-2, dithymoquinone formed two strong hydrogen bonds with Asn375 and Arg376, residues located near the catalytic channel that accommodates arachidonic acid, the natural substrate for prostaglandin synthesis. These interactions, together with extensive van der Waals contacts involving key residues such as His226 and Ser143, are likely to stabilize dithymoquinone within the hydrophobic active site and hinder access of arachidonic acid to the catalytic tyrosyl radical, thereby suppressing prostaglandin production. A complementary mechanism was observed for 5-LOX, where dithymoquinone formed hydrogen bonds with Arg246 and Arg370, residues known to play roles in substrate positioning and catalytic iron coordination during leukotriene biosynthesis. The additional π–alkyl interactions further enhance binding stability within the lipophilic pocket of 5-LOX, potentially interfering with the oxygenation of arachidonic acid to leukotriene intermediates. Collectively, these interactions suggest that dithymoquinone acts as a competitive or mixed-type inhibitor by occupying the active sites of COX-2 and 5-LOX, preventing effective enzyme–substrate interaction and downstream inflammatory mediator production. This mechanistic insight provides a molecular basis for its dual inhibitory activity and observed binding stability (Du et al., 2016).

Comparison with other compounds in black cumin oil revealed varying activity profiles. Thymoquinone and thymohydroquinone displayed notable affinities toward 5-LOX (–7.2 and –7.0 kcal/mol, respectively), but their strong binding to COX-1 reduced their selectivity profile. Conversely, p-cymene and thymol exhibited moderate activity across all target enzymes. Collectively, these results highlight dithymoquinone as the most promising compound among the bioactive constituents of black cumin oil.

From a therapeutic perspective, the ability of dithymoquinone to simultaneously inhibit COX-2 and 5-LOX presents a strategic advantage. This dual-inhibition approach may disrupt the inflammatory cascade through two critical pathways; prostaglandin synthesis mediated by COX-2 and leukotriene production mediated by 5-LOX, while reducing compensatory shunting of arachidonic acid metabolism that is often observed with selective COX-2 inhibitors (El-Malah et al., 2022). Such a mechanism is particularly relevant in chronic inflammatory

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diseases such as rheumatoid arthritis, where multiple, interconnected inflammatory pathways contribute to disease progression. Celecoxib, in contrast, exhibited distinct interaction profiles, including π–sigma and π–sulfur bonds with COX-2 and π–alkyl interactions with 5-LOX, reflecting its structure-based design for high affinity and selectivity toward defined binding pockets. These differences highlight a fundamental mechanistic distinction between natural and synthetic inhibitors: synthetic drugs are typically optimized to achieve strong, highly specific interactions with a single target enzyme, whereas natural compounds such as dithymoquinone often display broader, multi-target binding through flexible hydrophobic and polar interactions. This multi-target modulation is increasingly recognized as advantageous in complex inflammatory conditions, as it may enhance therapeutic efficacy while lowering the risk of side effects associated with excessive single-pathway inhibition (Mukhopadhyay et al., 2023).

The data underscored the potential of dithymoquinone as a selective dual COX-2/5-LOX inhibitor, with in silico evidence indicating higher predicted effectiveness based on its strong binding affinities and favorable selectivity profile. Effectiveness in this context is reflected by its lower Gibbs free energy (ΔG) values toward COX-2 and 5-LOX compared with COX-1, which suggests a higher likelihood of suppressing pro-inflammatory prostaglandin and leukotriene pathways while minimizing gastrointestinal toxicity associated with COX-1 inhibition (Bošković et al., 2022). The docking results further indicate that dithymoquinone achieves this effect through stable hydrophobic and π-based interactions within the active sites of COX-2 and 5-LOX, comparable to or exceeding those of celecoxib, thereby supporting its predicted anti-inflammatory potency at the molecular level. In silico studies offer valuable guidance by identifying key molecular features that influence enzyme binding and selectivity, thereby supporting the development and optimization of dithymoquinone. However, experimental validation through in vitro, in vivo, and pharmacokinetic studies is still required to confirm its efficacy and safety. Overall, the favorable selectivity of dithymoquinone compared with celecoxib, together with its natural origin and potential synergistic effects, highlights its promise as a lead compound for safer and more effective anti-inflammatory therapies.

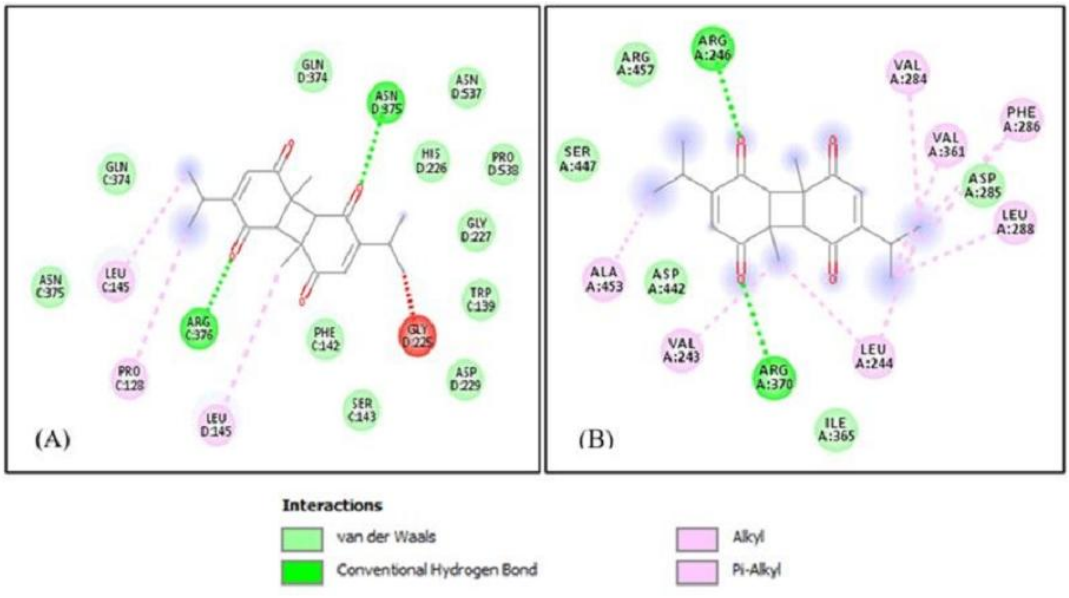

Figure 2. Interaction of Dithymoquinone with COX-2 (A) dan 5-LOX (B)

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Nevertheless, some limitations must be acknowledged. Computational predictions do not fully account for long-term molecular dynamics or protein conformational variability in vivo. Moreover, pharmacokinetic aspects such as hepatic metabolism and tissue distribution cannot be fully captured through silico approaches. Thus, experimental validation is a critical next step.

In summary, this study not only identified dithymoquinone as a promising selective dual COX-2/5-LOX inhibitor but also provided detailed insights into its molecular interaction mechanisms, as revealed by docking analysis. Specifically, dithymoquinone exhibited stable binding within the active sites of COX-2 and 5-LOX through favorable hydrophobic contacts and π-based interactions, which contributed to its low binding free energy and preferential affinity toward pro-inflammatory enzymes over COX-1. These interaction patterns suggest a molecular basis for its dual inhibitory activity and selectivity, supporting modulation of both prostaglandin and leukotriene pathways (Mukhopadhyay et al., 2023). Collectively, these findings establish an important foundation for translational research aimed at developing natural compound-based anti-inflammatory therapies with enhanced efficacy and improved safety profiles (Ahmadi et al., 2022).

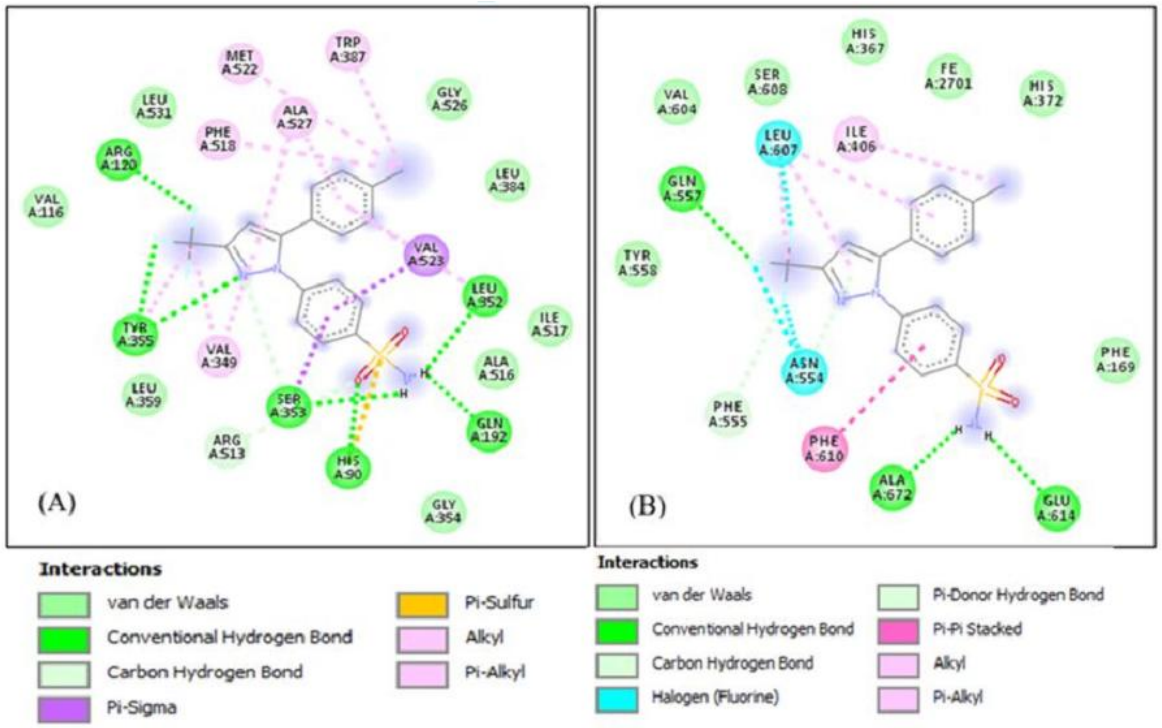

Figure 3. Interaction of Celecoxib with COX-2 (A) dan 5-LOX (B).

CONCLUSION

Based on the in silico findings, this study concludes that bioactive compounds from black cumin (Nigella sativa) oil, particularly dithymoquinone, exhibit strong potential as selective anti-inflammatory agents through dual inhibition of the COX-2 and 5-LOX enzymes. Molecular docking analysis demonstrated that dithymoquinone showed high binding affinities toward COX-2 (–9.3 kcal/mol) and 5-LOX (–8.4 kcal/mol), while exhibiting weaker interaction with COX-1 (–7.5 kcal/mol), indicating superior selectivity compared with the reference drug celecoxib. This selective binding behavior was supported by a distinct molecular

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interaction profile, involving stabilizing hydrogen bonds and van der Waals interactions with key catalytic residues within the active sites of COX-2 and 5-LOX. Such a dual-target mechanism suggests effective suppression of both prostaglandin and leukotriene inflammatory pathways, which may translate into improved anti-inflammatory efficacy with a reduced risk of gastrointestinal side effects commonly associated with non-selective NSAIDs. Nevertheless, comprehensive experimental validation remains essential. Further in vitro enzyme assays, in vivo animal studies, and pharmacokinetic evaluations are required to confirm the predicted biological activity, therapeutic efficacy, and safety profile of dithymoquinone. Additionally, these findings highlight the potential for exploring synergistic interactions among multiple bioactive constituents of black cumin oil as a holistic strategy for the management of inflammatory diseases.

ACKNOWLEDGMENTS

The authors would like to thank all team members for their contributions in developing the research idea and for their collaboration throughout this study.

AUTHOR'S DECLARATION

Syarifatul Mufidah: conceptualization, docking, analysis, writing. Putri Rachma Novitasari: compound screening, analysis. Prita Anggraini Kartika Sari: analysis, discussion, inflammation pathway. Hani Badriyah Hidayati: conceptualization, analysis.

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