Libyan Journal of Medical and Applied Sciences LJMAS
Online ISSN: 3006-1113
Volume 3, Issue 3, 2025, Page No: 26-35
Website: https://ljmas.com/index.php/journal/index

Investigation of the Analgesic and Anti-Inflammatory Potential of Moringa oleifera: Molecular Docking Analysis

Abdelkarim N Sasi [1 *], Hafsa A Abu-Fanas [2], Khadija A Al-Shawin [3], Safa M Ataalib [4]
[1,2,3,4] Faculty of Pharmacy, Misurata University, Misurata, Libya
[*]*Corresponding author: kareem.sasi@gmail.com*

Received: June 06, 2025 Accepted: July 09, 2025 Published: July 14, 2025

Cite this article as: A, N, Sasi., H, A, Abu-Fanas., K, A, Al-Shawin., S, M, Ataalib. (2025). Investigation of the Analgesic and Anti-Inflammatory Potential of Moringa oleifera: Molecular Docking Analysis. Libyan Journal of Medical and Applied Sciences (LJMAS). 2025;3(3):26-35.

Abstract

Medicinal plants have maintained a long-standing relationship with drug discovery, with numerous species being systematically screened for bioactive components responsible for their therapeutic effects. *Moringa oleifera*, recognized for its rich phytochemical profile and traditional analgesic applications, represents a particularly promising candidate for pain management research. This study evaluates the analgesic and anti-inflammatory activity of *Moringa oleifera* through molecular docking investigations examining binding affinity between its phytochemicals and cyclooxygenase-2 (COX-2) (PDB ID: 5IKR), a key enzyme in pain and inflammation pathways. Utilizing Molecular Operating Environment (MOE) software, we systematically screened a comprehensive 300 compound library of *Moringa* phytochemicals to identify bioactive compounds responsible for therapeutic effects. The molecular docking analysis revealed that several *Moringa oleifera* phytochemicals exhibit remarkable COX-2 inhibitory potential, outperforming the redocked native ligand mefenamic acid and demonstrating binding affinities comparable to established nonsteroidal anti-inflammatory drugs (NSAIDs). These computational findings, considered alongside extensive traditional use and previous experimental validations, underscore *Moringa*'s significant promise as a source of novel analgesic agents. The research demonstrates efficient identification of high value therapeutic leads through computational natural product exploration, the complete 300 compound library is accessible as a searchable online database at (https://www.libnpdb.ly/moringa-oleifera-database).

Keywords: Moringa oleifera, Analgesic Activity, Anti-Inflammatory, Molecular Docking.

تقييم الخصائص المسكنة والمضادة للالتهاب للمورينجا أوليفيرا: تحليل قائم على الالتحام الجزيئي

عبد الكريم نور الدين ساسي[1*]، حفصة أحمد ابوفاناس[2]، خديجة علي الشوين[3]، صفاء مفتاح الطالب[4]
[1,2,3,4] قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة مصراتة، مصراتة، ليبيا

الملخص

لطالما شكلت النباتات الطبية مصدراً غنياً لاكتشاف الأدوية، حيث تخضع أنواعها المختلفة لفحص منهجي دقيق بهدف عزل المكونات الحيوية المسؤولة عن فعاليتها العلاجية. وفي هذا السياق، يبرز نبات المورينجا أوليفيرا(Moringa oleifera) ، المشهور بثرائه بالمركبات الكيميائية النباتية واستخداماته التقليدية في تسكين الآلام، كمرشح واعد لأبحاث تطوير مسكنات الألم. تهدف هذه الدراسة إلى تقييم النشاط المسكّن والمضاد للالتهاب لنبات المورينجا من خلال محاكاة الإلتحام الجزيئي(Molecular Docking) ، بين مركبات الكيميائية للمورينجا وانزيم (COX-2) الذي يعد إنزيماً محورياً في مسارات الشعور بالألم والالتهاب. باستخدام برنامج محاكاة الإلتحام الجزيئي (MOE)، تم إجراء مسح منهجي لمكتبة شاملة تضم 300 مركب كيميائي نباتي مستخلص من نبات المورينجا أوليفيرا، بهدف تحديد المركبات الحيوية المسؤولة عن التأثيرات العلاجية. وقد كشفت نتائج محاكاة الإلتحام الجزيئي أن العديد من هذه المركبات أظهرت قدرة تثبيطية فائقة لإنزيم COX-2، متفوقة بذلك على أداء حمض الميفيناميك، كما أبدت ألفة ارتباط تضاهي تلك التي تتمتع بها مضادات الالتهاب غير الستيرويدية (NSAIDs) المعروفة. إن هذه النتائج الحاسوبية، مقترنة بالاستخداماتها التقليدية والدراسات التجريبية السابقة، تبرز بوضوح الإمكانات الكبيرة لنبات المورينجا كمصدر واعد لمركبات علاجية مبتكرة لتسكين الألم. علاوة على ذلك، يثبت هذا البحث فعالية توظيف المنهجيات الحاسوبية في استكشاف المنتجات الطبيعية كوسيلة سريعة لتحديد مركبات رائدة ذات قيمة علاجية عالية. وتجدر الإشارة إلى أن المكتبة الكاملة للمركبات التي تم جمعها و فحصها (300 مركب) متاحة للباحثين كقاعدة بيانات إلكترونية قابلة للبحث على الرابط التالي: (https://www.libnpdb.ly/moringa-oleifera-database).

الكلمات المفتاحية: المورينجا أوليفيرا، الخصائص المسكنة، مضاد للالتهاب، الالتحام الجزيئي.

Introduction

Medicinal plants have been a cornerstone of therapy for millennia, with their traditional use providing a rich foundation for modern drug discovery. This ancient knowledge, accumulated through empirical observation, continues to guide the scientific search for bioactive compounds. Today, a significant portion of the global population still relies on natural remedies, highlighting their enduring cultural and practical relevance. Natural products (NP), particularly from plants, are the bedrock of pharmacology due to their unparalleled structural diversity and biological activity [1]. This has yielded landmark drugs such as penicillin, artemisinin, and Taxol, demonstrating their proven clinical value [2]. Consequently, a substantial percentage of modern pharmaceuticals are either directly derived from plants or are synthetic analogues inspired by these natural leads, affirming the critical role of botanical research in contemporary medicine. The therapeutic effects of plants are attributed to phytochemicals like alkaloids and flavonoids, which exhibit analgesic and anti-inflammatory properties. These natural products offer unique chemical scaffolds and multi-target activities, providing historically validated starting points that significantly accelerate drug discovery and expand its molecular repertoire [3], [4]. For over 5000 years, ancient civilizations have used plants to manage pain and inflammation. This historical success proves plants produce compounds that effectively target these pathways. Notably, two major drug classes opioids and anti-inflammatories originate from botanical sources. Foundational discoveries include morphine from the opium poppy and salicin from willow bark, the precursor to aspirin, underscoring the profound legacy of plant-based medicine in modern pharmacology [5]. A wide array of phytochemicals combat inflammation by targeting multiple molecular pathways. Compounds such as curcumin from *turmeric*, resveratrol from *grapes*, and gingerols from *ginger* inhibit key regulators like the NF-κB pathway and pro-inflammatory cytokines. Some, like gingerols, also suppress both cyclooxygenase and lipoxygenase enzymes. Boswellic acids from frankincense provide a distinct advantage by specifically inhibiting the 5-lipoxygenase enzyme, offering an alternative to traditional NSAIDs. This variety highlights how plant derived molecules can influence complex biological processes through specific or multi target actions [4], [6], [7].

The search for safer anti-inflammatory drugs is driven by the significant side effects of conventional NSAIDs, creating a need for novel COX-2 inhibitors with improved safety profiles. To meet this challenge, computer-aided drug design (CADD), particularly molecular docking, has become invaluable. This computational technique efficiently predicts how potential drugs bind to protein targets like COX-2, using scoring functions to estimate affinity. By prioritizing the most promising candidates for laboratory testing, CADD accelerates the discovery process, saving time and resources while focusing efforts on compounds with the highest therapeutic potential [8], [9]. *Moringa oleifera*, known as the "miracle tree," is a prime candidate for translational research, serving as a cornerstone of traditional medicine for its analgesic and anti-inflammatory properties in treating ailments like arthritis. Its extensive traditional use is scientifically supported by an exceptionally rich phytochemical profile, including flavonoids, alkaloids, and polyphenols [10]. Modern studies are now validating this reputation, attributing *Moringa's* therapeutic value to bioactive compounds that inhibit pro-inflammatory enzyme like COX. Furthermore, these constituents modulate critical signaling cascades such as the NF-κB pathway and suppress mediators like Tumor Necrosis Factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). This multifaceted mechanism of action establishes *Moringa* as a promising natural alternative to conventional anti-inflammatory drugs, making it a compelling subject for scientific investigation [11], [12]. This study employs molecular docking to investigate the anti-inflammatory potential of Moringa oleifera. We evaluated a comprehensive 300-compound library against the COX-2 enzyme to provide a molecular basis for its traditional use, aiming to validate Moringa as a source for developing safer pain and inflammation therapeutics.

Materials and Methods

A library of *Moringa oleifera* Compounds (MOC) was compiled from scientific literature and the PubChem database, with 2D structures standardized using ChemDraw® 13.0. Molecular docking was performed against the Cyclooxygenase-2 (COX-2) crystal structure, obtained from the Protein Data Bank (PDB), using Molecular Operating Environment (MOE) 2013. Protein ligand interaction poses were visualized in both MOE and Discovery Studio® 0.4. Finally, physicochemical and ADME (absorption, distribution, metabolism and excretion) properties were calculated and visualized using Python, leveraging the RDKit and Pandas libraries for all cheminformatics analysis and data management [13], [14].

Data Mining and Collection Strategy

The search strategy for data collection involved an extensive and systematic approach across multiple sources. A thorough literature review was conducted, focusing on journal articles, theses, and textbooks that documented the extraction of compounds from *Moringa oleifera*. Key databases such as PubMed, ScienceDirect, Google scholar and other Databases were extensively utilized to identify relevant publications. Additional literature was uncovered through targeted Google searches and by tracing references cited in previously discovered materials, then all data will be collected and organized in excel worksheet [15].

Database Structure

The development of the data entry tool and database structure was initiated by creating a dedicated "Data" worksheet in Excel. The headers were precisely defined to capture all essential fields, these headers include essential data points such as Compound Name, PubChem CID, SMILES Strings, InChIkey Code, Compound Class, Species Name, Family, Known Uses, Biological Activity, Mode of Action, Source Country, GPS, Collection Date, Authors, and Reference information. All data were linkage to at least one referenced source, ensuring that reference information was captured before any other details were added. To streamline data input, a user friendly interface was developed using a Visual Basic for Applications (VBA) UserForm, which organized input fields into three logical frames: Compound, Plant, and Reference [15].

Ligand Preparation

The first step in molecular docking involves preparing the ligand molecules and seven compounds representing standard NSAID drugs. This process typically begins with obtaining the structural models of the compounds. A comprehensive library of MOC and standard NSAID drugs (Celecoxib, Indomethacin, Mefenamic acid, Diclofenac, Naproxen, Ibuprofen, and Aspirin) will be compiled from various literature sources, chemical databases (e.g., PubChem), and public repositories. For molecular docking, the 2D structures of these compounds will be generated or imported into MOE using their corresponding SMILES or InChI codes. The 3D structures were subsequently generated and subjected to energy minimization using the MMFF94x force field implemented in MOE. Potential ionization states and tautomers relevant at physiological pH (approximated at pH 7.4) were considered and generated within MOE's database or builder tools to account for different possible forms of the ligands. The prepared ligand library was then saved in a suitable format for docking [14].

Structural Classification

The structural classification of MOC was accomplished using NPClassifier, deep neural network based tool designed for the categorization of NP. This system organizes compounds into a three tiered hierarchy consisting of pathway, superclass, and class levels. To effectively visualize the distribution of dominant chemical structures across each of these three hierarchical categories, pie charts were generated using the Python programming language (version 3.12), providing a clear quantitative representation of the structural diversity within the database [16].

Physicochemical Properties and Drug likeness

The "ADME & Drug-Likeness Calculator" was developed in Python to enable high throughput computation of key physicochemical properties and drug-likeness metrics from chemical structures. Built with the Flask web framework and Pandas for data handling, the tool uses RDKit to compute descriptors such as SlogP, MW, TPSA, Rb, HBA, and HBD. Drug-likeness is evaluated using Lipinski's Rule of 5, calculating violations based on MW > 500, LogP > 5, HBD > 5, and HBA > 10, different charts will be generated using Python script utilizes the Plotly library (6.2.0) to generate charts to visualize the distribution of each property [14], [17].

Protein Preparation

Protein preparation, a critical first step for molecular docking, began with the COX-2 crystal structure (PDB ID: 5IKR). Using MOE, we selected one protein subunit (A) and prepared it by removing the co-crystallized ligand (mefenamic acid) and other non-essential components. Hydrogen atoms were added, and protonation states were assigned to residues to reflect physiological pH. A brief energy minimization using the MMFF94x force field was then performed to resolve any steric clashes. This process yielded a clean structure of 3D protein model, which was saved and optimized for the subsequent docking simulations, ensuring a reliable foundation for evaluating ligand interactions [14], [17].

Docking Protocol

Molecular docking simulations were conducted using the MOE Dock program with default parameters. The prepared COX-2 protein (PDB: 5IKR) served as the receptor, while a library of 300 MOC and seven standard NSAIDs were docked as ligands. The active site was defined using the coordinates of the co-crystallized mefenamic acid. For each ligand, multiple binding poses were generated and ranked by their free energy of binding (ΔG_b), with the ten most favorable conformations retained for subsequent analysis. The database file generated from the docking procedure was further analysed, with the binding mode (interactions) of the highest ten (10) conformations for each docked molecule in the active site visualized and studied with the help of MOE visualization window. Green stick rendering was added to the native ligands (Mefenamic acid) obtained from the PDB (5IKR) file, while the MOC were marked in yellow stick style for better contrast and to enable the study of the interactions of these docked compounds within the receptor active site. Among the visualization of the

conformation generated from the docking for each molecule the conformation with the best binding mode (interactions) with the lowest binding energy (ΔG_b) was selected for further analysis [13], [14], [17].

Accuracy of the docking protocol

Before docking the MOC the molecular docking (MD) with MOE was performed between the receptors and their native ligand (Mefenamic acid) to validate the docking protocol by calculating the root mean square deviation (RMSD). The RMSDs values between the re-docked poses and the original poses of the native ligands are indicative of whether the docking protocol is accurate, with values under 2 Å indicative of an accurate protocol. If the RMSD of the best docked conformation of the native ligand is 2.0 Å or less from the experimental one (native ligand), the used scoring function (protocol) is successful [14], [17], [18]. The obtained pose from re-docking the native ligands with their receptors were well correlated with the original poses, with small RMSD values. The binding energy (ΔG_b) and the binding mode (interaction) for the native ligands will also be calculated and analysed.

Statistical Analysis

This study employed descriptive and comparative statistics. Frequencies and percentages were used to analyze the compound library's structural classification and drug-likeness. The docking protocol was validated using Root Mean Square Deviation (RMSD). Binding energies were evaluated using descriptive statistics (median, range) and a comparative analysis, where compounds were ranked against standard NSAIDs and median binding energies of the phytochemical and reference groups were compared.

Result and discussion

Moringa oleifera as searchable online database

A comprehensive library of 300 MOC and standard NSAID drugs (Celecoxib, Indomethacin, Mefenamic acid, Diclofenac, Naproxen, Ibuprofen, and Aspirin) was compiled from various literature sources and databases (e.g., PubChem). This dataset, organized in Excel format, included detailed information for each compound, such as compound name, PubChem ID, SMILES/InChI codes, compound class, and reported biological activities. Each compound was assigned a unique identifier for tracking throughout the study [15]. Moreover, the library has been made available as a searchable online database (https://www.libnpdb.ly/moringa-oleifera-database) for ease of access and further research.

Ligand Preparation Outcomes

For molecular docking, the 2D structures corresponding to the collected SMILES or InChI codes were processed within MOE. Three dimensional structures were successfully generated for 300 compounds and for the standard known NSAID drugs. These 3D structures were then subjected to energy minimization using the MMFF94x force field to obtain stable conformers suitable for docking. Furthermore, relevant potential ionization states and tautomeric forms at approximate physiological pH (e.g., pH 7.4) were considered and generated within MOE, ensuring the representation of forms likely to interact with the protein [14], [17]. The final prepared ligand library, comprising 300 molecules, was saved and used for the subsequent docking simulations.

Structural Classification

Analysis of the *Moringa* compound library reveals a distinct distribution of compounds across various biochemical classifications. The constituents were categorized into seven biosynthetic pathways, thirty eight superclasses, and seventy eight classes, with the data indicating a significant concentration within a few major categories. A review of the biosynthetic pathways shows that a vast majority of the compounds originate from three predominant routes: shikimates and phenylpropanoids, which represent the largest group at 32.5%; fatty acids, at 21.6%; and terpenoids, at 15.5%. At the superclass level, the most prominent groups are flavonoids (19.3%), fatty acids and conjugates (10.6%), and saccharides (6.69%). The classification becomes more granular at the class level, where the most prevalent classes identified include flavonols (9.64%), cinnamic acids and their derivatives (6.4%), unsaturated fatty acids (4.8%), and monosaccharides (4.4%). This hierarchical breakdown highlights both the extensive chemical diversity of the *Moringa* library and the dominance of particular structural scaffolds, underscoring the plant's rich biosynthetic repertoire.

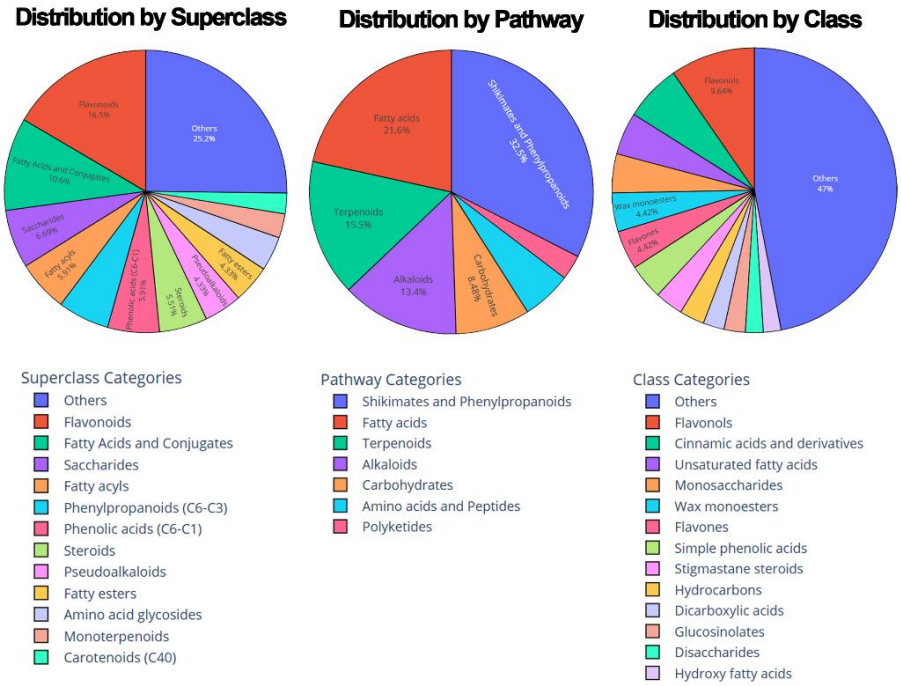

Figure 1. Structural classification of the LibNPDB compounds.

Drug Likeness

To evaluate its potential for drug discovery, the physicochemical properties of the MOC were analyzed. This comparative assessment utilized six key drug-likeness descriptors: MW, SlogP, TPSA, Rb, HBA and HBD to validate the library of MOC as a high quality screening library, referencing established thresholds like Lipinski's Rule of Five [14], [17]. As shown in (Figure 3) the findings demonstrate that *Moringa* phytochemicals compounds are predominantly situated within drug-like chemical space: 83.96% meet the MW range, 85.53% fall within the HBA range, and 79.87% for HBD. Additionally, 85.22% comply with Rb guidelines, 81.45% with SlogP, and 76.1% with TPSA thresholds. These high percentages reflect strong alignment with physicochemical parameters favourable for oral bioavailability and membrane permeability, underscoring significant enrichment in molecules suitable for orally administered drug development.

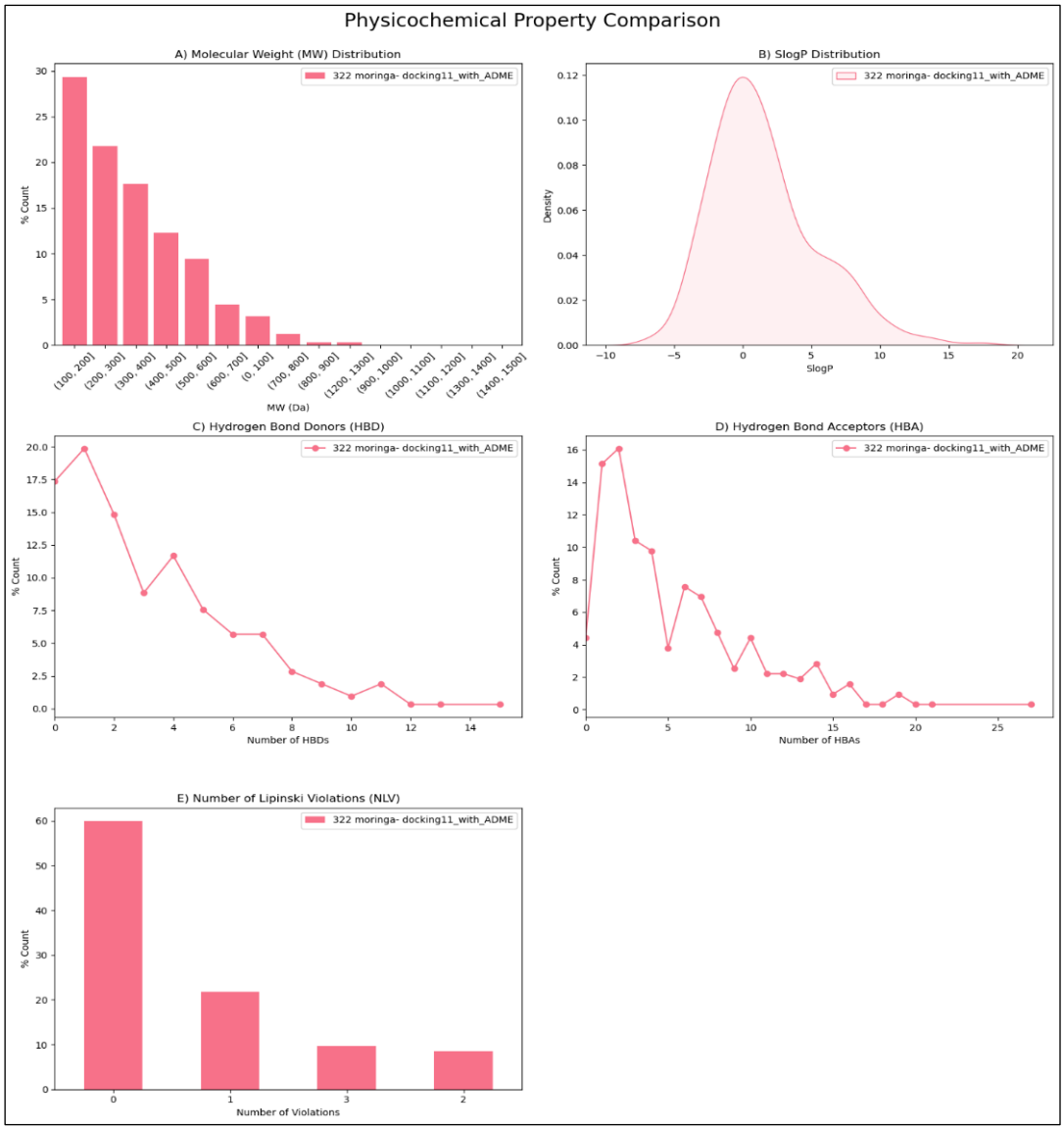

Figure 2. Physicochemical analysis of Moringa phytochemicals. The majority of compounds (~60%) show zero violations of Lipinski's Rule of Five (E). Panels (A-D) detail the istributions of molecular weight, SlogP, and hydrogen bond donors/acceptors.

Protein Preparation Outcomes

The crystal structure of Cyclooxygenase-2 (COX-2) with PDB ID: 5KIR was successfully retrieved from the Protein Data Bank and prepared for docking simulations (Figure 3). The preparation protocol within MOE involved several critical steps to ensure a suitable receptor structure. These included the removal of non-essential crystallographic water molecules and the co-crystallized ligand (Mefenamic acid) from the active site. Potential structural errors were assessed and addressed. Explicit hydrogen atoms were added, and appropriate protonation states for titratable residues were assigned based on an estimated physiological pH. A light energy minimization was performed to refine the structure and alleviate steric clashes. The resulting prepared 3D protein structure provided a refined and ready receptor model for docking the ligand library[14], [17].

Validation of Molecular Docking Protocol

The native co-crystallized ligand (Mefenamic acid) was re-docked within the inhibitor binding cavity of COX-2 (PDB ID: 5IKR). The RMSD value was subsequently determined by comparing the docked pose to the crystal structure conformation.

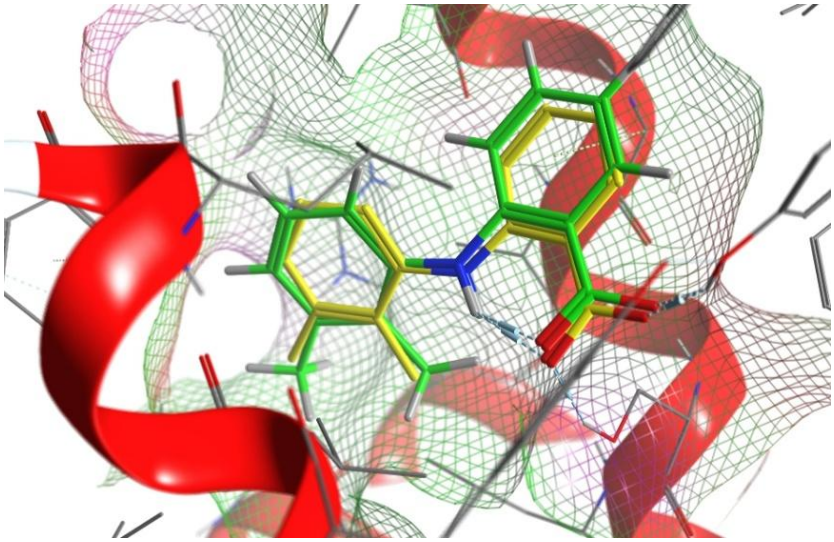

Figure 3. Superposition of the original (yellow) and redocked (green) poses of the native ligand mefenamic acid within the active site of COX-2 (PDB: 5IKR). The (RMSD) between the two poses is 0.329 Å. The (ΔGb) obtained for the redocked pose was 6.876 kcal/mol.

The RMSDs values between the re-docked poses and the original poses of the native ligands are indicative of whether the docking protocol is accurate, with values under 2 Å indicative of an accurate protocol [14], [17]. If the RMSD of the best docked conformation of the native ligand is 2.0 Å or less from the experimental one (native ligand), the used scoring function (protocol) is successful. As shown in Figure 3, the predicted docking pose (green) of Mefenamic acid displayed RMSD value of 0.329 Å, providing hardy evidence for the reliability and reproducibility of scoring function implemented in MOE docking protocol, used in current study. The binding energy (ΔG_b) obtained for the redocked Ligand (Mefenamic acid) pose was 6.876 kcal/mol.

Docking Simulation Results and Analysis

Moringa oleifera has established traditional medicinal use and proven *in-vitro* anti-inflammatory effects [12]. This study employed molecular docking to investigate binding affinities of selected compounds against COX-2, a key inflammatory target, elucidating potential molecular mechanisms. Using MOE Dock, we performed simulations on the COX-2 receptor (PDB: 5IKR), defining the binding site with the native ligand, mefenamic acid. Ligand poses were generated and scored based on their binding free energy (ΔG_b). For each molecule, the most probable binding conformation was identified as the pose with the lowest (ΔG_b) and the most significant hydrogen and hydrophobic interactions with essential residues in the active site. Table 1 summarizes the screening results of an initial library of 300 compounds. The (ΔG_b) of the redocked native ligand, Mefenamic acid, was established as a benchmark at -6.876 kcal/mol. Based on this, 60 compounds with binding energies equal to or better than this value were selected for further detailed analysis against the COX-2 crystal structure (PDB ID: 5IKR).

Table 1. Top 60 of MOC Docked to COX-2 (PDB: 5IKR) Ranked by Binding Energy.

No	MC ID	(ΔG_b)	No	MC ID	(ΔG_b)	No	MC ID	(ΔG_b)
1	MC063	-6.968	21	MC146	-7.082	41	MC277	-7.790
2	MC064	-7.232	22	MC162	-7.001	42	MC278	-7.398
3	MC067	-7.221	23	MC171	-7.375	43	MC279	-7.215
4	MC068	-7.204	24	MC172	-6.738	44	MC282	-7.483
5	MC069	-6.947	25	MC185	-6.911	45	MC283	-7.076
6	MC072	-6.932	26	MC186	-6.812	46	MC289	-7.153
7	MC093	-7.014	27	MC191	-7.641	47	MC290	-7.780
8	MC095	-7.185	28	MC192	-6.941	48	MC296	-7.282
9	MC097	-7.686	29	MC193	-7.178	49	MC297	-7.239
10	MC098	-6.945	30	MC200	-6.979	50	MC298	-7.193
11	MC099	-6.964	31	MC205	-7.792	51	MC300	-7.282
12	MC101	-7.381	32	MC217	-7.628	52	MC301	-8.132
13	MC106	-8.129	33	MC254	-7.160	53	MC303	-6.836
14	MC117	-6.821	34	MC261	-6.920	54	MC304	-7.328
15	MC119	-7.315	35	MC265	-7.264	55	MC306	-7.592

16	MC123	-6.954	36	MC271	-7.221	56	MC307	-6.802
17	MC128	-7.015	37	MC273	-7.352	57	MC308	-7.367
18	MC139	-7.368	38	MC274	-7.172	58	MC310	-7.505
19	MC140	-7.268	39	MC275	-7.346	59	MC316	-7.710
20	MC143	-6.992	40	MC276	-7.771	60	MC317	-7.618

Note: MC ID- *Moringa oleifera* Compound unique Identification number. **(ΔG_b)-** Binding Energy.

The results from the docking of these 60 selected compounds are highly encouraging. Remarkably, as shown in Table 1, all 60 *Moringa oleifera* compounds analyzed exhibited binding energies more favorable (i.e., more negative) than the study's redocked Mefenamic acid (-6.876 kcal/mol). This primary observation strongly suggests that these phytochemicals possess a greater predicted affinity for the COX-2 active site compared to this known inhibitor [14], [17]. The therapeutic potential of the top 60 MOC becomes particularly evident when compared to a panel of standard non-steroidal anti-inflammatory drugs (NSAIDs) presented in Table 2. This comparative analysis reveals that 47 compounds (78.3%) demonstrated superior binding energies compared to Mefenamic acid (-6.876 kcal/mol), while 43 compounds (71.7%) exceeded the binding affinity of Diclofenac. Most significantly, 15 compounds (25%) achieved binding energies greater than -7.500 kcal/mol, approaching or surpassing the performance of Celecoxib, which serves as the reference COX-2 selective inhibitor.

Table 2. Standard NSAID drugs Docked to COX-2 (PDB: 5IKR) Ranked by Binding Energy.

	Name	Binding Energy (ΔG_b)
1	Celecoxib	-7.772
2	Indomethacin	-7.101
3	Mefenamic acid (redocked)	-6.876
4	Diclofenac	-6.751
5	Naproxen	-6.652
6	Ibuprofen	-6.636
7	Aspirin	-5.590

The establishment of binding affinity benchmarks among standard NSAIDs revealed a distinct performance hierarchy, with Celecoxib demonstrating the highest binding energy at -7.772 kcal/mol, followed by Indomethacin at -7.101 kcal/mol, while Diclofenac, Naproxen, Ibuprofen, and Aspirin exhibited progressively weaker binding energies of -6.751, -6.652, -6.636, and -5.590 kcal/mol, respectively. This reference framework enabled systematic evaluation of *Moringa oleifera's* therapeutic potential through direct comparative analysis. The remarkable performance of MOC against these established standards demonstrates their exceptional anti-inflammatory potential, with notable proportions surpassing the binding affinities of widely used NSAIDs including Aspirin, Diclofenac, Naproxen, and Ibuprofen. The superior binding affinity of the 60 selected MOC to the COX-2 enzyme, which surpassed that of aspirin and other established NSAIDs, highlights their significant therapeutic potential. These computational findings are strongly corroborated by experimental evidence in which *moringa* extracts demonstrated anti-inflammatory effects comparable to aspirin and even twice as effective as diclofenac [19], [20]. Furthermore, MOC were reported to provide strong anti-inflammatory effects similar to pure ibuprofen, but with better protection against gastric ulcers, positioning them as highly promising candidates for safer, novel anti-inflammatory drugs [21].

Detailed statistical analysis reinforces the exceptional nature of these findings, as the top decile of MOC achieved binding energies ranging from -8.132 to -7.628 kcal/mol, indicating consistent high-affinity interactions throughout the leading compounds. The median binding energy among all 60 compounds reached approximately -7.200 kcal/mol, substantially exceeding the -6.714 kcal/mol median observed for standard NSAIDs. This distribution pattern indicates that *Moringa oleifera* contains multiple structurally diverse compounds capable of achieving superior COX-2 binding compared to conventional therapeutics. These findings suggest that *Moringa oleifera* represents an exceptionally rich source of potential anti-inflammatory therapeutics [10], [12], [20].

Expanding the comparative perspective, systematic evaluation against seven standard NSAIDs confirmed that *Moringa* derived compounds consistently match or surpass clinically established anti-inflammatory drugs, with 78% outperforming Mefenamic acid and 72% exceeding Diclofenac binding affinity [19], [20], [21]. These outcomes are particularly significant given that reference NSAIDs represent decades of intensive medicinal chemistry optimization and rigorous clinical validation, yet multiple MOC demonstrate superior computational binding profiles, suggesting untapped therapeutic potential within natural product chemistry. The identification of high affinity compounds across the entire MOC identification range suggests that COX-2 inhibitory activity is distributed throughout diverse chemical scaffolds present in *Moringa oleifera*. The presence of compounds indicates that multiple distinct chemical families within *Moringa* (Figure 1) possess anti-inflammatory potential.

This diversity provides numerous opportunities for lead optimization and development of compounds with varying pharmacological profiles.
While the computational predictions demonstrate compelling evidence for therapeutic potential, several important limitations must be acknowledged. The docking calculations represent idealized binding scenarios that may not fully account for protein flexibility, solvation effects, or allosteric considerations that influence actual biological activity. Additionally, the current analysis focuses exclusively on binding affinity without considering critical factors such as COX-1/COX-2 selectivity ratios, which are essential for minimizing gastrointestinal and cardiovascular side effects associated with NSAID therapy [22]. These *in-silico* findings, corroborated by traditional use and existing *in-vitro* data, strongly support further investigation of *Moringa oleifera's* anti-inflammatory potential. Compounds with the lowest binding energies warrant priority for: (1) *in-vitro* enzymatic assays to confirm COX-2 inhibition and determine IC50 values; (2) cell-based assays to evaluate effects on PGE2 production; (3) molecular dynamics and *in-vivo* potential studies; and (4) detailed structural analysis to identify key molecular interactions for lead optimization.

Conclusion

This molecular docking study identified several phytochemicals from *Moringa oleifera* with strong COX-2 inhibitory potential. Notably, these compounds exhibited binding energies superior to the redocked native ligand, mefenamic acid, and were comparable or superior to those of standard non-steroidal anti-inflammatory drugs (NSAIDs). When considered alongside *Moringa oleifera's* long standing use in traditional medicine and previous experimental data, our findings underscore the plant's promise as a source for novel analgesic and anti-inflammatory agents. Ultimately, the significant activity observed by multiple *Moringa oleifera* constituents (MOC) highlights the uncharted chemical diversity that traditional remedies possess. Our curated database is publicly available at https://www.libnpdb.ly/moringa-oleifera-database as a resource for researchers. Given the well documented adverse effects of current NSAIDs, continued experimental validation is essential. Such efforts may yield safer, more effective anti-inflammatory drugs and reinforce the strategic importance of natural product research in modern drug discovery.

References

1. A. Gurib-Fakim, "Medicinal plants: Traditions of yesterday and drugs of tomorrow," *Mol. Aspects Med.*, vol. 27, no. 1, pp. 1–93, Feb. 2006, doi: 10.1016/j.mam.2005.07.008.
2. I. I. Abdallah, H. Pramastya, R. van Merkerk, Sukrasno, and W. J. Quax, "Frontiers | Metabolic Engineering of Bacillus subtilis Toward Taxadien1000en 0#7"/. mml]#0 Biosynthesis as the First Committed Step for Taxol Production", doi:, m====10.3389/fmicb.2019.00218.
3. D. J. Newman and G. M. Cragg, "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019," *J. Nat. Prod.*, vol. 83, no. 3, pp. 770–803, Mar. 2020, doi: 10.1021/acs.jnatprod.9b01285.
4. J. B. Calixto, "The role of natural products in modern drug discovery.," *Acad Bras Cienc*, 2019.
5. P. M. Finch and P. D. Drummond, "Topical Treatment in Pain Medicine: From Ancient Remedies to Modern Usage," *Pain Manag.*, vol. 5, no. 5, pp. 359–371, Sep. 2015, doi: 10.2217/pmt.15.23.
6. L. Zhang, X. Wang, and H. Si, "Synergistic anti-inflammatory effects and mechanisms of the combination of resveratrol and curcumin in human vascular endothelial cells and rodent aorta," *J. Nutr. Biochem.*, vol. 108, p. 109083, Oct. 2022, doi: 10.1016/j.jnutbio.2022.109083.
7. A. M. A. Ali *et al.*, "Chemical profile, anti 5-lipoxygenase and cyclooxegenase inhibitory effects of ginger (*Zingiber officinale*) rhizome, callus and callus treated with elicitors", doi: https://doi.org/10.1590/0103-8478cr20210372.
8. Md. S. Hussain *et al.*, "Anti-inflammatory, analgesic and molecular docking studies of Lanostanoic acid 3-O-α-D-glycopyranoside isolated from Helichrysum stoechas," *Arab. J. Chem.*, vol. 13, no. 12, pp. 9196–9206, Dec. 2020, doi: 10.1016/j.arabjc.2020.11.004.
9. X. Deng, Y. Luo, M. Lu, T. Guan, Y. Li, and X. Guo, "Unraveling the Mechanism of Zhibaidihuang Decoction against IgA Nephropathy Using Network Pharmacology and Molecular Docking Analyses," *Tohoku J. Exp. Med.*, vol. 259, no. 1, pp. 37–47, 2023, doi: 10.1620/tjem.2022.J088.
10. K. Aburas, A. Misbah, H. Fehelbum, and A. Abukhdir, "Analgesic effect by using hot Plat and tail flick test in rats models for aqueuos moringa oleifer extract," *Libyan J. Med. Res.*, vol. 17, no. 1, pp. 107–119, Jun. 2023, doi: 10.54361/ljmr.17-21.
11. T. Tshabalala *et al.*, "Scribbling the Cat: A Case of the 'Miracle' Plant, Moringa oleifera," *Plants*, vol. 8, no. 11, p. 510, Nov. 2019, doi: 10.3390/plants8110510.
12. A. Pareek *et al.*, "Moringa oleifera: An Updated Comprehensive Review of Its Pharmacological Activities, Ethnomedicinal, Phytopharmaceutical Formulation, Clinical, Phytochemical, and Toxicological Aspects," *Int. J. Mol. Sci.*, vol. 24, no. 3, p. 2098, Jan. 2023, doi: 10.3390/ijms24032098.

13. Mm. R. E. Wuryandari, N. Yuliati, E. Sutikno, and S. A. Mohamed, “Interaction Affinity Between Flavonoids Against Cytokines Interleukin 12 in Diabetes Mellitus,” *MEDIA ILMU Kesehat.*, vol. 12, no. 2, pp. 217–223, Sep. 2023, doi: 10.30989/mik.v12i2.1060.
14. K. Obikeze, A.-A. Sasi, and I. Raji, “In-silico and in-vivo evaluation of the Cardiovascular effects of five Leonotis leonurus diterpenes,” *Sci. Afr.*, vol. 19, p. e01510, Mar. 2023, doi: 10.1016/j.sciaf.2022.e01510.
15. M. Sorokina, P. Merseburger, K. Rajan, M. A. Yirik, and C. Steinbeck, “COCONUT online: Collection of Open Natural Products database,” *J. Cheminformatics*, vol. 13, no. 1, p. 2, Jan. 2021, doi: 10.1186/s13321-020-00478-9.
16. H. L. Barazorda-Ccahuana *et al.*, “PeruNPDB: the Peruvian Natural Products Database for in silico drug screening,” *Sci. Rep.*, vol. 13, no. 1, p. 7577, May 2023, doi: 10.1038/s41598-023-34729-0.
17. Y. M. Abd-Elhakim *et al.*, “Investigation of the In-Vivo Cytotoxicity and the In Silico-Prediction of MDM2-p53 Inhibitor Potential of Euphorbia peplus Methanolic Extract in Rats,” *Toxins*, vol. 11, no. 11, p. 642, Nov. 2019, doi: 10.3390/toxins11110642.
18. R. Wang, Y. Lu, and S. Wang, “Comparative evaluation of 11 scoring functions for molecular docking,” *J. Med. Chem.*, vol. 46, no. 12, pp. 2287–2303, Jun. 2003, doi: 10.1021/jm0203783.
19. N. Panova *et al.*, “Metabolic Profile, Antioxidant, Antimicrobial, Contractile, and Anti-Inflammatory Potential of Moringa oleifera Leaves (India) - Consensus”, Accessed: Jul. 20, 2025. [Online]. Available: https://consensus.app/papers/metabolic-profile-antioxidant-antimicrobial-ivanov-todorova/041f71ef972a5d799990d8cc13bfa2c4/
20. A. Jaja-Chimedza *et al.*, “Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract - Consensus”, Accessed: Jul. 20, 2025. [Online]. Available: https://consensus.app/papers/biochemical-characterization-and-antiinflammatory-kim-pauli/73d55ae6914b5769b6d152e705680efd/
21. T. Gugu, G. C. Agu, E. Uronnachi, and S. Chime, “Enhanced anti-inflammatory and ulcerogenicity of Ibuprofen microsphere formulations using Irvingia wombolu fat (IRW) and moringa oil (MO) as co-lipids - Consensus”, Accessed: Jul. 20, 2025. [Online]. Available: https://consensus.app/papers/enhanced-antiinflammatory-and-ulcerogenicity-of-uronnachi-agu/d30de2f7586d5178b055d5a3f4855ede/
22. J. M. Paggi, A. Pandit, and R. O. Dror, “The Art and Science of Molecular Docking,” Aug. 2024, doi: 10.1146/annurev-biochem-030222-120000.