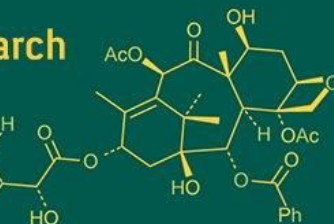
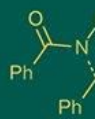
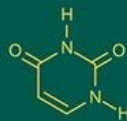
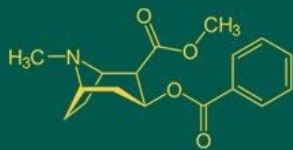


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In silico evaluation of Eugenol binding to COX-2 and TRPV1: Supporting traditional use in toothache relief

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Abstract

Clove (*Syzygium aromaticum*) is commonly used in traditional remedies for tooth pain, mainly because of eugenol-a key active compound it contains. Although studies show eugenol can reduce pain and swelling, how exactly it affects major pain-related proteins isn't fully clear yet. This research looks at how eugenol binds to two vital signaling proteins linked to inflammation and pain perception: COX-2 and TRPV1 channels. Computational docking methods were applied to explore these interactions in detail. Instead of ibuprofen, which served as control substance, eugenol was processed following routine ligand preparation steps. Protein structures (COX-2 from PDB ID 5IKR; TRPV1 from PDB ID 7LPB) underwent refinement before being analyzed. Active binding pockets within them were detected using Molegro Virtual Docker software tools. Docking results showed eugenol binds moderately-but meaningfully-to COX-2 (score: -74.46), mainly via contacts with Arg120, Tyr355, and Ser530. Meanwhile, it also engaged strongly with the TRPV1 site (score: -61.07), where Glu684, Ala680, and Gly683 helped stabilize the complex. By contrast, ibuprofen achieved a deeper binding effect on COX-2 (-99.99), aligning with its known therapeutic strength. Because eugenol affects both inflammation-related targets and pain signaling routes, it may explain its historical use in easing toothaches. Overall, these data position eugenol as a viable plant-derived candidate for pain relief, warranting future studies using refined simulations, energy analysis, along with lab testing.

Keywords: Eugenol, molecular docking, COX-2, TRPV1, natural analgesics, traditional medicine

1. Introduction

Clove (*Syzygium aromaticum*) has played a key role in Indian folk healing for generations, mainly to ease tooth pain and mouth swelling. In villages and tribal areas, people commonly use the fragrant buds at home for dental issues-either straight on the affected spot or through clove oil-to reduce sudden pain episodes. These uses appear throughout different parts of India, showing how clove remains a low-cost option for pain relief without drugs, particularly where professional dentistry isn't easily reached. Its ability to relieve pain and fight microbes comes mostly from eugenol-the main active compound-which acts fast to numb tissue, calm inflammation, and block harmful germs in the mouth.

Toothache usually comes from microbes invading, inflamed pulp, disrupted immunity, or triggered pain-sensing nerves. Instead of just bacteria like *Streptococcus mutans*, *Lactobacillus* species along with acid-loving germs cause decay; this gradually irritates the pulp, damages tissue, and uncovers nerve endings. When infection worsens, substances such as prostaglandins, bradykinin, plus cytokines build up in the pulp-making pain-sensitive nerves easier to trigger while increasing discomfort (Ahmad *et al.*, 2025) [2]. This heightened sensitivity grows stronger due to certain cellular signals that respond to harmful chemicals or temperature changes.

A main molecule involved in tooth pain is the TRPV1 ion channel, found on nerve cells in the dental pulp; this pathway lets positive ions flow when triggered. It responds to inflammation, low pH, or heat-factors common during cavities-which links it closely to sharp tooth discomfort. Computer-based studies suggest certain plant-derived substances-with structures like eugenol-affect TRPV1's active sites, reducing nerve activity and possibly numbing local areas (Santana *et al.*, 2024) [6]. In addition, large-scale modeling shows eugenol doesn't act on just one site: it alters both pain signals tied to TRPV1 and broader immune-related processes (Ahmad *et al.*, 2025) [2].

Alongside TRPV1-related pain signaling, inflammation from tooth diseases is largely controlled by the COX-2 protein. When pulp tissue becomes inflamed, COX-2 levels rise-leading to production of prostaglandins that cause blood vessel widening, swelling, plus increased sensitivity in nerve endings (Sharif *et al.*, 2025) [2]. Artificial NSAIDs, especially ibuprofen, are commonly given in dental care because they effectively block COX-2 while offering fast symptom reduction. For painful dental conditions linked to swelling, ibuprofen serves as a primary treatment option; sometimes paired with acetaminophen for discomfort after root canal procedures. Because of this, analyzing how eugenol interacts at the molecular level compared to ibuprofen offers a logical reference point when assessing its pain-relieving capacity.

Although widely used in traditional medicine and supported by increasing lab data, how exactly eugenol reduces pain and swelling is still unclear. To explore this, computer-based methods can help uncover its effects on major pain-linked proteins. Earlier work hints that eugenol attaches to certain enzymes and ion gates tied to inflammation and pain signals-however, side-by-side comparisons with standard painkillers are rare (Santana *et al.*, 2024; Ahmad *et al.*, 2025) [6, 2].

In this context, the current research uses molecular docking to explore how eugenol interacts with COX-2 and TRPV1-key players in inflammation and heat-related pain perception. As a benchmark, ibuprofen is used, offering a point of comparison for drug effects. Through analyzing binding strength, contact profiles, together with fit inside the target site, the study seeks to explain why clove has historically been applied for dental pain. It also supports the idea of eugenol as a plant-based option for pain relief.

2. Materials and Methods

2.1 Ligand Retrieval and Preparation

The chemical makeup of eugenol (PubChem CID: 3314) along with ibuprofen (PubChem CID: 3672), used as a comparison, came from the PubChem database. These

models were then moved into ACD/ChemSketch to create flat diagrams and check their structure. Their three-dimensional shapes were built next, followed by energy adjustments through the software's standard settings so they would reach stable forms ready for docking studies.

2.2 Protein Retrieval and Preprocessing

Two proteins linked to pain were chosen for docking studies Cyclooxygenase-2 (COX-2): PDB ID 5IKR

Transient Receptor Potential Vanilloid 1 (TRPV1): PDB ID 7LPB

Protein models came from the Protein Data Bank (PDB). Water molecules, ligands present during crystallization, along with extra heteroatoms, were deleted. Hydrogen atoms missing in structure files got inserted; their ionization forms adjusted accordingly. Binding pockets on proteins were detected via Molegro Virtual Docker's cavity prediction module.

2.3 Molecular Docking Procedure

Docking tests used Molegro Virtual Docker (MVD) software. Every ligand was placed into identified binding pockets through the tool's guided evolutionary search method. The procedure included these settings:

Iterations: 1500

Population size: 50

Amount of poses created for each ligand: 10

Scoring functions: MolDock Score and ReRank Score

After docking, the most stable position of every ligand was picked to study shape and binding behavior-using energy levels as a guide.

2.4 Binding Interaction and Visualization Analysis

Using MVD software, 3D binding modes of eugenol and ibuprofen in COX-2 and TRPV1 were displayed. Interaction diagrams in two dimensions helped spot hydrogen bonds, along with hydrophobic and van der Waals contacts. Important amino acids contributing to ligand stability were noted so that eugenol could be contrasted with ibuprofen.

Table 1: Overview of Materials and Methods Used in the Study

Step	Description	Tools/Databases Used
Ligand Retrieval	Chemical structures of eugenol and ibuprofen collected from public chemical databases.	PubChem
Ligand Preparation	2D drawing, verification, 3D generation, and energy minimization.	ACD/ChemSketch
Protein Retrieval	Download of COX-2 (5IKR) and TRPV1 (7LPB) structures.	Protein Data Bank (PDB)
Protein Preprocessing	Removal of water, heteroatoms; hydrogen addition; cavity identification.	Molegro Virtual Docker (MVD)
Docking Protocol	Docking using differential evolution and pose generation.	MVD Docking Engine
Scoring and Evaluation	MolDock and ReRank scoring; interaction analysis.	MVD Scoring Tools
Visualization	3D pose viewing and 2D interaction mapping.	MVD Visualization Tools

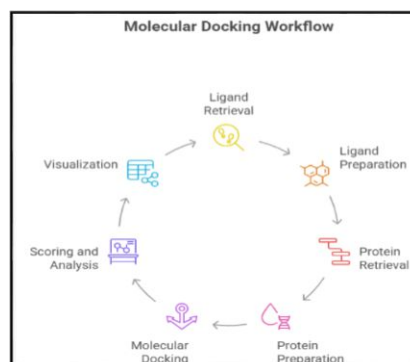


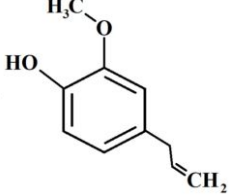
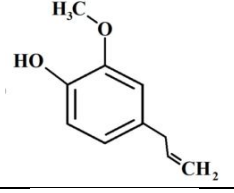
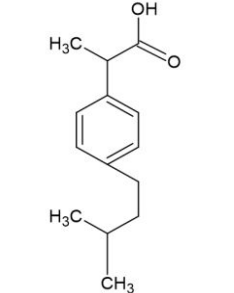
Fig 1: Molecular docking workflow for the evaluation of eugenol and ibuprofen against COX-2 and TRPV1.

The diagram shows the step-by-step process applied in this work-starting with obtaining ligands, followed by refining them, then acquiring the protein structure. Next comes preparing the protein, locating the binding site, performing docking simulations, ending with evaluation and visual analysis.

3. Results

The molecular docking results revealed how eugenol and ibuprofen bind to two major pain-related proteins, COX-2 and TRPV1. As shown in Table 2, the data includes binding scores, contact points, along with hydrogen bond details.

Table 2: Docking scores and interaction profiles of eugenol and ibuprofen with COX-2 and TRPV1

Ligand	Structure	Protein Target	MolDock Score	ReRank Score	H-Bond Score	Interacting Residues
Eugenol		COX-2 (PDB: 5IKR)	-74.4613	-58.4224	-4.03721	Arg120, Tyr355, Ser530
Eugenol		TRPV1 (PDB: 7LPB)	-61.0728	-61.0728	-2.5	Glu684, Ala680, Gly683
Ibuprofen		COX-2 (PDB: 5IKR)	-99.992	-82.3327	-4.78727	Tyr511, Glu600, Ser512

The docking data showed ibuprofen had the strongest attraction to COX-2, seen through its lowest MolDock value (-99.99) along with ReRank score. Although eugenol displayed weaker binding to COX-2 (-74.46), it still interacted with TRPV1 (-61.07), pointing toward activity across multiple targets. Important residues forming

hydrogen bonds or hydrophobic contacts within each ligand-protein pair are listed in Table 2. For better understanding of these molecular relationships, both 3D positioning diagrams and 2D interaction patterns for every complex appear afterward.

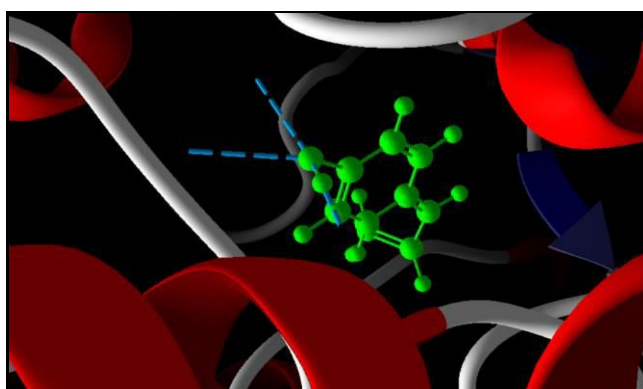


Fig 2A: 3D binding pose of eugenol within the COX-2 active site (PDB 5IKR).

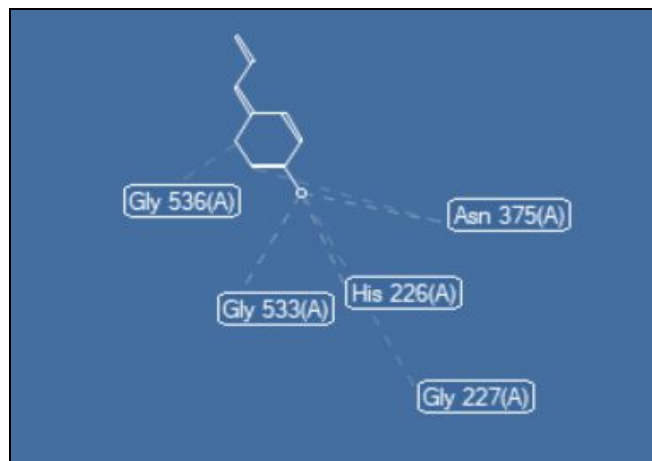


Fig 2B: 2D interaction map of the eugenol-COX-2 complex showing interactions with Arg120, Tyr355, and Ser530.

Eugenol fit into the catalytic pocket of COX-2, forming hydrogen bonds and hydrophobic interactions with Arg120, Tyr355, and Ser530, which contribute to ligand stabilization and explain its moderate binding score (MolDock: -74.46).

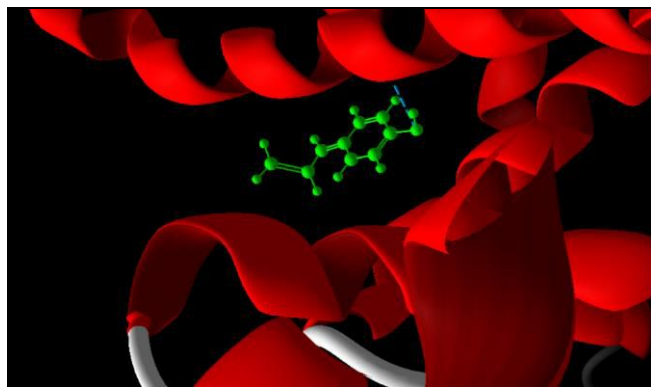


Fig 3A: 3D binding pose of eugenol within the TRPV1 binding pocket (PDB 7LPB).

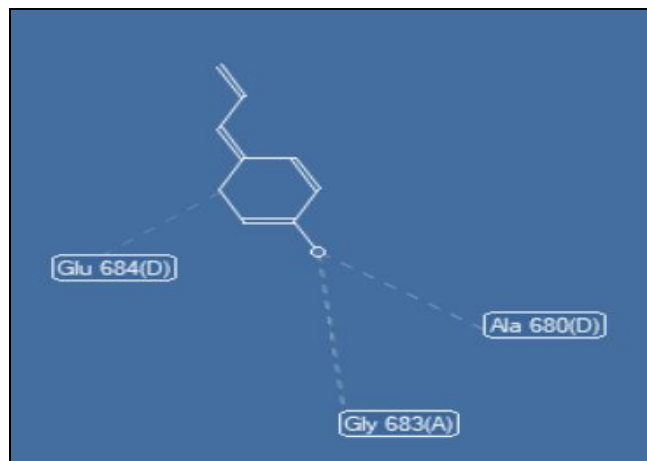


Fig 3B: 2D interaction map of eugenol-TRPV1 showing contacts with Glu684, Ala680, and Gly683.

Eugenol exhibited a favorable orientation within the vanilloid-binding region of TRPV1, forming stabilizing interactions that support its nociceptive-modulating effect.

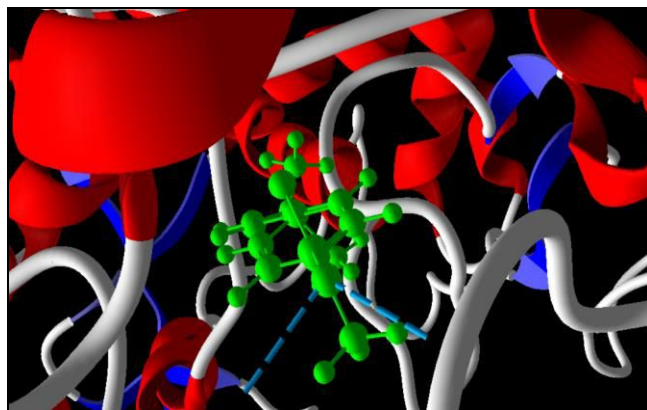


Fig 4A: 3D binding pose of ibuprofen within the COX-2 binding pocket (PDB 5IKR).

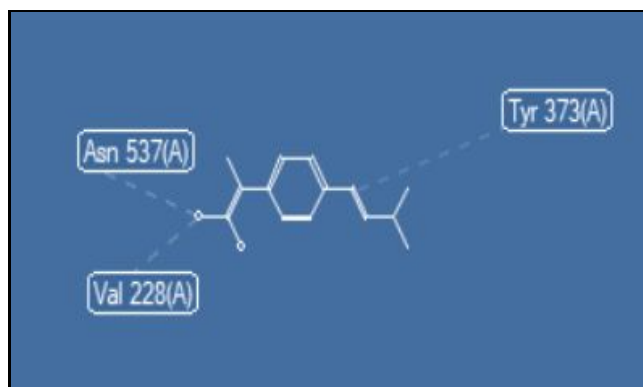


Fig 4B: 2D interaction map of ibuprofen-COX-2 showing interactions with Tyr511, Glu600, and Ser512.

Ibuprofen demonstrated the strongest affinity toward COX-2, consistent with its clinical potency as an NSAID. The interaction pattern supports deep insertion into the active site with multiple stabilizing contacts.

4. Discussion

The current molecular docking study helps explain why cloves have been traditionally used in Indian homes to relieve tooth pain. Instead of just tradition, eugenol-the main active ingredient in clove-shows measurable interaction with COX-2 and TRPV1, proteins linked to inflammation and

pain signals. While not extremely strong, this binding suggests a scientific basis for its effects. Therefore, the pain-relieving properties seen in everyday use are now backed by computer-based modeling.

In COX-2 docking, eugenol interacted with key amino acids-Arg120, Tyr355, Ser530-helping stabilize binding, which might block prostaglandin production; this could lessen inflammation-related discomfort. While its MolDock value fell short compared to ibuprofen's, the observed binding behavior matches earlier findings on anti-inflammatory effects. Thus, despite weaker strength than

man-made drugs such as ibuprofen, eugenol may still influence COX-2 activity naturally.

Besides reducing inflammation, eugenol attaches well to the TRPV1 channel, involved in sensing heat, pain signals, and harmful stimuli. The docking study detected contact points at Glu684, Ala680, and Gly683, suggesting it might influence the vanilloid site, explaining the quick numbness noted in folk remedies. Instead of just one pathway, combining COX-2 blocking with TRPV1 adjustment offers a clear reason for both fast and lasting relief when cloves are used for dental pain.

When compared to ibuprofen, results offered a useful reference point. Although ibuprofen showed much higher binding strength to COX-2, which aligns with its known role as an NSAID, it did not engage TRPV1 at all. In contrast, eugenol interacted with both targets. Because of this dual activity, natural substances may offer complex biological effects, suggesting why they've been used traditionally in healing practices.

The findings suggest traditional treatments aren't just cultural habits-they involve biological processes linked to current pain management methods. Instead of treating them separately, combining computer-based molecule analysis with folk medicine helps connect age-old insights with today's science.

5. Conclusion

The computer-based analysis from this work backs up historical use of clove and eugenol for relieving dental pain. Instead of just linking to one target, molecular modeling shows eugenol attaches to COX-2 along with TRPV1, hinting at combined pain-blocking and inflammation-reducing effects. Although ibuprofen binds more tightly to COX-2, eugenol also engages TRPV1, which adds another layer to how it may act. These results confirm that traditional Indian household treatments have a measurable biological basis while pointing to eugenol as a useful starting point for developing natural painkillers. Additional lab research is needed to test whether these benefits hold up in real-world medical settings.

6. Limitations and Future Scope

This research relies entirely on computer-based methods-offering predictions yet falling short in capturing full biological reality. While binding strength may suggest activity, it doesn't guarantee real-world effectiveness; instead, factors like uptake, availability, breakdown, or harmful effects remain unexamined. Further studies ought to explore these aspects

In vitro validation of COX-2 inhibition and TRPV1 modulation

In living organisms, research confirms pain-relieving effects as well as reduced inflammation

Improving plant compounds boosts how tightly they bind and work better as treatments

Assessment alongside alternative plant-based pain relievers

These studies could verify eugenol's healing effects while guiding its use in science-backed plant-derived treatments for pain.

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