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Anti-Inflammatory Potential of Active Compounds in Moringa Leaves (*Moringa Oleifera*) Against Osteoarthritis Through in Silico Methods

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Keywords:
osteoarthritis; 5-lipoxygenase; Moringa oleifera; in silico; molecular docking

Abstract

Osteoarthritis (OA) is a degenerative joint disease with a high global burden. Available therapies remain palliative, and selective COX-2 inhibition risks shifting arachidonic acid metabolism to the 5-lipoxygenase (5-LOX) pathway, which increases proinflammatory leukotrienes. Furthermore, no approved 5-LOX inhibitors exist for OA. This study aims to analyze the anti-inflammatory potential of active metabolites in moringa leaves as 5-LOX inhibitors in osteoarthritis using in silico methods. This study was conducted using in silico methods, including preparation of ligand and target protein structures, identification of binding sites, docking validation, molecular docking, and visualization of molecular interactions. Molecular docking was performed using AutoDock Vina software via PyRx to evaluate binding energy (ΔG). All moringa leaf metabolites bound stably to both the orthosteric and allosteric pockets of 5-LOX, with binding affinities competitive with those of the reference ligands particularly ellagic acid (ΔG = −7.7 kcal/mol) at the orthosteric site and isorhamnetin (ΔG = −8.3 kcal/mol) at the allosteric site. Docking validation confirmed the reliability of the method, supporting the feasibility of active metabolite compounds from moringa leaves as potential 5-LOX inhibitor candidates in osteoarthritis. Active metabolites of moringa leaves show potential as 5-LOX inhibitors with competitive affinity at orthosteric and allosteric sites, and may thus be developed as complementary anti-inflammatory agent candidates in osteoarthritis. However, further validation through in vitro and in vivo testing is required.

INTRODUCTION

Osteoarthritis (OA) is one of the most common chronic inflammatory joint diseases and remains a significant health problem worldwide (Coaccioli et al., 2022; Perhimpunan Reumatologi Indonesia, 2021). According to the Indonesian Rheumatology Association in 2021, OA is defined as an articular degenerative disease characterized by persistent pain that can cause disability, loss of function, and a decreased quality of life in affected individuals (Perhimpunan Reumatologi Indonesia, 2021). In 2021, Steinmetz *et al.* reported that the number of global OA cases increased by 132.2% from 1990 to 2020, with an estimated 595 million people currently affected (Courties et al., 2024). In terms of age, OA ranks 7th as the leading cause of disability in individuals over 70 years of age globally, particularly involving knee and lower limb defects (Courties et al., 2024; Steinmetz et al., 2023). Based on data from the Basic Health Research (*Riset Kesehatan Dasar*/Riskesdas) in 2018, the prevalence of diagnosed joint diseases was 7.3% of the general population (Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan, 2019). Elderly individuals with OA in Indonesia are estimated to number 55 million, accounting for approximately 24.7% of the population (Ika Wardojo et al., 2021).

To date, OA therapy has remained palliative, focusing on pain control, improvement of joint mobility, and enhancement of patients' quality of life (Perhimpunan Reumatologi Indonesia, 2021; Courties et al., 2024). In its management, non-steroidal anti-inflammatory drugs (NSAIDs) remain the first-line treatment recommended by the Indonesian Rheumatology Association (Perhimpunan Reumatologi Indonesia, 2021). Most current NSAIDs are designed to inhibit cyclooxygenase-2 (COX-2) in the arachidonic acid metabolic pathway; however, selective COX-2 inhibition alone may produce unintended downstream effects (Khalil et al., 2024; Lee & Katz, 2021; Marcum & Hanlon, 2010).

Selective inhibition of COX-2 can redirect arachidonic acid metabolism toward the 5-lipoxygenase (5-LOX) pathway, leading to increased production of leukotrienes and other pro-inflammatory mediators that may exacerbate local inflammation. Pharmacological evidence suggests that simultaneous inhibition of both COX-2 and 5-LOX can suppress the formation of prostaglandins and leukotrienes while preserving the production of pro-resolution inflammatory mediators. This dual-inhibition approach offers superior protection of synovial tissue compared to selective COX-2 inhibition alone (Tee & Berezovsky, 2024). Despite the widely recognized importance of 5-LOX as a therapeutic target in inflammatory disease research, no 5-LOX inhibitor has been approved or clinically adopted for the treatment of OA (Fiorucci et al., 2021; Mukhopadhyay et al., 2023; Rudrapal et al., 2023). Consequently, the development of 5-LOX inhibitory agents has gained considerable interest, particularly those derived from natural sources with established anti-inflammatory properties (Mukhopadhyay et al., 2023).

One such natural source that has been extensively studied as an alternative anti-inflammatory therapy is moringa leaves (*Moringa oleifera*) (Chiș et al., 2023; Herman-Lara et al., 2024; Manjunath et al., 2023; Mthiyane et al., 2022; Panova et al., 2025; Ramamurthy et al., 2022). Moringa leaves have been reported to exhibit anti-inflammatory activity, attributed to the presence of diverse active metabolite compounds capable of modulating inflammatory pathways (Chiș et al., 2023; Prabowo et al., 2024; Kumar et al., 2021). Several metabolites identified in moringa leaves have demonstrated anti-inflammatory activity, including quercetin, kaempferol, isorhamnetin, luteolin, chlorogenic acid, ferulic acid, ellagic acid, niazimnin, and moringin (Kandemir et al., 2022; Pop et al., 2022; Darekar et al., 2023; Tao et al., 2025; Ndou et al., 2023; Kashyap et al., 2022).

Although these compounds have previously been investigated for their anti-inflammatory potential, no *in silico* study has specifically evaluated moringa leaf metabolites as 5-LOX inhibitors (Mourão et al., 2024; Parvin et al., 2025; Rudrapal et al., 2025). The *in silico* molecular docking approach enables the identification and validation of ligand–protein interactions, facilitating the visualization of binding modes at the known orthosteric and allosteric sites of 5-LOX. This study is expected to provide robust computational evidence supporting the development of moringa leaf-based phytopharmaceuticals as innovative therapeutic agents with a more comprehensive anti-inflammatory mechanism and favorable safety profile.

This study was designed to investigate the anti-inflammatory potential of active compounds in moringa leaves (*Moringa oleifera*) as inhibitors of 5-lipoxygenase (5-LOX) in osteoarthritis through an *in silico* approach. The primary objective was to analyze the binding affinity and interaction patterns of active metabolites from moringa leaves at the orthosteric

and allosteric sites of the 5-LOX protein. Specifically, this study evaluated the strength of molecular interactions and the characteristics of the bonds formed as indicators of potential 5-LOX inhibitory activity. The findings of this study are expected to offer academic contributions by enriching the scientific literature on the use of *Moringa oleifera* as an anti-inflammatory candidate and serving as a model for the application of *in silico* methods in natural drug discovery. For the community, this research provides a scientific basis for the use of moringa leaves as a safe and affordable complementary therapy for OA patients. For policymakers, it supports evidence-based herbal medicine research and the sustainable utilization of local biodiversity.

METHOD

Types & Research Design

This study is an exploratory *in silico* study employing a molecular docking approach, designed to evaluate the potential molecular interactions between active metabolite compounds of moringa leaves (*Moringa oleifera*) and the target protein 5-lipoxygenase (5-LOX). The research was conducted entirely using bioinformatics software and publicly available databases, without involving any biological subjects.

Population and Sample

The study population comprised all active metabolite compounds present in moringa leaves (*Moringa oleifera*) as well as all 5-LOX protein structures available in public databases. The study sample consisted of nine active compounds identified in moringa leaves namely quercetin, kaempferol, isorhamnetin, luteolin, chlorogenic acid, ferulic acid, ellagic acid, niazicin, and moringin along with two 5-LOX target protein structures retrieved from the RCSB Protein Data Bank, with PDB IDs 6N2W and 6NCF.

Data Collection Techniques

Ligand and protein structure data were retrieved through searches of publicly accessible online databases, specifically PubChem for ligand structures and the RCSB Protein Data Bank for target protein structures. Ligand and protein preparation, method validation through redocking, molecular docking simulations, and visualization of molecular interaction patterns were subsequently performed using AutoDock Vina, PyRx, PyMOL, and Biovia Discovery Studio.

Data Analysis Techniques

Data analysis was conducted descriptively and comparatively based on key molecular docking parameters, including the root mean square deviation (RMSD) value obtained from validation redocking, binding affinity (ΔG, kcal/mol), and the type and number of amino acid residue interactions at the binding site. Docking results for each moringa leaf active compound were compared against those of the reference ligands to assess their potential as 5-LOX inhibitor candidates.

RESULT AND DISCUSSION

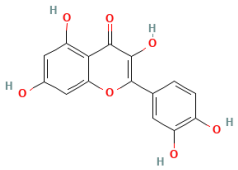
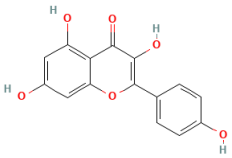
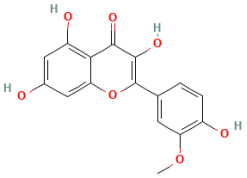
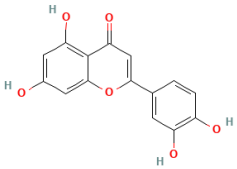
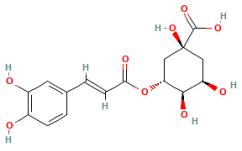
A. Results

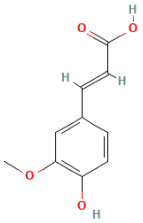
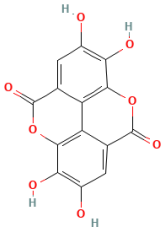
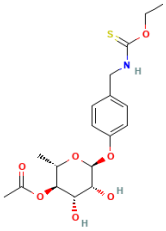
1. Preparation of the ligand structure of the active compound *Moringa oleifera*

The test ligand structure of the active compound Moringa *oleifera* that has been studied has *drug-likeness* properties and has previously been studied to have inhibitory properties

obtained from PubChem in (.sdf) format. The form and number of compounds used from the PubChem database can be seen in Table 1. The downloaded test compound is then converted to a format (.pdb) via OpenBabel. This is done so that the downloaded ligands can be used in *molecular docking* in Autodock Vina via PyRx.

Table 1. Table of ligand structure of the active compound test of *M. Oleifera* (16,52,58)

Compound Name	CID PubChem	Chemical Structure
Quercetin	5280343	
Kaempferol	5280863	
Isorhamnetin	5281654	
Luteolin	5280445	
Asam chlorogenic ate	1794427	

Compound Name	CID PubChem	Chemical Structure
Ferulic acid	445858	
Ellagic acid	5281855	
Niaziminin	10023860	

Compound Name	CID PubChem	Chemical Structure
Moringin	153557	

Source: PubChem database; compound structures were retrieved using the respective CID numbers and processed by the authors

2. Preparation of the target structure of the 5-LOX protein

At this stage, the 5-LOX protein bound to its original ligand at the active and allosteric sites is downloaded for separation and the protein is prepared for *molecular docking*. The 5-LOX structure with a PDB ID of 6N2W is used to determine the coordinates of the active binding site, whereas the PDP ID 6NCF is used to determine the coordinates of the allosteric binding site of the 5-LOX protein. Both PDB IDs use the structure of the 5-LOX protein derived from humans (*homo sapiens*). Protein preparation is performed using AutoDockTools 1.5.7 by removing non-residual amino acid compounds, water molecules, adding hydrogen, and charges, and separating the original ligand from the target protein. This is done so that the *docking atmosphere* is close to the physiological pH. The results of this protein preparation are visualized in Figure 1.

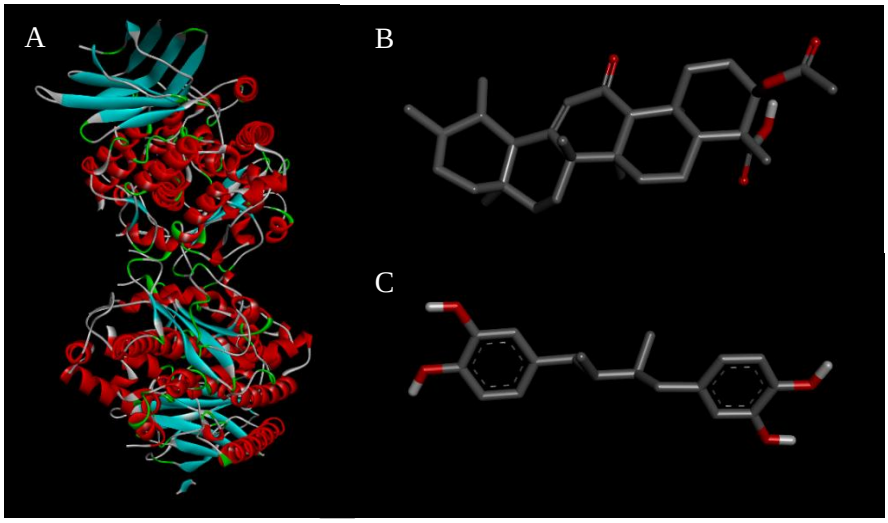


Figure 1. Visualization of the results of preparation of 5-LOX target proteins separated by the original ligand

Source: Authors' visualization using PyMOL based on protein structures obtained from RCSB Protein Data Bank

Ket: (A) 5-LOX protein; (b) AKBA ligan; (C) NDGA ligand

3. Identification of binding sites

This stage aims to determine the specific interaction space on the protein (*gridbox*) that will be used as the *docking site*. The determination *of the docking location* is carried out by determining the coordinates and size of the *gridbox* to be used. The *gridbox coordinates* are taken from the center of the original ligand and the determination of the *size of the gridbox* is adjusted to the size of the binding site and the test ligand. The center and dimensional size of the *gridbox* are listed in Table 2.

Table 2. Center coordinates and gridbox dimensions of the binding site

PDB ID	*Gridbox*					
	%			Dimensions (Å)		
	X	And	Z	X	And	Z
6N2W	35,944	65,322	38,344	25	25	25
6NCF	11,277	-21,891	-18,408	25	25	25

Source: Authors' calculation based on native ligand coordinates from RCSB Protein Data Bank structures 6N2W and 6NCF

4. Docking validation

The results of *the docking* validation using Autodock Vina through PyRx showed that the *docking* process carried out succeeded in producing *a pose* with the affinity value of each original ligand well. *The best pose* of each *docking result was* then revalidated using PyMOL by comparing the original structure position with the docking result structure position visualized in Figure 2. The results of the validation resulted in an RMSD value of 1.418 Å in the 6N2W structure and 0.348 Å in the 6NCF structure. Results with RMSD values below 2 Å show that the position of the docked ligand is very close to its crystallographic position. The value confirms that the method used is valid and can be used for further analysis.

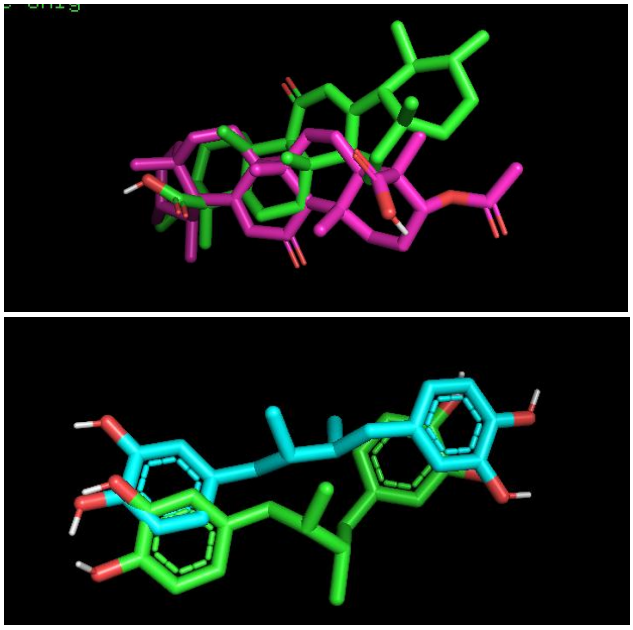


Figure 2. Visualization of the comparison of the docking structure with the original position of the original ligand
Source: Authors' redocking validation and visualization using AutoDock Vina, PyRx, and PyMOL.

5. Hasil *Molecular Docking*

The analysis of *the results of molecular docking* between ligands and receptors was carried out based on the free bond energy value obtained through Autodock Vina via PyRx. Based on the free binding energy (ΔG) values in Table 3, the lowest ΔG values of the test ligands were obtained by ellagatic acid (-7.7 kcal/mol) at the active site and isorhamnetin (-8.3 kcal/mol) at the allosteric site. At the active site, kaempferol, luteolin, chlorogenic acid, and ellaic acid have lower ΔG values than the original ligand, namely NDGA, which has a ΔG value of -7.2 kcal/mol. At the allosteric site, no ligand has a lower ΔG value than the original ligand, namely AKBA with a ΔG value of -9.5 kcal/mol.

Table 3. *Affinity binding* value of molecular docking results

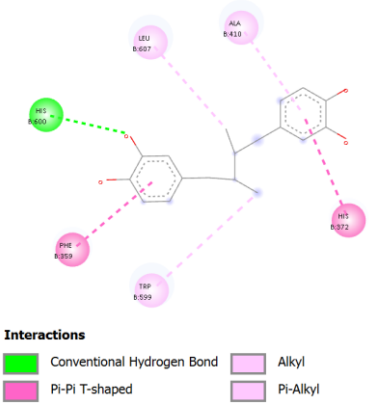
Ligand Names	Free Binding Energy (ΔG) (kcal/mol)	
	Active Sites	Situs Alosterik
NDGA (Active Site Ligand)	-7,2	-
AKBA (Allosteric Site Ligand)	-	-9,5
Quercetin	-7,0	-8,2
Kaempferol	-7,4	-8,0
Isorhamnetin	-7,2	-8,3
Luteolin	-7,4	-8,1
Asam chlorogenic ate	-7,3	-7,9
Phenolic acids	-5,7	-5,8
Ellagic acid	-7,7	-7,8
Niaziminin	-6,8	-7,4
Moringin	-6,2	-7,4

Source: Authors' molecular docking analysis using AutoDock Vina through PyRx

6. Visualization of Molecular Interactions

Visualization of *the molecular docking* results between the test ligand and the 5-LOX protein at the active site shown in Figure 3 and the allosteric site in Figure 3. This visualization was carried out using the Discovery Studio Visualizer 2025 software to facilitate the analysis of the bonds formed. The amino acid bonds and interactions formed between ligands and receptors can be seen by using color coding to visualize the interactions that are formed. The dark green color indicates a hydrogen bond, bright green indicates a hydrophobic bond, and light green represents the Van der Waals interaction, providing information about the type of interaction that occurs and its effect on the affinity and stability of the bond formed.

A



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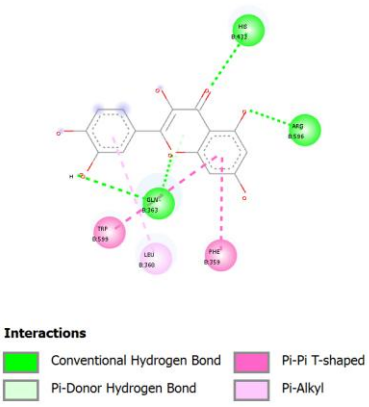

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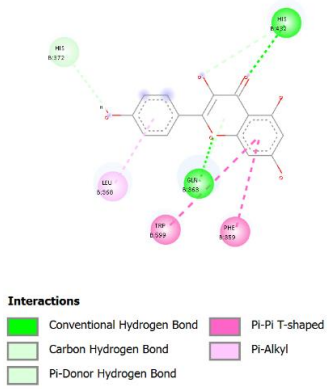

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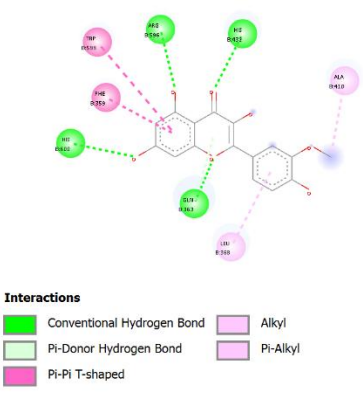

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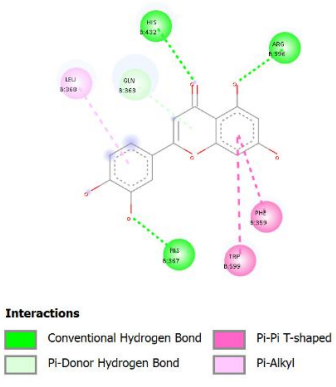

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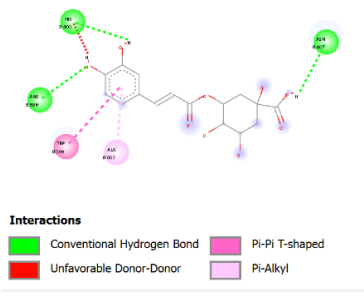


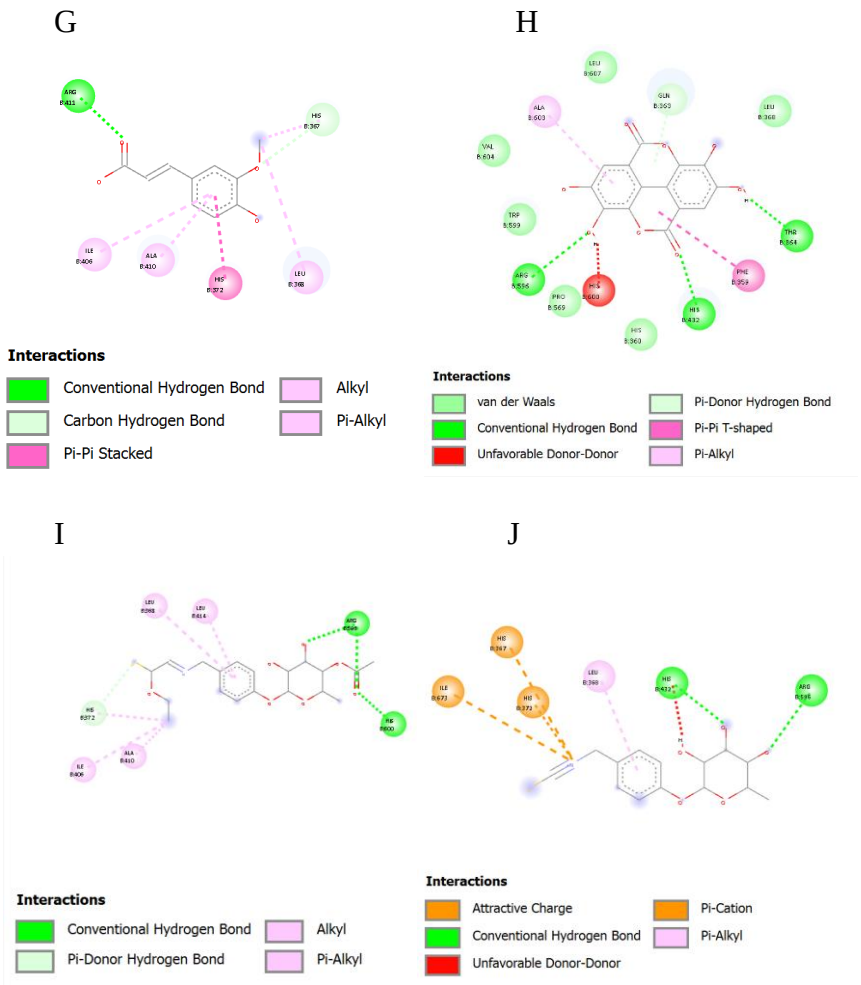


Figure 3. Visualization of *molecular docking* results at active sites

Source: Authors' visualization using Discovery Studio Visualizer based on docking results from AutoDock Vina/PyRx.

Ket: (A) NDGA; (B) Quercetin; (C) Kaempferol; (D) Isorhamnetin; (E) Luteolin; (F) Asam klorogenat; (G) Asam ferulat; (H) Asam ellagat; (I) Niaziminin; (J) Morigin

Based on the *docking* results in Table 5, the original ligand of NDGA as a comparator bonded to the active site residue via His600 hydrogen bond at a distance of 2.94 Å. The *docking* results showed that all test ligands could bind strongly via hydrogen bonding. Morigin has the shortest distance (1.58 Å) with His 432 and additional non-hydrogen contacts to Arg 596 and Leu 368. Quercetin forms three hydrogen bonds (1.91–3.19 Å) with His 432, Arg 596, Gln 363 as well as hydrophobic interactions at Phe 359, Leu 368, Trp 599. Kaempferol, isorhamnetin, and luteolin also bind to His 432/His 600 and show a range of 1.97–2.59 Å. Chlorogenic acid, ferulic, and ellagate exhibit hydrogen bonds at 2.05–2.90 Å with key residues (His 600, Arg 596) as well as extensive non-hydrogen contacts.

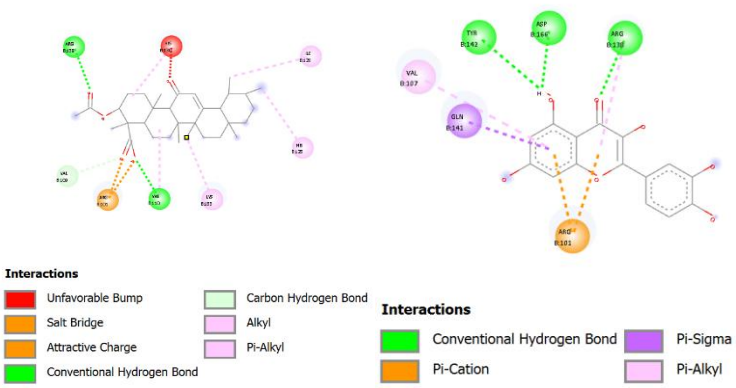
Table 4. Results of visualization of residual acids at active sites

In La Liga	Ikatan Hidrogen		Non-Hydrogen Interactions
	AA	Jarak (Å)	
NDGA (native ligand)	His600	2,94 Å	Ala410, Phe359, Trp599, His 372, Leu607
Quercetin	His432 Arg596 GLN363	2,38; 3,19; 2,86 1,91 2,33	Phe359, Leu368, Trp599
Kaempferol	His432 GLN363	2,37; 3,79 3,04; 2,22	His372, Leu368, Trp599, Phe359
Isorhamnetin	His600 His432 Arg596 GLN363	2,59 2,20 2,13 2,20	Ala410, Trp599, Phe359, Leu368
Luteolin	His367 His432 Arg596	2,56 2,30 1,97	Gln363, Leu368, Trp599, Phe359
Asam chlorogenic ate	His600 Arg596 Asn407	2,57 2,90 2,76	Ala603,Trp599
Ferulic acid	Arg411	2,10	Ala410, His372, Ile406, Leu368, His267
Ellagic acid	Thr364 His432 Arg596	2,05 2,19 2,80	Ala603, Arg596, His432, His360, Leu607, Leu368, Gln363, Phe359, Pro569
Niaziminin	His600 Arg596	2,01 2,03; 2,10,2,99	Ala410, Leu414, Leu368, His372, Ile406
Moringin	His432 Arg596	1,58; 2,38 2,46	Leu368, His367, His372, Ile673

Source: Authors' analysis of ligand–protein interactions using Discovery Studio Visualizer

At the allosteric site, the original comparator ligand, AKBA, showed the formation of a hydrogen bond with Arg138 residues at distances of 3.15 Å and 3.25 Å. Hydrogen bonds are also formed with Val110 residues at a distance of 2.72 Å. In addition, AKBA also forms non-hydrogen interactions with Arg101, Lys133, Ile126, His125, and Val109 which play a role in the stabilization of binding complexes. This visualization can be seen in Figure 14 and a list of interacting residues can be seen in Table 5.

A B



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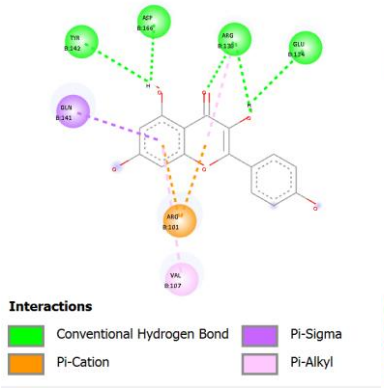
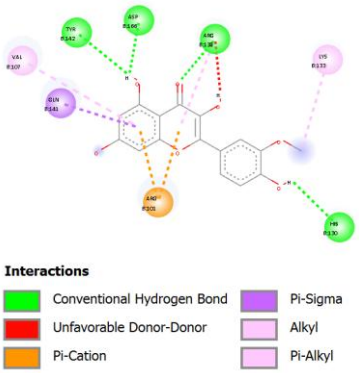
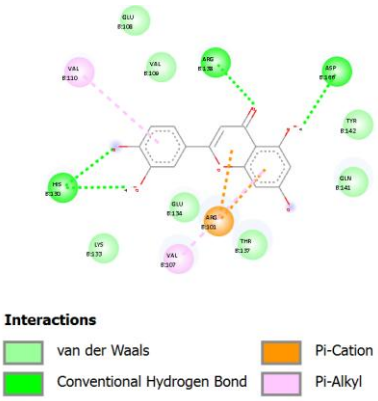

Interactions
Conventional Hydrogen Bond
Pi-Sigma
Pi-Cation
Pi-Alkyl

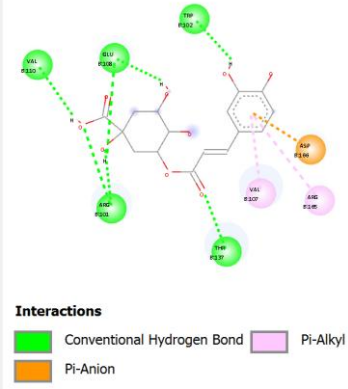
D


Interactions
Conventional Hydrogen Bond
Pi-Sigma
Unfavorable Donor-Donor
Alkyl
Pi-Cation
Pi-Alkyl

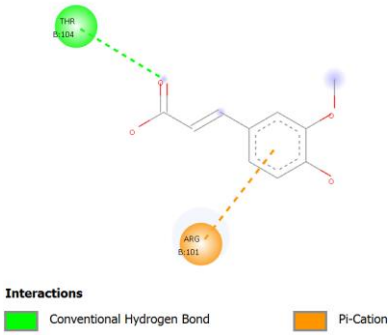
And


Interactions
van der Waals
Pi-Cation
Conventional Hydrogen Bond
Pi-Alkyl

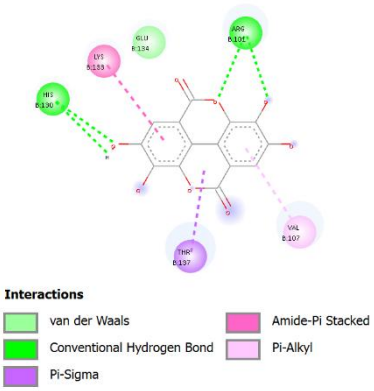
F


Interactions
Conventional Hydrogen Bond
Pi-Alkyl
Pi-Anion

G


Interactions
Conventional Hydrogen Bond
Pi-Cation

H


Interactions
van der Waals
Amide-Pi Stacked
Conventional Hydrogen Bond
Pi-Alkyl
Pi-Sigma

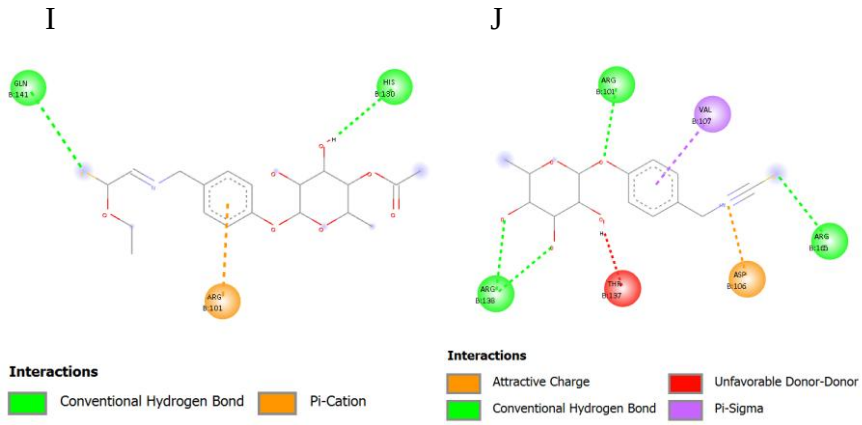


Figure 4. Visualization of molecular docking results at allosteric sites
Source: Authors' visualization using Discovery Studio Visualizer based on docking results from AutoDock Vina/PyRx
Ket: (A) AKBA; (B) Quercetin; (C) Kaempferol; (D) Isorhamnetin; (E) Luteolin; (F) Asam klorogenat; (G) Asam ferulat; (H) Asam ellagat; (I) Niaziminin; (J) Morigin

In general, all the compounds tested formed an extensive bonding network, including hydrogen bonds and non-hydrogen bonds with key residues at allosteric sites. The compounds kaempferol and isorhamnetin exhibit very strong hydrogen bonds with Asp166 residues, at distances of 1.93 Å and 1.92 Å, respectively. Quercetin also forms short hydrogen bonds with Asp166 (1.86 Å), Arg138 (2.14 Å), and Tyr142 (2.82 Å). In addition, Luteolin exhibits a strong hydrogen bond with Arg138 at a distance of 1.98 Å. Ellagic acid forms the most diverse interactions, forming hydrogen bonds with Val110 (2.43 Å), Arg101 (2.60 Å), Glu108 (2.06 Å and 2.39 Å), Trp102 (2.19 Å), and Trp137 (2.40 Å) The involvement of residues such as Asp166, Arg138, Arg101, Gln141, and Val107 in this binding pattern suggests that all test ligands successfully bond stably in the allosteric sac, although the specific interaction patterns vary depending on their chemical structure.

Table 5. Results of visualization of residual acids at allosteric sites

In La Liga	Ikatan Hidrogen		Interaction Non-Hydrogen
	AA	Jarak (Å)	
AKBA (original ligand)	Arg138 Val110	3,15; 3,25 2,72	Arg101, Lys133, Ile126, His125, Val109
Quercetin	Asp166 Arg138 Tyr142	1,86 2,14; 2,90; 5,43 2,82	Arg101, Gln141, Val107
Kaempferol	Asp166 Arg138 Glu134 Tyr142	1,93 2,14; 2,90; 5,41 2,74 2,83	Arg138, Gln141, Val107
Isorhamnetin	Asp166 Arg138 His130 Tyr142	1,92 2,15; 2,92; 5,42 2,69	Arg101, Lys133, Gln141, Val107

In La Liga	Ikatan Hidrogen		Interaction Non-Hydrogen
	AA	Jarak (Å)	
		2,78	
Luteolin	Arg138	2,43; 1,98	Arg101, Val107, Val10
	Asp166	2,96	
	His130	2,44; 2,53	
Asam chlorogenic ate	Arg101	2,08; 2,35	Asp166, Arg165, Val107
	Glu108	2.06; 2,39	
	Val110	2,43	
	Trp102	2,19	
	Thr137	2,40	
Ferulic acid	Thr104	2,74	Arg101
Ellagic acid	Val110	2,43	Asp166, Val107, Arg165
	Arg101	2,60	
	Glu108	2,06; 2,39	
	Trp102	2,19	
	Trp137	2,40	
Niaziminin	GLN141	3,56	Arg101
	His130	2,37	

In La Liga	Ikatan Hidrogen		Interaction Non-Hydrogen
	AA	Distance	
Moringin	Arg138	2,01; 2.12	Asp108, Val107
	Arg101	2,18	
	Arg165	2,83	

Source: Authors' analysis of ligand–protein interactions using Discovery Studio Visualizer

Inhibition of the enzyme 5-Lipoxygenase (5-LOX) is a key strategy to reduce the production of pro-inflammatory mediators of leukotriene that play a role in inflammation and joint damage in osteoarthritis (OA) (Fiorucci et al., 2021). 5-LOX inhibitors can act on either the active site (orthosteric) or the allosteric site. Orthosteric NDGA inhibitors compete directly with arachidonic acid to bind to iron-containing catalytic sites (Fe^{2+}) in 5-LOX, stopping the conversion of LTA4 which is a leukotriene precursor like LTB4. In contrast, AKBA allosteric inhibitors bind secondary sites away from active sites. This binding alters the conformation of the 5-LOX enzyme to stabilize inactive conformation or block interaction with activation proteins, thereby preventing enzyme activation and halting the entire leukotriene biosynthesis pathway. By suppressing the production of leukotrienes, especially LTB4 which is pro-inflammatory, inhibition of 5-LOX helps suppress inflammation, reduces cartilage degradation, promotes chondrogenic regeneration, and overall offers potential as a disease-modifying therapy for OA (Fiorucci et al., 2021).

Molecular docking is one of the computational research methods that has been widely used in the early stages of drug discovery. This method serves to simulate the interactions between micromolecules (ligands) and macromolecules (enzymes or receptors) that help in identifying binding interactions, bond affinities, and binding mechanisms formed. This makes it possible to provide an overview of a compound's ability to affect an enzyme, without direct testing (Chiș et al., 2023; Kumar et al., 2021). Before performing the molecular docking ligand test, the docking protocol is validated by redocking the original ligand on a 5-LOX structure. The RMSD result of 1.418 Å for 6N2W GDP (active site) and 0.348 Å for 6NCF GDP

(allosteric site) showed that the docked ligand pose was almost identical to the crystallographic pose, so the method could be declared valid (RMSD < 2 Å).

The results of the study showed that some active compounds of moringa leaves have the potential as anti-inflammatory agents through the inhibition mechanism of the 5-LOX enzyme. Molecular docking showed a stable interaction between ligands and key residues at orthotic and allosteric sites of 5-LOX, which plays a role in the inflammatory process through the production of leukotrien. The active compounds tested showed varying affinity binding values. The lower the bond energy value (kcal/mol), the more stable the ligand–receptor interaction and the greater the potential of the compound as an inhibitor (Kumar et al., 2021). The results of this study show that some moringa leaf metabolites have binding affinity values that are close to or even surpass control ligands, indicating competitive inhibition potential.

The results of molecular docking of nine active metabolites of moringa leaves at the 5-LOX active site showed that most of the metabolites had binding affinities equal to or even exceeding NDGA. Ellagic acid showed the best affinity with a value of -7.7 kcal/mol, surpassing NDGA (-7.2 kcal/mol) by 0.5 kcal/mol. The compound forms an extensive interaction network involving 12 amino acid residues, including hydrogen bonds with His432 (2.19 Å), Thr364 (2.05 Å), and Arg596 (2.80 Å), as well as non-hydrogen bonds with Ala603, His360, Leu607, Leu368, Gln363, Phe359, and Pro569. This broad and precise binding pattern suggests ellagatic acid has a much more stable bond within the binding pouch than NDGA, which supports its potent potential as a 5-LOX active site inhibitor.

At the 5-LOX allosteric site, the AKBA primary comparison ligand showed the highest overall affinity ΔG = -9.5 kcal/mol. However, some metabolites show quite strong allosteric affinity such as isorhamnetin as low as ΔG = -8.3, quercetin as low as ΔG = -8.2, and luteolin as low as ΔG = -8.1. These values close to AKBA suggest that these compounds have the potential to trigger conformational changes at 5-LOX located about 30 Å from the active site that can alter enzyme activity (Fiorucci et al., 2021). Ellagic acid, which is dominant at the active site, also retains a strong affinity at the allosteric site ΔG = -7.8 kcal/mol making it a prime candidate as a 5-LOX inhibitor.

Analysis of amino acid residue interactions showed the involvement of important residues that play a role in the catalytic activity of 5-LOX. Interactions in the form of hydrogen bonds, hydrophobic bonds, and electrostatic strengthen the prediction that moringa leaf compounds are able to bind well and inhibit enzyme activity (Chiș et al., 2023; Kumar et al., 2021). At active sites, these metabolites tend to interact with residues that form 5-LOX hydrophobic sacs such as Phe359, Trp599, Leu368 and involve catalytic residues His432, His600, and Arg596. At allosteric sites, interactions often involve Arg138, Asp166, and Val110 residues. This interaction pattern is very similar to the original ligand of AKBA which suggests that the conformational modulation mechanism of these metabolites is similar.

The results of this study are in line with the research conducted by Pauzan in 2025, where the results of his research show that moringa leaves appear as a promising natural agent with potential anti-inflammatory effects. Preclinical studies have shown that moringa leaves may reduce inflammation in various disease models, while several clinical trials have shown that moringa leaf supplementation may reduce inflammatory markers and improve symptoms in individuals with chronic inflammatory conditions such as osteoarthritis (Prabowo et al., 2024). Literature research conducted by Rambe and Yuniarti concluded that the use of moringa leaves

has a significant effect on inflammation. This is due to the content of flavonoids, quercetin, alkaloids, and saponins that have anti-inflammatory properties in moringa leaves. Moringa leaves have many benefits so it is recommended to be a candidate for natural ingredients developed into phytopharmaceuticals (Kashyap et al., 2022).

Overall, these findings suggest that the active metabolites of moringa leaves in the form of quercetin, kaempferol, isorhamnetin, luteolin, chlorogenic acid, ferulic acid, ellagaic acid, niazimnin, and moringin have potential as anti-inflammatory agents for OA through inhibition of the 5-LOX enzyme. In particular, ellagaic acid, kaempferol, isorhamnetin, and luteolin show high affinity at 5-LOX binding sites. However, the docking method is static in nature that only predicts the affinity of bonds in one ideal thermodynamic configuration and ignores the dynamic motion of proteins. In addition, molecular docking generally models ligand–receptor interactions under very ideal conditions with a simplified environment. This can lead to results that differ from the actual biological conditions within the cell or tissue. Therefore, experimental validation is needed to confirm the therapeutic potential of these moringa leaf metabolites against 5-LOX specifically, mainly through in vivo and in vitro assays.

CONCLUSION

Based on the results of the research that has been obtained, it can be concluded that: The active metabolite compounds of moringa leaves have a competitive affinity to orthosistic sites and pro-inflammatory protein allosteric sites 5-LOX. The best affinity at orthostatic sites was shown by ellagatic acid with a value of -7.7 kcal/mol lower than NDGA, while the best affinity at allosteric sites was shown by isorhamnetin with a value of -8.3 kcal/mol, but not exceeding AKBA. The active metabolite compounds of moringa leaves interact with the 5-LOX protein through hydrogen bonding and hydrophobic interactions with key residues that can inhibit catalytic activity. The interactions formed at the orthostec site were Phe359, Trp599, Leu368, His432, His600, and Arg596, while the interactions formed at the allosteric site were Arg138, Asp166, and Val110.

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