World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

In Silico Analysis of BMP-2 And MMP-9 Interaction with Epicatechin, Cocamide, and Theobromine from Cocoa Bean (*Theobroma cacao L.*) Extract for Orthodontic Movement

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World Journal of Advanced Research and Reviews, 2026, 29(03), 220-228

Publication history: Received on 25 January 2026; revised on 03 March 2026; accepted on 05 March 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.29.3.0547

Abstract

Background: The high prevalence of malocclusion in Indonesia (80%) necessitates orthodontic treatment involving alveolar bone remodeling regulated by Bone Morphogenetic Protein-2 (BMP-2) and Matrix Metalloproteinase-9 (MMP-9). Bioactive compounds in cocoa beans (*Theobroma cacao L.*), such as epicatechin, theobromine, and clovamide, may modulate these proteins, offering potential therapeutic applications.

Objective: This in silico study aimed to predict interactions between cocoa-derived compounds (epicatechin, theobromine, clovamide) and target proteins (BMP-2, MMP-9).

Methods: A computational chemistry approach was employed. Ligand structures were minimized using PyRx 0.8, while protein structures (BMP-2 PDB: 6OMN; MMP-9 PDB: 5TH6) were prepared via PyMol. Molecular docking analyzed binding affinities (kcal/mol) and interaction types (hydrogen bonds, hydrophobic, van der Waals) using Discovery Studio v20. Simvastatin served as control.

Results: Bone Morphogenetic Protein-2 (BMP-2) activation: clovamide showed the strongest affinity (-6.5 kcal/mol), near simvastatin (-6.8 kcal/mol), forming hydrogen bonds with Trp31/Tyr103 and van der Waals interactions with key residues (Met89, Leu92). MMP-9 inhibition: clovamide (-7.3 kcal/mol) and epicatechin (-6.8 kcal/mol) outperformed simvastatin (-6.3 kcal/mol), binding critical residues (Arg51, Thr96, Gly186) via hydrogen bonds and electrostatic interactions.

Conclusion: Cocoa beans extract such a clovamide exhibits dual potential to activating BMP-2 and inhibiting MMP-9—making it a candidate for adjunctive orthodontic therapy. Further in vitro/in vivo validation is required to confirm these computational predictions.

Keywords: Epicatechin; Theobromine; Clovamide; Bmp-2; Mmp-9

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1. Introduction

Malocclusion remains one of the most prevalent dental problems in Indonesia, with an incidence reaching approximately 80%, making orthodontic treatment an essential therapeutic need. Orthodontic tooth movement involves a complex biological process characterized by alveolar bone remodeling regulated by molecular mediators such as Bone Morphogenetic Protein-2 (BMP-2) and Matrix Metalloproteinase-9 (MMP-9). BMP-2 plays a critical role in osteoblast differentiation and new bone formation at the tension site, whereas MMP-9 contributes to extracellular matrix degradation on the pressure side during tooth movement. The balance between the activation of BMP-2 and inhibition of MMP-9 is therefore crucial to ensure controlled remodeling and prevent unwanted complications during orthodontic treatment [1, 2, 3, 4].

Natural bioactive compounds from cocoa beans (*Theobroma cacao L.*) including epicatechin, theobromine, and clovamide have shown promising biological activities relevant to bone remodeling [5, 6]. Epicatechin has been reported to enhance osteoblastic activity through the upregulation of BMP-2 expression while simultaneously suppressing osteoclastic pathways associated with MMP-9. Clovamide exhibits strong antioxidant and anti-inflammatory properties, enabling potential inhibition of proteolytic enzymes such as MMP-9, whereas theobromine contributes to mineralization and osteoblast stimulation. These properties indicate that cocoa-derived compounds may support optimal periodontal tissue responses required during orthodontic tooth movement [7, 8].

In silico approaches, particularly molecular docking, provide an efficient preliminary method to predict the interaction strength and binding characteristics between ligands and target proteins prior to in vitro or in vivo experimentation [7]. Simvastatin, a widely studied molecule known to activate BMP-2 via the TGF-β/Smad pathway and inhibit MMP-9 expression, is commonly used as a positive control in molecular docking studies. Comparative docking analysis between cocoa bioactive compounds and simvastatin enables the prediction of their potential roles in modulating osteogenesis and matrix remodeling during orthodontic treatment. Such computational predictions can facilitate the identification of natural compounds with favorable binding affinities for future therapeutic applications. The purpose of this study is to analyze the predicted binding affinity and molecular interactions of epicatechin, clovamide, and theobromine from cocoa bean extract with BMP-2 and MMP-9 using in silico molecular docking to evaluate their potential in modulating osteogenesis and matrix remodeling in comparison with simvastatin [8, 9].

2. Material and methods

This study employed a descriptive analytic non-experimental design using a computational chemistry approach through in silico analysis. Molecular docking simulations were performed to evaluate the interaction between cocoa bean bioactive compounds epicatechin, clovamide, and theobromine—and the target proteins BMP-2 and MMP-9. The workflow included ligand preparation, protein selection, energy minimization, docking simulation, and visualization of molecular interactions. Ethical approval for this study was not required because the research did not involve human subjects, animals, or biological samples, and utilized only publicly available computational databases.

Ligand structures of epicatechin, clovamide, theobromine, and the control drug simvastatin were retrieved from the PubChem database in .sdf format. Each ligand was converted into .pdb format using PyMol and subsequently subjected to energy minimization using Open Babel integrated in PyRx 0.8 to obtain the most stable conformer. The ligand preparation process ensured that all chemical structures were optimized before docking to increase accuracy in binding affinity prediction. The final optimized ligands were then imported into AutoDock Vina within PyRx for docking analysis.

Protein structures of Bone Morphogenetic Protein-2 (BMP-2; PDB ID: 6OMN) and Matrix Metalloproteinase-9 (MMP-9; PDB ID: 5TH6) were obtained from the RCSB Protein Data Bank All water molecules and non-essential heteroatoms were removed using PyMol to ensure that only the active protein chains were used for docking. Grid box parameters were determined based on the active binding site of simvastatin, allowing docking simulations of all ligands to occur within the same active region. Docking results were analyzed using Discovery Studio Visualizer to identify binding affinity values, hydrogen bonds, hydrophobic interactions, electrostatic interactions, and amino acid residues involved.

3. Results

This study evaluated the binding affinity and molecular interactions of epicatechin, clovamide, and theobromine with BMP-2 and MMP-9 through molecular docking. The docking results showed that all ligands successfully bound to the same active site as the control drug simvastatin, indicating that the selected grid box accurately captured the functional binding region of both target proteins. Clovamide demonstrated the strongest overall binding activity among the tested

cocoa compounds, particularly against MMP-9, where it exceeded the affinity of simvastatin. The complete binding affinity values obtained from docking simulations are presented in Table 1.

Table 1 Binding Affinity (kcal/mol) of Ligands Against BMP-2 and MMP-9

Ligan (compound)	**Binding affinity (kcal/mol)**	
	BMP-2	MMP-9
Simvastatin	-6.8	-6.3
Clovamide	-6.5	-7.3
Epicatechin	-5.7	-6.8
Theobromine	-4.1	-5.7

Docking simulations with BMP-2 revealed that clovamide had the most favorable interaction, with a binding affinity of −6.5 kcal/mol, approaching the control drug simvastatin. Visualization results showed that clovamide, epicatechin, and theobromine shared several key amino acid residues in the BMP-2 active site, including Ile32, Met89, Tyr91, and Trp31, which contributed to hydrophobic and hydrogen bonding interactions. Clovamide formed both hydrogen bonds and van der Waals interactions with residues such as Trp31, Leu92, Tyr103, Met89, Tyr91, and Val33, as seen in Figure 1. Meanwhile, epicatechin and theobromine also interacted with similar residues, although with fewer stabilizing hydrogen bonds compared to clovamide.

Table 2 Binding Site Visualization of BMP-2

Protein	**Type of bond**	**Simvastatin**	**Clovamide**	**Epicatechin**	**Theobromine**
BMP-2	Van der Waals	Asp30 Met89 Glu94 Asp93 Lys101 Tyr103	Ile32 Val33 Met89 Tyr91 Leu92 Tyr103	Ile32 Met89 Tyr91	Trp31 Ile32 Met89 Tyr91
	Conventional hydrogen bond	-	Trp31	Leu92	Tyr103
	Carbon hydrogen bond	Trp31	-	-	-
	Pi-Pi T-shaped	-	Trp28	-	-
	Pi-Sigma	Trp28	-	-	Trp28
	Pi-Alkyl	Ile32 Tyr91	-	-	-
	Unfavorable Donor-Donor/Aceptor-Aceptor	-	-	Trp31	-
	Pi-Donor Hydrogen	-	-	Trp28 Tyr103	-

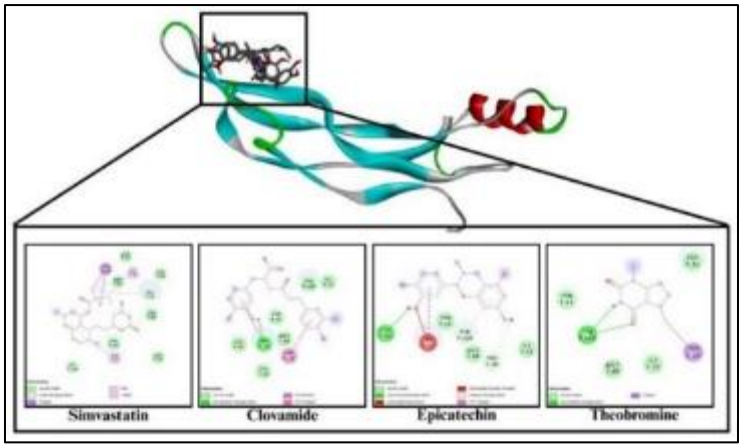

Figure 1 Visualization of Docking simulations with BMP-2

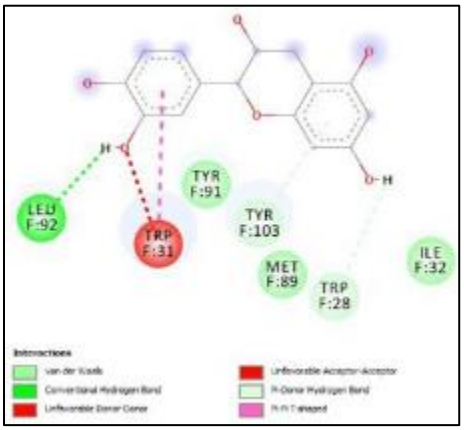

Figure 2 Docking simulations BMP-2 With Epicatechin

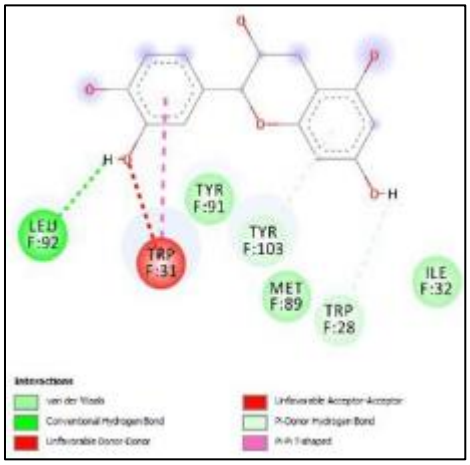

Figure 3 Docking simulations BMP-2 With Cocamide

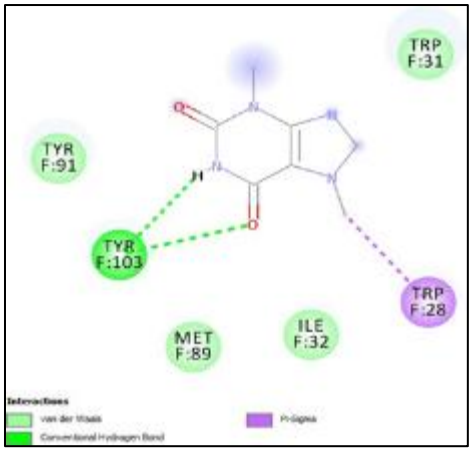

Figure 4 Docking simulations BMP-2 With Theobromine

Docking results with MMP-9 demonstrated that clovamide exhibited the strongest binding affinity (–7.3 kcal/mol), followed by epicatechin (–6.8 kcal/mol), both outperforming simvastatin. Clovamide interacted extensively with multiple active-site residues, including Glu47, Tyr52, Met94, Pro97, Asp182, Asp185, Lys184, Arg51, and Gly186, forming both hydrogen bonds and hydrophobic interactions. Epicatechin similarly bound to critical catalytic residues such as Arg51, Tyr48, Thr96, Pro97, and Gly186, contributing to its strong affinity. Theobromine exhibited the weakest binding among the ligands, with limited hydrogen bonding and dominance of van der Waals interactions, as illustrated in Figure 2.

Table 3 Binding Site Visualization of MMP-9

Protein	Type of bond	Simvastatin	Clovamide	Epicatechin	Theobromine
MMP-9	Van der Waals	Glu47 Arg51 Met94 Pro97 Asp182 Lys184 Asp185 Leu187	Leu44 Glu47 Tyr52 Met94 Arg95 Pro97 Arg98 Asp182 Gly183 Asp185	Leu44 Tyr48 Tyr50 Met94 Arg95 Thr96 Pro97 Arg98 Gly186 Leu187	Leu44 Glu47 Tyr48 Arg98 Gly186
	Conventional hydrogen bond	-	Arg51 Thr96 Lys184 Gly186	Glu47 Tyr52	Arg51 Tyr52 Asp182 Leu187
	Carbon hydrogen bond	Gly186	Tyr48	-	Met94
	Pi-Sigma	-	-	-	-
	Pi-Alkyl	Tyr48 Tyr52	-		-
	Pi-Cation	-	-	Arg51	-

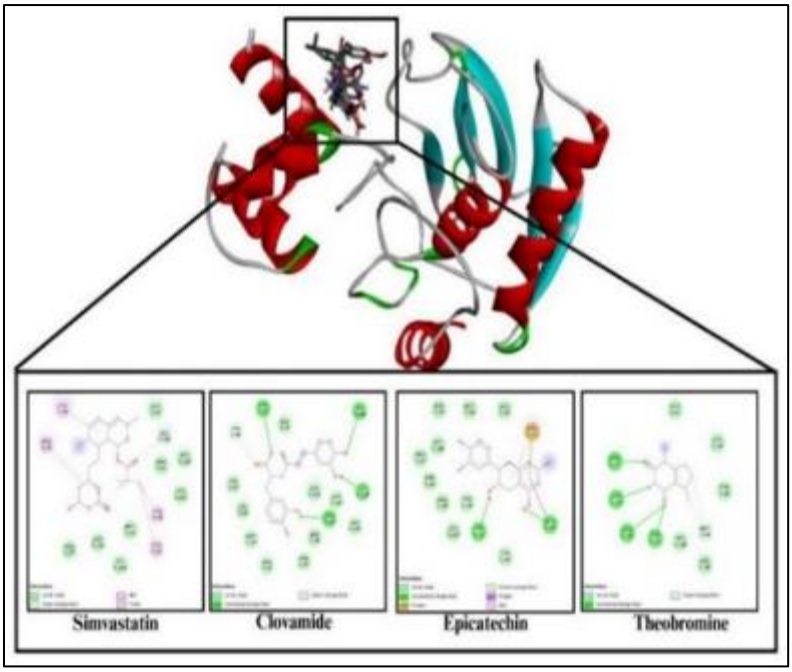

Figure 5 Visualization of Docking simulations with MMP-9

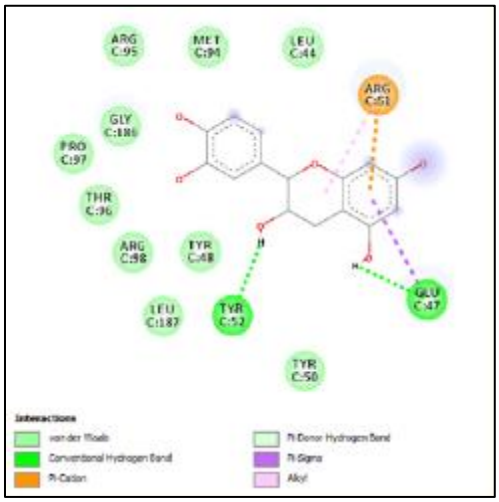

Figure 6 Docking simulations MMP-9 With Epicatechin

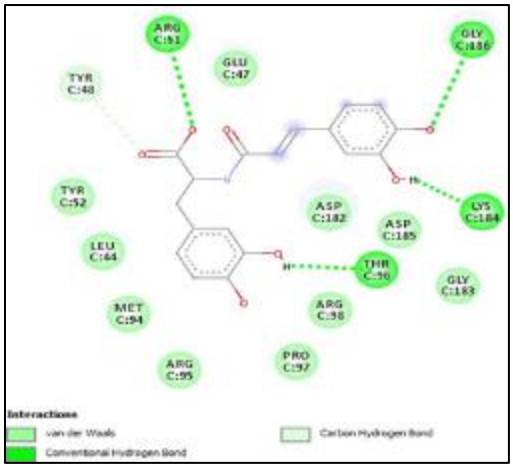

Figure 7 Docking simulations MMP-9 With Cocamide

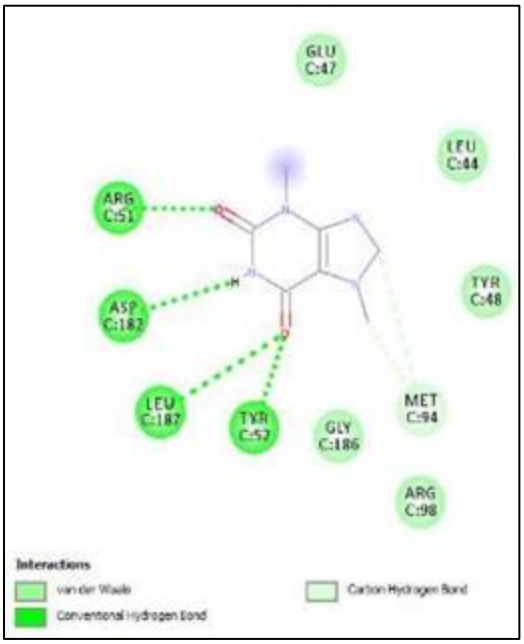

Figure 8 Docking simulations MMP-9 With Theobromine

4. Discussion

The results of this study demonstrate that clovamide, epicatechin, and theobromine from cocoa bean extract exhibit varying degrees of molecular interaction with BMP-2 and MMP-9, indicating their potential biological relevance in orthodontic bone remodeling [10]. Clovamide consistently showed the strongest binding affinity among the tested ligands, particularly toward MMP-9, suggesting its superior capability to modulate proteolytic and inflammatory processes during periodontal tissue remodeling. The shared interaction of these ligands with key amino acid residues in BMP-2 and MMP-9 active sites further supports their structural compatibility with both protein targets. These findings highlight the significance of cocoa-derived phytocompounds as potential adjunctive agents in optimizing orthodontic tooth movement [11, 12].

Clovamide's strong affinity toward MMP-9 may be attributed to its phenolic structure, which facilitates hydrogen bonding and stabilizing hydrophobic interactions within the enzyme's catalytic region [13]. This ability to bind deeply within the active pocket, especially at residues such as Arg51, Glu47, Met94, and Gly186, implies a potential inhibitory effect that could reduce excessive matrix degradation during orthodontic force application [14]. Epicatechin also

displayed high binding affinity to MMP-9, reinforcing previous evidence that flavonoids can suppress osteoclastic activity through modulation of matrix metalloproteinases [15]. Meanwhile, theobromine showed weaker interactions and limited hydrogen bonding, indicating that its bioactivity may be less pronounced in pathways involving MMP-9 regulation [16, 17].

Regarding BMP-2 interaction, clovamide exhibited the highest affinity among the cocoa compounds by forming hydrogen bonds and van der Waals interactions with residues critical for osteogenic signaling. Although its affinity was slightly lower than simvastatin, clovamide's interaction pattern suggests it may emulate osteogenic activation pathways similar to statins, particularly through stabilization of the BMP-2 binding domain [17, 18]. Epicatechin and theobromine also exhibited favorable interactions with BMP-2, although with fewer stabilizing bonds, reflecting their supportive yet milder potential in modulating osteoblast activity [19]. These findings imply that cocoa phytocompounds, especially clovamide, may contribute to enhanced osteogenesis and balanced remodeling when used as natural adjuncts in orthodontic therapy [20]. The findings of this study conclude that clovamide, epicatechin, and theobromine from cocoa bean extract exhibit notable binding interactions with BMP-2 and MMP-9, with clovamide demonstrating the strongest dual potential for enhancing osteogenesis and inhibiting matrix degradation, suggesting that cocoa-derived bioactive compounds may serve as promising natural candidates to support controlled and efficient orthodontic tooth movement [21].

5. Conclusion

The bioactive compounds epicatechin, theobromine, and clovamide in cocoa bean extract (*Theobroma cacao L.*) bind to BMP-2 through molecular docking, with clovamide exhibiting the strongest binding and theobromine exhibiting the weakest. Cocoa seed extract has strong potential to interact with BMP-2, approaching the efficacy of simvastatin in activating the osteogenesis pathway. The bioactive compounds epicatechin, theobromine, and clovamide in cocoa bean extract (*Theobroma cacao L.*) exhibit binding with MMP-9 via molecular docking, with the strongest binding observed for clovamide and epicatechin and the weakest for theobromine. Clovamide and epicatechin demonstrate very strong potential as MMP-9 inhibitors, even surpassing simvastatin, and indicate the potential of these compounds in reducing excessive bone resorption during orthodontic treatment.

Compliance with ethical standards

Acknowledgments

This study was supported by an internal grant from the University of Jember.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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