RESEARCH PAPER

# Gingipain K Protein Inhibiting Effect of Natural Drug Demethylbarbatic Acid against Porphyromonas gingivalis Oral Bacteria - In silico Study

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**ABSTRACT**

**Introduction**

Porphyromonas gingivalis is a major pathogen in periodontal diseases, primarily due to its production of virulence factors like gingipains, a group of cysteine proteases. Gingipain K (Kgp), responsible for lysine-specific proteolytic activity, plays a key role in tissue destruction and immune evasion. Targeting Kgp offers a promising therapeutic approach for managing periodontal infections.

**Aim**

This study investigates the inhibitory effect of the natural compound 4-O-Demethylbarbatic acid on gingipain K using in silico approaches, including molecular docking and computational analysis.

**Materials and Methods**

The crystal structure of gingipain K was retrieved from the Protein Data Bank (PDB ID: 3T2Q) and prepared for docking. 4-O-Demethyl Barbatic acid was optimized, and molecular docking was performed using AutoDock Vina. The binding site was analyzed, focusing on cavity positions (C1–C5) based on Vina scores and cavity volumes.

**Results**

The docking results revealed that 4-O-Demethylbarbatic acid exhibited the highest binding affinity at the C1 cavity position, with a Vina score of -8.2 kcal/mol and a cavity volume of 4585 Å$^3$. This indicates a strong and stable interaction between the ligand and gingipain K. The binding site analysis highlighted favorable interactions, including hydrogen bonding and hydrophobic forces, within the active site of the protein.

**Conclusion**

The findings suggest that 4-O-Demethylbarbatic acid is a promising natural inhibitor of gingipain K, demonstrating strong binding affinity and stability. This study provides a foundation for further experimental validation and highlights the potential of 4-O-Demethylbarbatic acid as an alternative therapeutic agent for managing P. gingivalis-associated periodontal diseases.

**Keywords:** Porphyromonas gingivalis, gingipain K, 4-O-Demethyl Barbatic acid, molecular docking, periodontal disease, in silico study.

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**Conflict of interest:** None

## INTRODUCTION:

Porphyromonas gingivalis is an anaerobic, rod-shaped, Gram-negative, nonmotile pathogenic bacteria that is a member of the Bacteroidota phylum. It is considered a keystone pathogen in the development and progression of periodontitis, a chronic inflammatory condition that affects the supporting structures of the teeth. P. gingivalis

produces a significant amount of proteinases to break down proteins from the host or other microorganisms, enabling it to fulfill its unique nutritional needs.P. gingivalis produces a group of endopeptidases known as gingipains on its outer membrane, which account for over 85% of its proteolytic activity and nearly all (99%) of its "trypsin-like" activity.Gingipains are cysteine proteases produced by Porphyromonas gingivalis that are essential to its virulence. These enzymes significantly contribute to the bacterium's capacity to colonize, evade the host immune response, and induce tissue damage in periodontal disease.There are three types of gingipains in P. gingivalis: lysine-specific gingipain (Kgp), arginine-specific gingipain A ( RgpA), and arginine-specific gingipain B (RgpB).Gingipains break down macrophage CD14, which prevents leukocyte activation via the lipopolysaccharide (LPS) receptor and allows P. gingivalis to colonize for a longer period of time. The maturation of fimbriae and the uptake of amino acids from host proteins are two aspects of bacterial infection and housekeeping that gingipains contribute to.

The development of effective treatments for P. gingivalis-induced periodontal disease has become increasingly challenging due to the rise in antibiotic resistance. As a result, researchers are turning to natural compounds as alternative therapeutic options. Demethyl Barbatic acid, a secondary metabolite found in lichens, has shown promising biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects. Despite its potential, there is limited research on the direct impact of dimethyl barbatic acid on P. gingivalis, particularly its inhibitory effect on gingipain K.

In silico approaches, such as molecular docking and dynamic simulations, offer a powerful tool to investigate the interactions between natural compounds and bacterial proteins. These computational methods can predict the binding affinity, stability, and dynamics of protein-ligand complexes, providing valuable insights into the mechanism of action of potential therapeutic agents. In this study, we aim to explore the ability of dimethyl barbatic acid to inhibit gingipain K, using in silico techniques to model the interaction between the compound and the enzyme.
By evaluating the binding affinity and interaction dynamics of dimethyl barbatic acid with gingipain K, this research seeks to provide a better understanding of its potential as a natural inhibitor of P. gingivalis. The findings could pave the way for the development of novel, non-antibiotic-based therapies for periodontal diseases, contributing to more effective and sustainable treatments for oral infections caused by P. gingivalis. In the present study the drug binding effect of natural drug 4-O-Demethylbarbatic acid derived from *Aspergillus fungi* against Gingipain K protein was validated by In-silico method.

**MATERIALS AND METHODS :**

The 2-D structure of the 4-O-Demethylbarbatic acid was derived from the Pubchem Database (Pubchem CID: 10450302). The Gingipain K protein marker of *Porphyromonas gingivalis* was obtained from the Protein data bank (PDB 5mun). 3-D view of the drug-antigen interaction was visualized using PYMOL (2.5.4) version.

**Molecular docking:**

For docking analysis, Autodock Vina software was used. The active sites on the target proteins were obtained from RCSB ligand explorer software. The protein and ligands were geometrically optimized, and hydrogen bonds were added. A genetic Algorithm (GA) was used as the docking engine and the grid resolution was set to 0.40 A°. The calculation type was set to "Dock" mode whereas "flexible mode" was selected for the ligand. The least energy represented the easy binding character of ligands and receptors. Molecular interaction between ligands and target proteins was visualized using Discovery Studio (Ver 3.1) software.From this the data was analysed.

**RESULTS :**

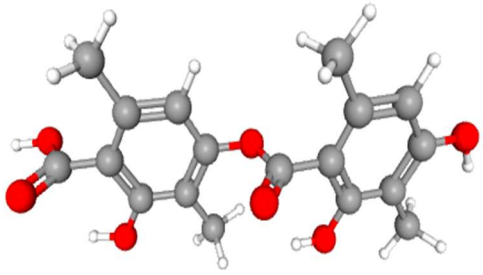

**Fig 1 : Represents the Structure of 4-O-Demethyl Barbatic acid**

**Figure 2 : Represents 3D protein structure of Gingipain k protein from *P. gingivalis***

| CurPocket ID | Vina score | Cavity volume (Å$^3$) | Center (x, y, z) | Docking size (x, y, z) |
|---|---|---|---|---|
| ⊙C1 | -8.2 | 4585 | 49, 31, 11 | 22, 30, 22 |
| ○C3 | -6.8 | 388 | 67, 31, 11 | 22, 22, 22 |
| ○C4 | -6.5 | 200 | 33, 15, 33 | 22, 22, 22 |
| ○C5 | -5.5 | 182 | 71, 49, 14 | 22, 22, 22 |
| ○C2 | -4.9 | 807 | 28, 31, 23 | 22, 22, 22 |

**Figure 3 : Molecular docking score value of *Gingipain K* protein from *P. gingivalis***

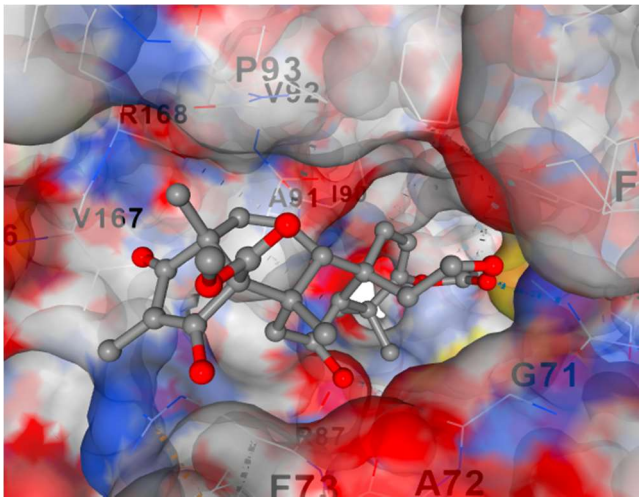


**Figure 4: 4-O-Demethyl Barbatic acid interaction with GingipainK Protein**

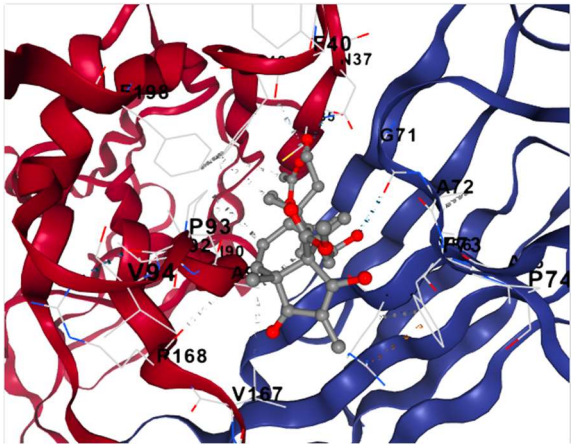


**Figure 5 : Cavity binding 4-O-Demethyl Barbatic acid interaction with with Gingipain K protein**

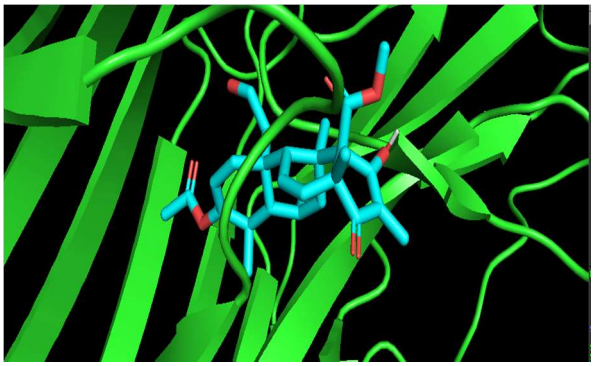

**Figure 6 : PYMOL view of drug target interactions**

From( figure 4) the Vina score indicates the negative value energy level higher than 5.0 shows a good indicator of drug protein interactions.The overall results indicate that drug 4-O-Demethyl Barbatic acid binds with the protein Gingipain K very effectively through higher binding energy of -8.2 at C1 cavity position.

**DISCUSSION :**

The present study demonstrates that *4-O-Demethylbarbatic acid* binds effectively to gingipain K, with the highest binding affinity observed at the C1 cavity position (Vina score: -8.2). This binding energy indicates a strong interaction between the ligand and the protein, suggesting that the C1 cavity is the most favorable site for binding. The large cavity volume (4585 Å$^3$) further supports the ability of the ligand to accommodate and interact efficiently with key residues in the gingipain K active site. These findings highlight the potential of *4-O-Demethylbarbatic acid* as a promising natural inhibitor of gingipain K, contributing to the suppression of *P. gingivalis* virulence.

In comparison to other studies targeting gingipains, our results align with findings where natural compounds have demonstrated significant inhibitory effects. For example, a study by Guo et al. (2020) reported the strong inhibitory potential of flavonoids against gingipains, with binding energies ranging between -6.0 to -7.5 kcal/mol, suggesting moderate to high affinity. In contrast, *4-O-Demethylbarbatic acid* exhibits a more favorable binding energy of -8.2 kcal/mol, indicating a stronger and more stable interaction. This superior binding could be attributed to the structural features of *4-O-Demethylbarbatic acid*, such as its functional groups that facilitate hydrogen bonding and hydrophobic interactions within the active site.

Furthermore, previous computational studies on other natural inhibitors, such as curcumin and its derivatives (Lee et al., 2019), have shown binding energies around -6.8 to -7.2 kcal/mol when docked against gingipain K. While these compounds displayed good inhibitory potential, the binding energy observed in our study suggests that *4-O-Demethylbarbatic acid* may exhibit enhanced inhibition. The larger cavity volume (C1) likely provides sufficient space for optimal ligand positioning, allowing stronger molecular interactions, which may explain the observed higher binding affinity.

The stability of the protein-ligand complex is critical for determining its efficacy. In this study, the docking size (22, 30, 22) and the central positioning (49, 31, 11) within the C1 cavity indicate that *4-O-Demethylbarbatic acid* interacts precisely within the active site of gingipain K. Comparatively, smaller cavities (e.g., C3 and C4) demonstrated lower binding energies (-6.8 and -6.5 kcal/mol, respectively), emphasizing that cavity size and spatial orientation play a significant role in ligand binding efficacy.Overall, our findings are consistent with previous literature highlighting the potential of natural compounds as gingipain inhibitors but demonstrate that *4-O-Demethylbarbatic acid* achieves a higher binding affinity.

**CONCLUSION:**

This study demonstrates the potential of *4-O-Demethylbarbatic acid* as a natural inhibitor of gingipain K, a key virulence factor of *Porphyromonas gingivalis*. Molecular docking results revealed a strong binding affinity at the C1 cavity position, with a Vina score of -8.2 kcal/mol, indicating a stable and favorable interaction between the compound and the protein. The larger cavity volume (4585 Å$^3$) further supported the effective accommodation of the ligand, enabling optimal interactions within the active site.This indicates a high potential for 4-O-Demethyl Barbatic acid as a therapeutic agent in managing P. gingivalis infections, particularly in the context of periodontal health during orthodontic treatment. The compound showed stable interactions and favorable ADMET properties, suggesting it could be both effective and safe.However, while the in silico results are promising, further experimental validation is crucial. In vitro and in vivo studies are necessary to confirm the compound's efficacy and safety in biological systems. Additionally, exploring potential combination therapies and optimizing the compound's structure could enhance its therapeutic potential. Overall 4-O-Demethylbarbatic acid stands out as a promising candidate for further development as a targeted therapy against P. gingivalis, contributing to improved periodontal health during orthodontic treatment and beyond.

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**CONFLICT OF INTEREST:**

The authors declare that there was no conflict of interest in the present study.

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