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In Silico Study of Black Garlic (*Allium sativum*) Against COX-2 in Anti-Inflammatory Therapy

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Abstract

Cyclooxygenase-2 (COX-2) is an inducible enzyme involved in prostaglandin biosynthesis during inflammatory processes and represents an important therapeutic target for anti-inflammatory drug development. Black garlic (Allium sativum) contains bioactive compounds such as S-allyl cysteine (SAC), 5-hydroxymethylfurfural, and flavonols with reported anti-inflammatory potential. This study aimed to investigate the potential of black garlic-derived compounds as COX-2 inhibitors using an in silico approach. Twenty-four compounds were evaluated through Lipinski's Rule of Five, ADMET prediction, ligand-based pharmacophore screening, and molecular docking using the COX-2 crystal structure (PDB ID: 3LN1), with celecoxib as the reference inhibitor. In contrast, 5-hydroxymethylfurfural demonstrated high intestinal absorption and strong pharmacophore similarity to celecoxib, although its binding affinity was comparatively low. Flavonols showed the best docking performance, characterized by binding energy of −8.18 kcal/mol, high pharmacophore fit scores, and acceptable pharmacokinetic profiles. However, toxicity prediction suggested that both 5-hydroxymethylfurfural and flavonols may possess mutagenic and carcinogenic potential. Overall, flavonols and 5-hydroxymethylfurfural may warrant further investigation as potential COX-2-targeting compounds, although molecular dynamics, in vitro, and in vivo studies are needed to validate their activity and safety.

Keywords: Anti-inflammatory, Inflammation, Black garlic, COX-2, Molecular docking

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Introduction

Inflammation represents the body's protective mechanism that activates in response to tissue injury, infection, or exposure to harmful agents (Abdulkhaleq et al., 2018; Medzhitov, 2021). Through this process, damaging substances are cleared from the affected area while tissue repair mechanisms are simultaneously initiated, allowing the body to maintain its physiological balance (Medzhitov, 2021). From a clinical standpoint, inflammatory conditions can typically be recognized through five cardinal signs: redness, swelling, pain, increased local temperature, and impaired function of the affected tissue (Latief et al., 2021). Based on its duration and the cellular populations involved, inflammation is generally classified into two main types, acute and chronic (Abdulkhaleq et al., 2018). Acute inflammation is predominantly characterized by neutrophil infiltration, whereas chronic inflammation involves the sustained presence of macrophages, plasma cells, and lymphocytes (Pahwa et al., 2023). Beyond its role as a transient defense response, persistent low-grade inflammation has also been associated with the development of various serious health conditions, including cancer, metabolic disorders, and cardiovascular disease (Vitale et al., 2024).

Within this inflammatory cascade, several molecular mediators play distinct yet

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interconnected roles, one of which is cyclooxygenase-2 (COX-2), an enzyme that has attracted considerable research interest due to its central role in prostaglandin biosynthesis (Alexanian & Sorokin, 2017; Wautier & Wautier, 2023). COX-2 is particularly relevant in this context because, unlike COX-1, which is constitutively expressed across many tissue types under normal physiological conditions, COX-2 expression is markedly upregulated specifically in response to inflammatory stimuli, leading to increased prostaglandin production that contributes to pain, edema, and localized inflammation (Alexanian & Sorokin, 2017; Wautier & Wautier, 2023). Functionally, COX-2 catalyzes the conversion of arachidonic acid into prostaglandin H_2, a key intermediate that subsequently gives rise to a range of prostanoids involved in inflammatory signaling (Gilman & Limesand, 2021). This pivotal role has established COX-2 inhibition as a key therapeutic strategy for managing various inflammatory disorders (Rayar et al., 2017).

Given the central role of COX-2 in driving the inflammatory cascade, pharmacological inhibition of this enzyme has become a primary therapeutic target, most notably through nonsteroidal anti-inflammatory drugs (NSAIDs), which are among the most widely prescribed agents for managing inflammation and pain via cyclooxygenase inhibition (Rayar et al., 2017). To reduce the gastrointestinal toxicity commonly associated with non-selective NSAIDs, selective COX-2 inhibitors such as celecoxib were subsequently developed (Alexanian & Sorokin, 2017). Despite their demonstrated anti-inflammatory efficacy, prolonged use of these agents has been linked to adverse effects that may ultimately limit their long-term clinical application (Ahmadi et al., 2022). This limitation underscores the continued need for alternative anti-inflammatory compounds that offer a more favorable balance between efficacy and safety, making it an important and ongoing focus within pharmaceutical research (Rayar et al., 2017).

In response to this need for safer and more effective anti-inflammatory alternatives, natural products have increasingly been recognized as a valuable source of bioactive molecules for the development of novel therapeutic agents (Tech et al., 2022). Among the natural products that have garnered growing scientific attention is black garlic (Allium sativum), a product derived from raw garlic through a controlled aging process carried out under elevated temperature and humidity conditions (Ahmed & Wang, 2021; Vecchi et al., 2025). Throughout this aging process, garlic undergoes extensive chemical transformations driven primarily by Maillard reactions, which alter both its physicochemical characteristics and phytochemical composition (Najman et al., 2021). As a result of these transformations, several bioactive compounds accumulate within black garlic, including S-allyl cysteine (SAC), 5-hydroxymethylfurfural, flavonoids, and various sulfur-containing compounds (Ahmed & Wang, 2021; Manoonphol et al., 2023).

Building on the growing interest in black garlic as a source of bioactive compounds, its biological properties have also been extensively documented in the literature (Ahmed & Wang, 2021). Reported effects include antioxidant, anti-inflammatory, cardioprotective, anticancer, and metabolic regulatory activities, all of which are believed to contribute to its potential health benefits in humans (Ahmed & Wang, 2021; Stępień et al., 2024). Among these bioactive compounds, S-allyl cysteine (SAC) is of particular interest because its concentration increases substantially during the aging process of black garlic and it has been associated with anti-inflammatory activity (Ahmed & Wang, 2021; Stępień et al., 2024). This activity has been attributed to SAC's ability to suppress nuclear factor-κB (NF-κB) activity, thereby downregulating the expression of downstream inflammatory mediators, including COX-2 itself, which positions SAC as a mechanistically relevant compound for mitigating COX-2-driven inflammation (Mong & Yin, 2012). Notably, the concentration of SAC has been shown to increase substantially during aging, establishing it as a major bioactive constituent in black garlic (Manoonphol et al., 2023). Similarly, 5-hydroxymethylfurfural (5-HMF), a compound generated through thermal processing, has been found to accumulate at levels considerably higher than those observed in fresh garlic and has been reported to exert

anti-inflammatory effects through modulation of NF-κB signaling, thereby potentially reducing COX-2 expression (Ahmed & Wang, 2021; Kong et al., 2019).

Considering the presence of these bioactive constituents, several studies have further demonstrated the anti-inflammatory potential of black garlic-derived compounds (Kong et al., 2019). Specifically, 5-hydroxymethylfurfural has been reported to suppress inflammatory responses by modulating the NF-κB, MAPK, and mTOR signaling pathways (Kong et al., 2019). Inhibition of these pathways, in turn, reduces the production of pro-inflammatory mediators and cytokines (Guo et al., 2020; Kong et al., 2019). In line with this, black garlic extracts have also been associated with decreased expression of key inflammatory markers, including TNF-α, IL-1β, and IL-6 (Stępień et al., 2024). Taken together, these findings suggest that black garlic contains several compounds with promising therapeutic potential for managing inflammation-related disorders (Ahmed & Wang, 2021; Stępień et al., 2024).

Despite this growing body of evidence supporting the anti-inflammatory properties of black garlic, information regarding the molecular interactions between its individual bioactive compounds and COX-2 remains limited (Stępień et al., 2024). Computational approaches offer an efficient means of evaluating ligand-target interactions and predicting the potential biological activity of compounds prior to experimental validation (Pantaleão et al., 2022). Therefore, this study aimed to investigate the potential of black garlic-derived bioactive compounds as COX-2 inhibitors using an integrated in silico approach that included Lipinski's Rule of Five evaluation, ADMET prediction, pharmacophore screening, and molecular docking analysis. The findings of this study are expected to provide preliminary molecular evidence to support the development of black garlic-derived compounds as prospective anti-inflammatory agents targeting COX-2.

Methods

Materials and Software

All computational analyses were conducted on a laptop equipped with an 11th Generation Intel® Core™ i3-1115G4 processor, 8 GB RAM, and a 64-bit Windows 11 operating system. Chemical structures of the bioactive compounds in black garlic and celecoxib were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Geometry optimization of ligands was carried out in Chem3D Pro 12.0 using the Molecular Mechanics 2 (MM2) force field. The crystal structure of Cyclooxygenase-2 (COX-2) complexed with its native ligand was obtained from the RCSB Protein Data Bank under accession code 3LN1.

Prediction of pharmacokinetic parameters and toxicity was performed using the PreADMET web server (https://preadmet.webservice.bmdrc.org). The active and distractor chemicals used to validate the pharmacophore model were obtained from the DUD.E database. Ligand-based pharmacophore models were generated and validated using Ligand Scout version 4.4. Molecular docking simulation was performed using AutoDock 4.2. Designing receptors and ligands and analyzing molecular interactions were done using BIOVIA Discovery Studio 2020. The criteria of oral drug similarity and physicochemical properties were calculated through Mcule Property Calculator (https://mcule.com/apps/property-calculator).

Predictions of Lipinski's Rule of Five

The appropriateness of the oral drug was analyzed using Lipinski's Five Rules (Benet et al., 2016; Lipinski et al., 2012). The compounds under study were obtained in SMILES format from PubChem and loaded into the Mcule Property Calculator. Calculated parameters are molecular weight, LogP value, number of hydrogen bond donors and number of hydrogen bond acceptors. The obtained values were evaluated against the established RO5 criteria for the suitability of each chemical for oral delivery.

ADMET Prediction

The pharmacokinetic behavior and toxicity profile of each compound were evaluated using the PreADMET platform. Molecular structures obtained from PubChem served as the input data. Absorption-related parameters consisted of Human Intestinal Absorption and Caco-2 permeability. Distribution characteristics were represented by Blood-Brain Barrier penetration and Plasma Protein Binding values. Toxicological evaluation included Ames mutagenicity prediction and carcinogenicity assessment in rodents. These analyses provided preliminary information on the safety and pharmacokinetic properties of the investigated compounds prior to further computational evaluation (Pantaleão et al., 2022).

Pharmacophore screening

Pharmacophore screening was performed to identify black garlic compounds with pharmacophoric features consistent with COX-2 inhibition. Initially, an active and decoy dataset for Cyclooxygenase-2 (COX-2; PDB ID: 3LN1) was generated using the DUD.E platform. Based on this dataset, ten ligand-based pharmacophore models were constructed in LigandScout 4.4. Each generated model was subsequently transferred to the screening perspective using the "Copy to Other Perspective" function and validated against the active and decoy datasets.

The predictive efficacy of the model was evaluated by Receiver Operating Characteristic (ROC) curve analysis. ROC values from the ten pharmacophore models were compared, and the model with the highest discriminative performance was selected for subsequent screening. After model selection, the database of test compounds was imported into the screening perspective using the "Load Screening Database" feature. The selected pharmacophore model was then transferred from a ligand-based perspective into the screening module, and virtual screening was performed to identify compounds that exhibited the highest compatibility with the pharmacophoric requirements of the COX-2 target (Muchtaridi et al., 2017; Opo et al., 2022).

Molecular docking simulation

Molecular docking study was used to study the interaction of bioactive compounds from black garlic with Cyclooxygenase-2 (COX-2) receptor using AutoDock 4.2.6. The protein complex was prepared for docking in BIOVIA Discovery Studio 2020 by removing the co-crystallized ligands and crystallographic water molecules. The receptor structure was then assigned the Kollman charges and polar hydrogens. The ligand was prepared by adding hydrogen atoms, computing Gasteiger charges, and adding non-polar hydrogen atoms.

Redocking of the native ligand into the active site of COX-2 was used to validate the docking method. Docking calculations were performed with a grid box of 30 × 30 × 30 grid points centered at x = 30.092, y = −22.559 and z = −15.758. The search algorithm was the Lamarckian Genetic Algorithm with 100 independent docking trials. The Root Mean Square Deviation (RMSD) values obtained from the redocking procedure were used to assess the protocol's reliability. An RMSD value of ≤ 2.0 Å indicates a successful docking method that can reproduce the experimentally observed binding conformation (Liu et al., 2020; Meng et al., 2011).

All test compounds were docked into the COX-2 active site using the validated procedure and established docking parameters. The binding affinity was assessed by the binding free energy (ΔG) and the predicted inhibition constant (Ki) generated by AutoDock 4.2.6. The ligand-receptor complexes obtained were further visualized and analyzed in BIOVIA Discovery Studio 2020 using two-dimensional and three-dimensional representations of the interactions to outline the amino acid residues involved in ligand binding.

Results and Discussion

Predictions of Lipinski's Rule of Five

The pharmacological properties of bioactive substances in black garlic were evaluated using Lipinski's Five Rules (RO5), a common preliminary screening method for assessing a compound's suitability for oral administration (Benet et al., 2016; Lipinski et al., 2012). The factors evaluated were molecular weight, lipophilicity (log P), number of

hydrogen-bond donors, and number of hydrogen-bond acceptors, all of which are related to membrane permeability and oral bioavailability (Shultz, 2019). All the compounds tested satisfy the criteria of the RO5, with a molecular weight of less than 500 Da, a log P value of less than 5, and fewer than five hydrogen-bond donors and ten hydrogen-bond acceptors, as shown in Table 1. The physicochemical properties of these chemicals suggest the potential to increase membrane permeability and oral absorption (Lipinski et al., 2012; Shultz, 2019).

Although RO5 is widely utilized during the early stages of drug discovery, compliance with these criteria alone does not ensure optimal pharmacokinetic performance or therapeutic efficacy (Pantaleão et al., 2022). Drug absorption and distribution are also influenced by factors such as metabolic stability, transporter interactions, plasma protein binding, and toxicity profiles (Harutyunyan et al., 2025; Pantaleão et al., 2022). Furthermore, numerous natural products have demonstrated significant biological activity despite violating one or more RO5 parameters, indicating that this rule should be considered a preliminary screening tool rather than a definitive predictor of drug performance (Pantaleão et al., 2022). To provide a more comprehensive molecular assessment, the present study did not rely on RO5 evaluation alone but complemented it with ADMET prediction to assess absorption, distribution, and toxicity profiles, ligand-based pharmacophore screening to evaluate structural compatibility with the COX-2 binding pocket, and molecular docking simulation to characterize the binding affinity and interaction pattern of each compound with the COX-2 receptor. This integrated computational approach allowed the physicochemical, pharmacokinetic suitability, target compatibility, and binding behavior of the tested compounds to be examined collectively, providing a more complete picture of their potential as COX-2 inhibitors beyond what RO5 compliance alone could indicate.

Table 1. Prediction outcomes of Lipinski's Rule of Five for bioactive chemicals in black garlic (*Allium sativum*)

Compound	Molecular Weight (Da)	Log p	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Lipinski violations
S-allyl-L-cysteine	161.2245	1.0178	2	3	0
Diallyl sulfide	114.2109	2.0916	0	0	0
Tryptophan	204.2248	1.8226	3	4	0
Histidine	155.1547	0.0644	3	5	0
Valine	117.1464	0.7546	2	3	0
Arginine	155.1547	0.0644	3	5	0
Aspartic acid	163.1717	0.5995	3	5	0
S-methyl-L-cysteine	135.1873	0.4616	2	3	0
S-allylmercaptocysteine	193.2920	1.6660	2	3	0
Pyruvic acid	88.0619	-0.3400	1	3	0
γ-glutamyl-s-allylcysteine	290.3384	0.7583	4	7	0
p-Coumaric acid	164.1574	1.4900	2	3	0
o-Coumaric acid	164.1574	1.4900	2	3	0
m-Coumaric acid	164.1574	1.4900	2	3	0
Fructosyl-arginine	336.3418	-2.4816	8	11	2
Fructosyl-phenylalanine	327.3291	-1.5357	6	8	1
Flavan-3-ols	226.2694	2.7237	1	2	0
Glycine	75.0667	-0.2700	2	3	0
Flavonols	238.2369	3.1656	1	3	0
Diallyl disulfide	146.2784	2.7398	0	0	0
Diallyl trisulfide	146.2784	2.7398	0	0	0
5-Hydroxymethyl furfural	126.1097	0.5844	1	3	0
Tetrahydroharman-3-carboxylic acid	230.2620	2.1566	3	4	0
Eleagnine 1,3-dicarboxylic acid	274.2714	1.3954	4	6	0

ADMET prediction

To determine their suitability as drug candidates, we carry out ADMET predictions to evaluate the pharmacokinetic properties and potential toxicity of the compounds studied. The analysis included parameters related to absorption, permeability, distribution, and toxicity. Based on EMA and FDA guidelines, human intestinal absorption (HIA) is generally categorized as low when the fraction absorbed (fa) is <50%, moderate when it ranges between 50–84%, and high when it reaches ≥85% (U.S. Food and Drug Administration, 2021). Meanwhile, the Caco-2 permeability was determined by the apparent permeability coefficient (Papp). The Papp less than 10 nm/s represents low permeability, 10–100 nm/s represents moderate permeability and Papp higher than 100 nm/s represents high permeability (Karaküçük et al., 2021). According on the predicted outcomes, several compounds exhibited favorable absorption characteristics, as indicated by moderate to high HIA values and acceptable Caco-2 permeability, suggesting a reasonable ability to cross intestinal epithelial membranes. These properties are important for orally administered drugs because adequate membrane permeability facilitates systemic drug exposure (Czub et al., 2023; Karaküçük et al., 2021).

Furthermore, plasma protein binding (PPB) is classified as high when binding exceeds 90% and low to moderate when it is below 90%. High PPB reflects a stronger affinity of compounds toward plasma proteins, which may reduce the concentration of free (pharmacologically active) drug in circulation, whereas lower PPB may support higher bioavailability and reduce the risk of protein-binding-related drug interactions (Hasan & Herowati, 2024). In this study, most compounds demonstrated high PPB, indicating strong interactions with plasma proteins that may influence the distribution and free concentration of the drug in systemic circulation. Although high PPB can reduce the fraction of active free drug, it may also prolong the drug's half-life in the bloodstream (Celestin & Musteata, 2021; Hasan & Herowati, 2024).

In terms of safety prediction, toxicity profiling indicated that substances such as S-allyl cysteine (SAC), S-allylmercaptocysteine (SAMC), and tryptophan were assessed as non-carcinogenic, suggesting a comparatively benign toxicological profile. However, certain compounds, including 5-hydroxymethylfurfural and flavonol derivatives, showed potential carcinogenicity and therefore require further experimental evaluation. Interestingly, mutagenicity predictions were observed for several compounds, possibly due to the presence of reactive functional groups commonly found in sulfur-containing phytochemicals. Since in silico toxicity models may produce false-positive results, these findings should be interpreted cautiously. In the present study, the mutagenicity and carcinogenicity results were based solely on computational predictions and were not experimentally validated. Therefore, these toxicity predictions should be considered preliminary and require confirmation through future in vitro and in vivo toxicity assays (Pantaleão et al., 2022).

Ten compounds produced from black garlic were found to fit the necessary pharmacophore properties using pharmacophore model 10. The pharmacophore fit score quantifies the spatial fit between the compound's chemical features and those of the pharmacophore model. The pharmacophore model describes the important ligand-target interaction properties such as hydrogen bond acceptors, hydrogen bond donors, hydrophobic features and aromatic features. Enhanced fit ratings indicate a higher probability of biological activity, which show a greater geometric and functional similarity with the reference ligand (Moussa et al., 2021; Muchtaridi et al., 2017). Tetrahydroharman-3-carboxylic acid and 5-hydroxymethylfurfural showed the highest pharmacophore fit scores among the screened compounds (33.75 and 33.57, respectively), indicating that these molecules share significant structural traits with the reference inhibitor celecoxib. The reliability of the generated pharmacophore model for identifying active compounds while excluding decoys was assessed using ROC curve analysis, as illustrated in Figure A. As illustrated in Figures B and C, the pharmacophore mapping results indicated that both selected compounds adequately satisfied the essential

pharmacophoric requirements of the COX-2 binding pocket. The observed correspondence with hydrogen-bonding features, hydrophobic regions, and aromatic centers supports their potential affinity toward the target receptor. (Muchtaridi et al., 2017).

Table 2. ADMET results of *Black Allium sativum* bioactive compounds

Compound	Human Intestinal Absorption (%)	Caco-2 (nm/sec)	Plasma Protein Binding (%)	Blood–Brain Barrier	Mutagen	Carcinogen	
						Mouse	Rat
S-allyl-L-cysteine	81.972219	6.65342	11.674627	0.229899	+	-	-
Diallyl sulfide	100.00000	23.614	78.046833	0.797718	+	+	+
Tryptophan	85.324638	0.253487	29.779419	0.42924	+	-	-
Histidine	59.681257	18.3714	0.0000000	0.286661	+	-	+
Valine	75.255083	20.0466	88.619012	0.410878	+	+	-
Arginine	17.476205	21.0457	15.878726	0.0660006	+	+	+
Aspartic acid	35.259079	8.81869	56.139579	0.101274	+	+	+
S-methyl-L-cysteine	74.077946	20.3452	0.684887	0.152756	+	+	-
S-allylmercaptocysteine	82.576618	1.60099	0.00000	0.190643	+	-	-
Pyruvic acid	70.598137	6.94955	71.348864	0.31138	+	+	+
γ-glutamyl-s-allylcysteine	33.683268	7.38661	7.373809	0.0702774	+	+	-
p-Coumaric acid	92.095876	21.1093	63.055072	0.694635	+	+	-
o-Coumaric acid	92.094712	20.6216	64.851493	0.694203	+	-	+
m-Coumaric acid	92.095857	20.6217	23.028748	20.6217	+	-	+
Fructosyl-arginine	1.952151	9.97903	6.630118	0.053302	+	-	+
Fructosyl-phenylalanine	29.908871	12.8095	20.380762	0.0423496	+	-	+
Flavan-3-ols	97.640207	54.2526	86.705503	1.58168	+	+	-
Glycine	68.343444	3.83796	87.307451	0.169765	+	+	-
Flavonols	95.337868	32.2662	86.590933	0.797284	+	+	+
Diallyl disulfide	98.169149	22.0171	98.037400	1.36944	+	+	+
Diallyl trisulfide	98.995694	57.6318	98.995694	2.27681	+	+	+
5-Hydroxymethyl furfural	88.682048	6.41666	49.465993	0.52231	+	-	+
Tetrahydroharman-3-carboxylic acid	86.009404	0.299422	65.605205	1.15217	+	-	-
Eleagnine 1,3-dicarboxylic acid	75.297471	0.319647	78.927285	0.337679	+	-	-

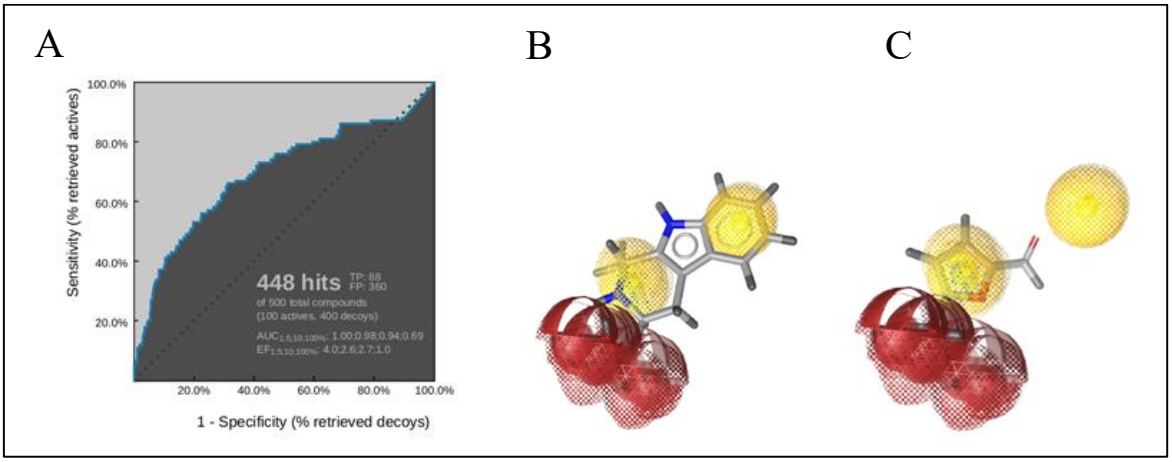

Figure 2. Results of pharmacophore screening: A) Validation curve for pharmacophore screening (ROC curve model) 2); B) Pharmacophore Visualization Tetrahydroharman-3-carboxylic acid; C) Visualization of pharmacophores 5-Hydroxymethyl furfural.

Molecular Docking Simulation

To assess the binding of black garlic constituents, the docking methodology was first validated by redocking the native ligand into the active site of cyclooxygenase-2 (COX-2). The validation process produced an RMSD value of 1.469 Å, indicating a close agreement between the experimentally determined ligand conformation and the pose generated by the docking simulation. A docking approach is usually considered reliable if the RMSD value is < 2.0 Å and it reflects the ability of the method to reproduce the binding orientation observed in the crystallographic structure (Allo et al., 2023; Meng et al., 2011). Based on these criteria, the docking parameters used in the present study were considered sufficiently accurate to predict interactions between COX-2 and black garlic bioactive compounds.

A total of twenty-four bioactive compounds identified in black garlic were subsequently docked against the COX-2 receptor to evaluate their binding affinity and interaction characteristics. The binding energy of celecoxib was -11.08 kcal/mol with projected inhibition constant (Ki) of 7.59 μM as a reference inhibitor. Interaction analysis revealed that celecoxib established conventional hydrogen bonds with Tyr341, Arg499, His75, Gln178, and Phe504. In addition to these, some other hydrophobic interactions like alkyl-alkyl, pi-alkyl and pi-sigma were also found in the binding pocket. The role of the interplay between hydrogen bonds and hydrophobic contacts is recognized as an important factor in ligand stabilization and can potentially improve the inhibition of the COX-2 catalytic activity (Kumaeum & Jaiyong, 2025).

The highest binding affinity was observed for flavonol among the tested substances, with a binding energy of −8.18 kcal/mol and a calculated inhibition constant of 1.01 μM, suggesting moderate binding to the enzyme's active site. However, these compounds did not form hydrogen bond interactions similar to those observed in the celecoxib complex. In contrast, smaller polar molecules such as 5-hydroxymethylfurfural showed weaker binding affinity (−4.43 kcal/mol; Ki 569.64 μM) but formed hydrogen bonds with several residues involved in the celecoxib interaction network, including Gln178, Phe504, His75, Arg499, and Tyr341. Similarly, S-allyl-L-cysteine (SAC) exhibited low binding affinity (−4.69 kcal/mol; Ki 362.42 μM) but maintained hydrogen bond interactions with residues Gln178, Arg499, and Phe504. Despite exhibiting lower binding energies than the reference inhibitor, critical hydrogen-bond interactions indicate that specific phytochemicals from black garlic may contribute to favorable interactions within the COX-2 binding pocket. These findings support their further investigation as potential COX-2-targeting compounds.

Table 3. Molecular docking results from black garlic (*Allium sativum*) bioactive compounds

Compound	Binding Energy (kcal/mol)	Inhibition Constant/Ki (uM)	Interactions with amino acids	
			Hydrogen Bonds (HB)	Other bonds
Celecoxib	-11.08	7.59	**Conventional HB** TYR A:341 ARG A:499 HIS A:75 GLN A:178 PHE A:504 **Carbon HB** SER A:339	**Alkyl** LEU A:338 LEU A:345 VAL A:335 ALA A:513 **Pi-Alkyl** MET A:508 TRP A:373 **Pi-Sigma** VAL A:509 **Unfavorable Donor-Donor** ILE A:503

Continue Table 3.

Compound	Binding Energy (kcal/mol)	Inhibition Constant/Ki (uM)	Interactions with amino acids	
			Hydrogen Bonds (HB)	Other bonds
S-allyl-L-cysteine	-4.69	362.42	**Conventional HB** LEU A:338 GLN A:178 ARG A:499 PHE A:504	**Alkyl** VAL A:335 HIS A:75 TYR A:341 VAL A:509 **Pi-Alkyl** VAL A:335 HIS A:75 TYR A:341 VAL A:509
Diallyl sulfide	-3.63	2.17	-	**Pi-Sulfur** TRP A:373 PHE A:367 **Alkyl** MET A:508 VAL A:509 PHE A:504 LEU A:307 LEU A:338 TYR A:371 VAL A:335 TYR A:334 **Pi-Alkyl** MET A:508 VAL A:509 PHE A:504 LEU A:307 LEU A:338 TYR A:371 VAL A:335 TYR A:334
Tryptophan	-6.89	8.85	**Conventional HB** ILE A:503 GLN A:178 LEU A:338 PHE A:504 ARG A:499	**Pi-Alkyl** ALA A:513 **Pi-Sigma** VAL A:509 **Unfavorable Donor-Donor** ILE A:503
Histidine	-5.16	164.99	**Conventional HB** LEU A:338 GLN A:178 PHE A:504 ARG A:499	-
Valine	-4.69	367.46	**Conventional HB** ARG A:499 PHE A:504 GLN A:178 LEU A:338	**Alkyl** VAL A:509 HIS A:75 **Pi-Alkyl** VAL A:509 HIS A:75

Continue Table 3.

Compound	Binding Energy (kcal/mol)	Inhibition Constant/Ki (uM)	Interactions with amino acids	
			Hydrogen Bonds (HB)	Other bonds
Arginine	-4.33	668.33	**Conventional HB** GLN A:178 LEU A:338 MET A:508 VAL A:509 ARG A:499 SER A:339	-
Aspartic acid	-3.71	1.92	**Conventional HB** LEU A:338 GLN A:178 TYR A:341 ARG A:499 PHE A:504 **Carbon HB** SER A:339	
S-methyl-L-cysteine	-4.41	589.71	**Conventional HB** ARG A:499 PHE A:504 GLN A:178 LEU A:338	**Alkyl** VAL A:509 **Pi-Alkyl** VAL A:509 **Unfavorable Donor-Donor** GLN A:178
S-allylmercaptocysteine	-5.20	155.07	**Conventional HB** SER A:339 LEU A:338 PHE A:504 ARG A:499	**Alkyl** MET A:508 VAL:509 **Pi-Alkyl** MET A:508 VAL:509 **Pi-Sulfur** PHE A:504
Pyruvic acid	-3.48	2.8	**Conventional HB** ARG A:499 PHE A:504 ILE A:503	-
γ-glutamyl-s-allylcysteine	-5.95	43.26	**Conventional HB** LEU A:338 GLN A:178 PHE A:504 ARG A:499	**Alkyl** TYR A:371 TRP A:373 MET A:508 **Pi-Alkyl** TYR A:371 TRP A:373 MET A:508
p-Coumaric acid	-5.79	56.67	**Conventional HB** GLN A:178 PHE A:504 **Carbon HB** SER A:339	**Pi-Alkyl** ALA A:502 **Pi-Sigma** VAL A:509 **Unfavorable Donor-Donor** ILE A:503

Continue Table 3.

Compound	Binding Energy (kcal/mol)	Inhibition Constant/Ki (uM)	Interactions with amino acids	
			Hydrogen Bonds (HB)	Other bonds
o-Coumaric acid	-5.66	70.93	**Conventional HB** PHE A:504 TYR A:341 GLN A:178	**Pi-Alkyl** VAL A:509 **van der Waals** SER A:339 **Amide-Pi Stacked** LEU A:338
m-Coumaric acid	-5.71	65.11	**Conventional HB** GLN A:178 PHE A:504 VAL A:335	**Pi-Alkyl** ALA A:502 ARG A:499 **Pi-Pi Stacked** HIS A:75 **Pi-Sigma** VAL A:509
Fructosyl-arginine	-4.33	665.76	**Conventional HB** PHE A:504 SER A:339 GLN A:178 LEU A:338	**Pi-Cation** TYR A:371
Fructosyl-phenylalanine	-5.23	147.58	**Conventional HB** TYR A:341 ARG A:499 LEU A:338 VAL A:335 **Carbon HB** SER A:339	**Pi-Alkyl** ALA A:513 **Pi-Pi Stacked** PHE A:504
Flavan-3-ols	-7.57	2.85	**Conventional HB** SER A:516 **Carbon HB** ALA A:513	**Alkyl** LEU A:338 VAL A:509 LEU A:370 **Pi-Alkyl** LEU A:338 VAL A:509 LEU A:370 **Pi-Pi T-Shaped** PHE A:504 GLY A:512 TYR A: 371 **Pi-Sigma** VAL A:335 **Pi-Sulfur** MET A:508
Glycine	-3.31	3.77	**Conventional HB** ARG A:499, PHE A:504, LEU A:338, GLN A:178	**Unfavorable donor-donor** GLN A:178 ILE A:503
Flavonols	-8.18	1.01	**Conventional HB** VAL A:335	**Pi-Alkyl** TRP A:378 **Pi-Pi T-shaped** TRP A:378 **Pi-Sigma** VAL A:509, LEU A:338

Continue Table 3.

Compound	Binding Energy (kcal/mol)	Inhibition Constant/Ki (uM)	Interactions with amino acids	
			Hydrogen Bonds (HB)	Other bonds
Diallyl disulfide	-4.08	1.01	-	**Alkyl** MET A:508, LEU A:338, VAL A:509, VAL A:335, ALA A:513 **Pi-Alkyl** PHE A:504, TYR A:371, TYR A:334 **Pi-Sulfur** TRP A:373, PHE A:367
Diallyl trisulfide	-4.63	402.15	-	**Alkyl** LEU A:370, LEU A:338, MET A:508 **Pi-Alkyl** TRP A:373, VAL A:509, ALA A:502, ARG A:499 **Pi-Sulfur** PHE A:504
5-Hydroxymethyl furfural	-4.43	569.64	**Conventional HB** GLN A:178 ILE A:503 PHE A:504 HIS A:75 ARG A:499 TYR A:341	**Alkyl** VAL A:509 **Pi-Alkyl** VAL A:509
Tetrahydroharman-3-carboxylic acid	-6.55	15.7	**Conventional HB** ARG A:106 TYR A:341	**Alkyl** LEU A:338 VAL A:335 ALA A:502 **Pi-Alkyl** LEU A:338 VAL A:335 ALA A:502 **Pi-Sigma** VAL A:509
Eleagnine 1,3-dicarboxylic acid	-6.94	8.23	**Conventional HB** ARG A:106 VAL A:509	**Pi-Alkyl** LEU A:338 ALA A:509 **Pi-Sigma** VAL A:509

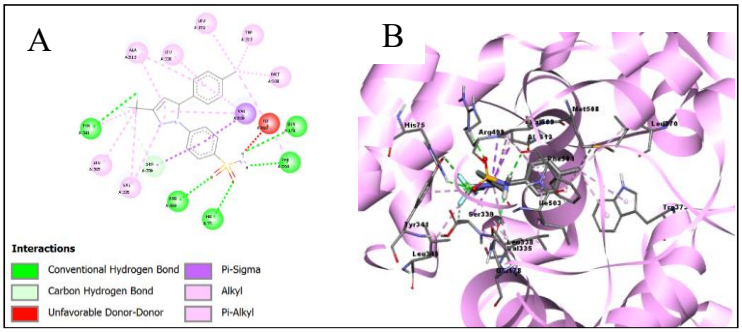

Figure 3. Visualization interactions between COX-2 and Celecoxib: A) 2D visualization interactions; B) 3D visualization interactions.

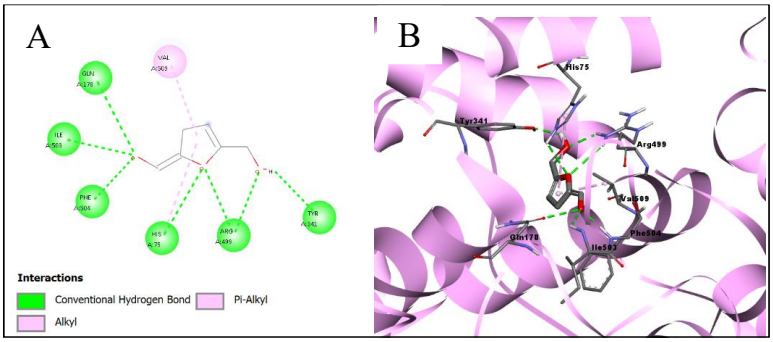

Figure 4. Visualization interactions between COX-2 and 5-Hydroxymethyl furfural: A) 2D visualization interactions; B) 3D visualization interactions.

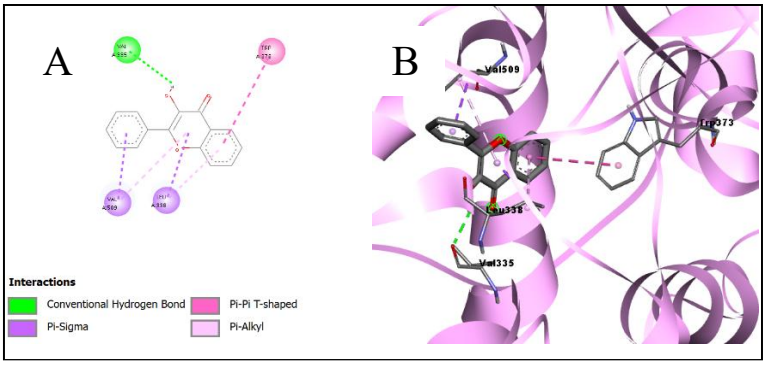

Figure 5. Visualization interactions between COX-2 and flavonols: A) 2D visualization interactions; B) 3D visualization interactions

CONCLUSION

The present study aimed to evaluate the potential of bioactive compounds from black garlic as COX-2-targeting compounds through an in silico approach including Lipinski's Rule of Five, ADMET predictions, pharmacophore modelling, and molecular docking. The docking protocol was successfully validated by redocking the native ligand, yielding an RMSD of 1.469 Å. Among the examined compounds, flavonols exhibited the strongest predicted binding affinity toward COX-2, with a binding energy of −8.18 kcal/mol. Simultaneously, 5-hydroxymethylfurfural exhibited significant hydrogen-bond interactions with essential residues in the COX-2 binding site, suggesting a potential interaction in the active site, similar to that of the reference inhibitor celecoxib. ADMET predictions suggested potential mutagenic and carcinogenic concerns for various chemicals, including 5-hydroxymethylfurfural and flavonols, necessitating further validation through experimental research; as this study was limited to a computational scope, these toxicity predictions were not experimentally validated and should therefore be interpreted with caution. These findings suggest that several bioactive compounds from black garlic may interact favorably with the COX-2 binding site and may warrant further investigation as potential COX-2-targeting compounds. The present findings are based on in silico predictions, and further molecular dynamics simulations, in vitro assays, and in vivo studies are needed to confirm their pharmacological activity and safety.

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