Molecular Docking Analysis of Major Bioactive Compounds from the Traditional Lom Pakang Formulation Against Cyclooxygenase-2 (COX-2)

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Abstract

Lom Pakang is a disorder recognized in Thai Traditional Medicine (TTM) and has traditionally been treated using a herbal formulation consisting of black pepper (Piper nigrum L.), fresh ginger (Zingiber officinale Roscoe), kaffir lime peel (Citrus hystrix DC.), Bermuda grass (Cynodon dactylon (L.) Pers.), and calcined alum. Although this formulation has long been used to relieve inflammatory symptoms, its molecular mechanism of action remains unclear. This study aimed to investigate the molecular interactions between major bioactive compounds from the traditional Lom Pakang formulation and cyclooxygenase-2 (COX-2) using molecular docking analysis. Eight major phytochemicals, namely piperine, piperettine, 6-gingerol, 6-shogaol, citronellal, limonene, apigenin, and luteolin, were selected based on previous phytochemical studies. Three-dimensional ligand structures were obtained from the PubChem database, whereas the crystal structure of COX-2 (PDB ID: 1CX2) was retrieved from the Protein Data Bank. Molecular docking was performed using ArgusLab 4.0.1, and ligand–protein interactions were analyzed using Discovery Studio Visualizer. All investigated compounds were successfully docked within the predicted binding pocket of COX-2. Among the tested compounds, 6-shogaol exhibited the lowest predicted binding energy (−12.70 kcal/mol), followed by piperettine (−11.30 kcal/mol) and 6-gingerol (−10.98 kcal/mol). Interaction analysis indicated that 6-shogaol formed hydrogen bonds with ALA527 and SER530 together with multiple hydrophobic interactions that may contribute to stabilization of the ligand–protein complex. These findings suggest that several bioactive compounds in the traditional Lom Pakang formulation, particularly 6-shogaol, possess favorable predicted interactions with the COX-2 active site and provide preliminary computational evidence supporting the anti-inflammatory potential of the formulation. Nevertheless, further experimental studies are required to validate these computational predictions.

Keywords: Lom Pakang formulation, Molecular docking, COX-2, Anti-inflammatory activity

Introduction

Lom Pakang is a disorder described in Thai Traditional Medicine (TTM), characterized by unilateral or temporal headache, dizziness, and a sensation of tightness around the head.

According to the Chowdara Scripture (Amatayakul, 2012), this condition has traditionally been treated using a herbal formulation consisting of black pepper (Piper nigrum L.), fresh ginger (Zingiber officinale Roscoe), fresh kaffir lime peel (Citrus hystrix DC.), Bermuda grass (Cynodon dactylon (L.) Pers.), and calcined alum. Although this traditional formulation has been widely used in Thai traditional medicine for generations, its molecular mechanism of action has not yet been clearly elucidated. Inflammation plays an important role in the pathophysiology of pain and headache. Cyclooxygenase-2 (COX-2) is an inducible enzyme responsible for catalyzing the conversion of arachidonic acid into prostaglandins, which are key mediators of inflammatory responses. Owing to its central role in inflammation, COX-2 has become one of the most extensively investigated molecular targets for the discovery of anti-inflammatory agents (Ricciotti & FitzGerald, 2011). Previous phytochemical and pharmacological studies have demonstrated that several constituents identified in the traditional *Lom Pakang* formulation possess anti-inflammatory properties. Piperine, the major bioactive alkaloid of *Piper nigrum*, has been reported to reduce oxidative stress and inflammatory responses in experimental models (Umar et al., 2013)**.** Likewise, the ginger constituents 6-gingerol and 6-shogaol have been shown to possess anti-inflammatory activity through the modulation of inflammatory signaling pathways associated with COX-2 and related mediators (Grzanna et al., 2005; Semwal et al., 2015)**.** In addition, limonene, one of the major volatile compounds of *Citrus hystrix*, has been reported to exhibit anti-inflammatory and antioxidant activities (Vieira et al., 2018). Furthermore, the flavonoids apigenin and luteolin have been widely reported to inhibit inflammatory responses through multiple cellular mechanisms (Kim et al., 2004). These findings suggest that the therapeutic effects of the traditional *Lom Pakang* formulation may arise from the synergistic actions of multiple bioactive compounds rather than from a single constituent.

Molecular docking is a widely used computational technique for predicting ligand–protein interactions and estimating the binding affinity of small molecules toward biological targets. It serves as an efficient preliminary screening tool for identifying potential bioactive compounds before experimental validation (Meng et al., 2011). Although molecular docking cannot replace biological experiments, it provides valuable molecular-level information regarding ligand binding orientation and interaction patterns within the active site of target proteins. Therefore, this study aimed to investigate the interactions between eight major bioactive compounds from the traditional Lom Pakang formulation and the COX-2 enzyme using molecular docking analysis. The findings are expected to provide preliminary computational evidence supporting the anti-inflammatory potential of the formulation and to contribute to future pharmacological and experimental investigations of Thai traditional herbal medicine.

Research Objectives

1. To investigate the molecular interactions between major bioactive compounds from the traditional Lom Pakang formulation and the cyclooxygenase-2 (COX-2) enzyme using molecular docking analysis.
2. To compare the predicted binding affinities and ligand–protein interaction patterns of the selected bioactive compounds in order to identify compounds with potential anti-inflammatory activity.

Scope of the Research

1. Population Scope

The study focused on eight major bioactive compounds identified from the traditional *Lom Pakang* formulation, namely piperine, piperettine, 6-gingerol, 6-shogaol, citronellal, limonene, apigenin, and luteolin. These compounds were selected based on previous phytochemical studies and investigated for their molecular interactions with the cyclooxygenase-2 (COX-2) enzyme using molecular docking analysis.

2. Variable Scope

The molecular interactions between the selected bioactive compounds and the COX-2 enzyme were evaluated using molecular docking analysis. The investigated variables included the predicted binding energy (binding affinity) and ligand–protein interaction patterns, including hydrogen bonding and hydrophobic interactions within the active site of COX-2.

3. Time Scope

The study was conducted from May to June 2026.

Literature Review

Traditional Thai herbal medicine has been extensively used for the treatment of pain and inflammatory disorders. The traditional *Lom Pakang* formulation, described in the *Chowdara Scripture*, consists of black pepper (*Piper nigrum* L.), fresh ginger (*Zingiber officinale* Roscoe), kaffir lime peel (*Citrus hystrix* DC.), Bermuda grass (*Cynodon dactylon* (L.) Pers.), and calcined alum. This formulation has long been prescribed in Thai Traditional Medicine for relieving symptoms associated with headache and inflammation. However, despite its long history of use, the molecular mechanisms underlying its therapeutic effects remain largely unexplored.

Previous phytochemical studies have identified several bioactive compounds in the medicinal plants used in the *Lom Pakang* formulation. Piperine and piperettine are the principal amide alkaloids found in *Piper nigrum*, whereas 6-gingerol and 6-shogaol are recognized as the major phenolic constituents of *Zingiber officinale*. Likewise, citronellal and limonene are major volatile compounds present in kaffir lime peel, while apigenin and luteolin are naturally occurring flavonoids that have been reported in several medicinal plants, including *Cynodon dactylon*. These compounds have been reported to possess antioxidant and anti-inflammatory activities through various biological mechanisms (Grzanna et al., 2005; Kim et al., 2004; Semwal et al., 2015; Umar et al., 2013; Vieira et al., 2018).

Cyclooxygenase-2 (COX-2) is an inducible enzyme that plays a central role in the biosynthesis of prostaglandins during inflammatory responses. Overexpression of COX-2 has been associated with numerous inflammatory disorders and pain-related conditions, making it an important molecular target for anti-inflammatory drug discovery. Several naturally occurring phytochemicals have been reported to interact with the COX-2 active site and may inhibit its catalytic activity through hydrogen bonding and hydrophobic interactions (Rajapaksha et al., 2024).

Molecular docking has become one of the most widely used computational approaches for investigating ligand–protein interactions in the early stages of drug discovery. This technique predicts the preferred binding orientation of small molecules within the active site of target proteins and estimates their binding affinity using computational scoring functions. Although molecular docking cannot confirm biological activity, it provides valuable preliminary information for prioritizing compounds for subsequent experimental studies (Meng et al., 2011).

Despite the growing number of studies on the anti-inflammatory properties of individual phytochemicals, molecular docking investigations focusing on the major bioactive compounds from the traditional *Lom Pakang* formulation remain limited. Therefore, this study aimed to evaluate the predicted interactions between eight major bioactive compounds from the formulation and the COX-2 enzyme using molecular docking analysis. The findings may provide preliminary computational evidence to support future pharmacological investigations of this traditional Thai herbal formulation.

Research Methodology

1. Research Methodology

This study employed an in silico molecular docking approach to investigate the molecular interactions between major bioactive compounds from the traditional *Lom Pakang* formulation and the cyclooxygenase-2 (COX-2) enzyme. Molecular docking analysis was performed using ArgusLab version 4.0.1 (Thompson, 2004) to predict the binding affinity and interaction patterns of the selected compounds with the COX-2 active site.

2. Research Steps

2.1 Eight major bioactive compounds, namely piperine, piperettine, 6-gingerol, 6-shogaol, citronellal, limonene, apigenin, and luteolin, were selected based on previous phytochemical studies of the traditional *Lom Pakang* formulation.

2.2 The three-dimensional (3D) structures of the selected ligands were obtained from the PubChem database (Kim et al., 2023), while the crystal structure of cyclooxygenase-2 (COX-2) (PDB ID: 1CX2) was retrieved from the Protein Data Bank (Berman et al., 2000). The crystal structure used in this study was originally reported by (Kurumbail et al.,1996).

2.3 The ligand structures and the COX-2 protein receptor were prepared prior to molecular docking. Water molecules and other non-essential molecules were removed from the receptor, and the binding pocket was defined according to the active site of the crystallographic ligand.

2.4 Molecular docking simulations were carried out using ArgusLab version 4.0.1 (Thompson, 2004) with the ArgusDock docking engine, employing the AScore scoring function in Regular Precision mode. The docking grid resolution was set to 0.4 Å, the binding site dimensions were 23.568 × 24.650 × 20.716 Å, and a maximum of 150 docking poses was generated for each ligand.

2.5 Ligand–protein interactions were visualized and analyzed using Discovery Studio Visualizer (Dassault Systèmes BIOVIA, 2021).

3. Data Collection

The molecular docking results were collected based on the predicted binding energy (kcal/mol) and ligand–protein interaction patterns. The recorded interactions included hydrogen bonds, hydrophobic interactions, and other non-covalent interactions between the ligands and amino acid residues within the COX-2 active site.

4. Data Analysis

The docking results were analyzed by comparing the predicted binding energy and ligand–protein interaction patterns of the selected compounds. Compounds exhibiting lower predicted binding energies together with favorable interactions with key amino acid residues in the COX-2 active site were considered to have greater potential for interaction with the target protein. The findings were interpreted as preliminary computational evidence and were not intended to confirm biological or pharmacological activity.

Research Results

Molecular docking analysis was successfully performed for all eight major bioactive compounds from the traditional *Lom Pakang* formulation against the cyclooxygenase-2 (COX-2) enzyme. The predicted binding energies obtained from ArgusLab are presented in **Table 1**. All compounds exhibited negative predicted binding energy values, indicating favorable predicted interactions with the COX-2 active site.

Among the investigated compounds, 6-shogaol exhibited the lowest predicted binding energy (−12.70 kcal/mol), followed by piperettine (−11.30 kcal/mol), 6-gingerol (−10.98 kcal/mol), limonene (−10.90 kcal/mol), luteolin (−10.33 kcal/mol), apigenin (−10.10 kcal/mol), piperine (−9.84 kcal/mol), and citronellal (−9.72 kcal/mol). These results suggest that all selected compounds have the potential to interact with the COX-2 binding pocket, although the predicted binding affinities varied among the compounds.

Table 1: Binding energy values of major bioactive compounds docked with the COX-2 enzyme.

Compound	Binding Energy (kcal/mol)
1. 6-Shogaol	-12.70
2. piperettine	- 11.30
3. 6-Gingerol	- 10.98
4. Limonene	-10.90
5. Luteolin	- 10.33
6. Apigenin	- 10.10
7. piperine	- 9.84
8. Citronellal	- 9.72

Ligand–Protein Interaction Analysis

The ligand–protein interaction patterns were further analyzed using Discovery Studio Visualizer**.** The results demonstrated that all compounds formed multiple non-covalent interactions within the COX-2 active site, including hydrogen bonds and hydrophobic interactions. Among the investigated compounds, 6-shogaol formed hydrogen bonds with ALA527 and SER530, together with several hydrophobic interactions involving amino acid residues surrounding the active site. These interactions may contribute to the stabilization of the ligand–protein complex. Similar interaction patterns were also observed for the other phytochemicals, although differences were found in both the number and type of molecular interactions.

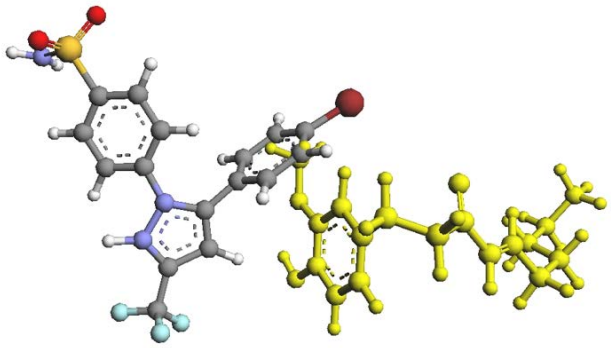
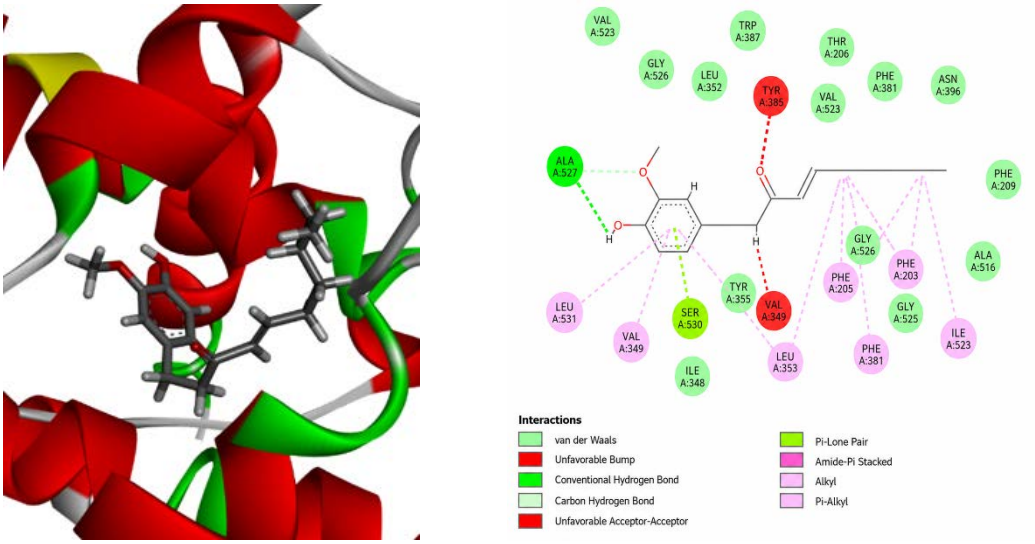


Figure 1: Three-dimensional docking pose of 6-shogaol within the active site of the cyclooxygenase-2 (COX-2) enzyme.

The three-dimensional docking pose showed that 6-shogaol was accommodated within the predicted binding pocket of the COX-2 enzyme (Figure 2). The ligand orientation was generally consistent with the interaction profile observed in the two-dimensional analysis, which may explain the favorable predicted binding energy obtained from the molecular docking simulation.



(A) (B)

Figure 2: Molecular docking analysis of 6-shogaol with the cyclooxygenase-2 (COX-2) enzyme showing (A) the three-dimensional binding pose within the active site and (B) the two-dimensional ligand–protein interaction diagram.

Table 2: Types of interactions between 6-shogaol and amino acid residues at the active site of the COX-2 enzyme.

Amino acid residue	Interaction type
ALA527	Conventional Hydrogen Bond
SER530	Carbon Hydrogen Bond, π-Lone Pair
VAL349	Alkyl, Unfavorable Acceptor–Acceptor
ILE348	Alkyl
LEU531	Amide–π Stacked
PHE203	π-Alkyl
PHE381	π-Alkyl
GLY520	π-Alkyl
GLY526	π-Alkyl
ILE523	π-Alkyl
TYR385	Unfavorable Bump

Table 2 summarizes the interactions between 6-shogaol and amino acid residues within the active site of the COX-2 enzyme. Hydrogen bonds with ALA527 and SER530, together with multiple hydrophobic interactions, may contribute to the stabilization of the ligand–protein complex and may explain the favorable predicted binding energy observed in the molecular docking analysis.

Overall, the molecular docking results suggest that 6-shogaol exhibited the lowest predicted binding energy among the investigated compounds and demonstrated favorable interaction patterns within the COX-2 active site. These findings provide preliminary computational evidence supporting its potential contribution to the anti-inflammatory activity of the traditional *Lom Pakang* formulation. However, further experimental studies are required to validate these computational predictions.

Discussion

The molecular docking analysis demonstrated that all eight major bioactive compounds from the traditional Lom Pakang formulation exhibited favorable predicted interactions with the cyclooxygenase-2 (COX-2) enzyme, as indicated by their negative predicted binding energy values. Among the investigated compounds, 6-shogaol exhibited the lowest predicted binding energy and favorable interaction patterns within the COX-2 active site, suggesting a stronger predicted binding affinity compared with the other selected compounds. These findings are consistent with previous studies reporting the anti-inflammatory properties of 6-shogaol and its ability to interact with inflammatory signaling pathways associated with COX-2.

The interaction analysis revealed that 6-shogaol formed hydrogen bonds with ALA527 and SER530, together with several hydrophobic interactions involving amino acid residues within the active site of COX-2. Hydrogen bonds are generally considered important for ligand recognition and binding orientation, whereas hydrophobic interactions contribute to stabilization of the ligand–protein complex. The combination of these interactions may explain the favorable predicted binding energy observed for 6-shogaol in the present study.

The remaining phytochemicals, including piperettine, 6-gingerol, limonene, luteolin, apigenin, piperine, and citronellal, also demonstrated favorable predicted binding energies and interaction patterns with the COX-2 enzyme. These findings suggest that the traditional Lom

Pakang formulation may exert its pharmacological effects through the combined actions of multiple bioactive constituents rather than a single active compound. Such multi-component interactions are consistent with the holistic principles of Thai Traditional Medicine, in which herbal formulations are designed to achieve complementary therapeutic effects through the combined activity of their constituents.

Although molecular docking is a valuable computational tool for predicting ligand–protein interactions (Meng et al., 2011), it has inherent limitations. The predicted binding energies and interaction patterns represent computational estimations and do not directly demonstrate biological activity or therapeutic efficacy. Therefore, the present findings should be regarded as preliminary computational evidence. Further validation through in vitro, in vivo, and clinical studies is required to confirm the anti-inflammatory activity and pharmacological mechanisms of the traditional Lom Pakang formulation.

Recommendations

1. Recommendations for the application of research results

The findings of this study provide preliminary computational evidence regarding the interactions between major bioactive compounds from the traditional Lom Pakang formulation and the COX-2 enzyme. These findings may serve as supporting information for future pharmacological studies and the development of traditional herbal formulations.

2. Recommendations for future research

Further studies are recommended to validate the molecular docking findings through in vitro, in vivo, and clinical investigations. In addition, advanced computational approaches, such as molecular dynamics (MD) simulations, may be employed to further investigate the stability of ligand–protein interactions.

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