Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Harnessing the antimicrobial potential of *Aegle marmelos* against Mfa1 fimbriae in *Porphyromonas gingivalis*: a new strategy for endodontic therapy

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Article Info

Article history:

Received: October 13, 2024
Accepted: December 18, 2024
Published: January 31, 2025

Use your device to scan and read the article online

Abstract

Endodontic infections, primarily caused due to microbial invasion into the root canal system, pose a significant challenge to dental health and management due to their complex etiology and resistance to conventional treatment. *Porphyromonas gingivalis* is a key bacterium pathogen that uses the Mfa1 fimbriae for its adhesion and biofilm formation contributing to its pathogenicity. The study explores the potential of Aegle marmelos leaf metabolites as a potential inhibitor of Mfa1 fimbriae using the molecular docking and simulation approach. We assessed the binding affinities of various metabolites with Rutin emerging as a promising candidate due to its strong and stable interactions within the Mfa1 active sites. The findings are also supported by existing literature that underscores the anti-microbial and anti-inflammatory properties of Aegle marmelos and its phytochemicals. The study also highlights the novelty of targeting Mfa1 fimbriae, a structure not addressed by current therapeutics.

Keywords: Endodontic, Mfa1, Aegle marmelos, Phytochemicals, Simulation, Therapeutics.

1. Introduction

Endodontic Infections are inflammatory diseases majorly caused due to microbial invasion into the oral root canal system, which results in a significant impact on dental health due to their diverse and complex etiology leading to severe complications [1]. Endodontic infection can be classified into two categories (i) Primary and (ii) Secondary infection with primary infection being caused when tooth pulp is infected and colonized by oral microbes, whereas secondary is mostly caused due to persistent root canal infection following endodontic treatment or extension to introduction or retention during the initial treatment of primary [2]. Among the disparate microflora responsible for these infections *Porphyromonas Gingivalis*, a Gram-Negative anaerobe commonly associated with periodontal infections, is known to play a crucial role in aggravating the endodontic infection. Its disease-causing potential is partly accredited to its fimbrial structure, especially Mfa1 fimbriae, which ensure adhesion to host tissues and other oral bacteria leading to biofilm formation and persistence in Oral Cavity [3,4].

The Mfa1 fimbriae is composed of various proteins, with it serving as main and crucial structural component [5]. These fimbriae play important role in its ability to adhere to synergistic species such as streptococci, through its interaction with antigen I/II [6]. This adhesion is crucial for its colonization and contributing role towards endodontic infection.3,4 Mfa1 is also known to play a crucial role in immunomodulation and potential impact on the progression of endodontic diseases by interacting with TLRs, especially TLR4 which is essential for immunomodulatory effect [7]. Therefore, understanding the role of Mfa1 is important as it attracts its potential as therapeutic target for intervention. Currently, there are no specific drugs or molecules known that target Mfa1. This gap in therapeutics necessitates research into new agents that can specifically inhibit Mfa1 function.

The developing resistance of oral pathogens to antibiotics treating periodontal infections also impacts the management of endodontic infections and demands increasing need for the exploration of alternative treatment strategies [8,9]. Bioactive molecules, especially those extracted from medicinal plants offer a promising direction for development of new antimicrobial drugs. *Aegle marmelos*

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Doi: http://dx.doi.org/10.14715/cmb/2025.70.1.10

is a plant with rich history in traditional medicines, the leaves are well known to contain a variety of bioactive phytochemicals, including phenols, flavonoids and terpenoids which have demonstrated significant antimicrobial, anti-inflammatory, and anti-oxidant activities [10,11]. These properties suggest *Aegle Marmelos* metabolites may serve as effective agents against endodontic pathogens such as *P.gingivalis*. The incorporation of bioactive molecules from natural products not only aligns with the current trend toward holistic and patient-centered care but also offers a viable solution for overcoming the challenges associated with existing therapeutic approaches.

The current study aims to use molecular docking and Dynamic simulations to investigate the potential of *Aegle marmelos* leaf metabolites as inhibitors of Mfa1 fimbriae. We seek to identify a potential candidate that could enhance the efficacy of endodontic treatments, due to lack of molecules that specifically target Mfa1 underscores the need for continued research in this area to improve treatment outcomes and combat antibiotic resistance.

2. Material and methods

2.1. Bioactive molecule of *Aegle Marmelos*, and structure preparation

The leaf bioactive molecules of *Aegle marmelos* were retrieved from IMPPAT database (https://cb.imsc.res.in/imppat/)[12]. The 3D and prepared using the Ligprep module of Schrodinger (LigPrep, Schrödinger, LLC, New York, NY, USA, 2024-1). Ligprep was used to correct improper bond distance and bond orders and generate ionization states and perform energy minimizations. The Three-dimensional crystal structure of Mfa1 (PDB ID: 5NF2). All the structures were optimized prior to docking and any structural inconsistencies were corrected for the same.

2.2. Molecular docking and MM-GBSA refinement

Molecular studies were performed using Schrödinger Maestro Suite 2024-1(Schrödinger, LLC, New York, NY). The active site for docking was predicted using the Schrödinger SiteMap module. The predicted site was further used to generate the receptor grid. Post docking MM-GBSA refinement was carried out on the docked poses with a flexible residue at a distance of 5Å.

The binding energy was calculated by the following formula:

$\Delta G = \Sigma(\Delta GBind\ coulomb + \Delta GBind\ covalent + \Delta GBind\ H\text{-}bond + \Delta GBind\ Lipo + \Delta GBindpacking + \Delta GBind\ Self\ cont + \Delta GBind\ Solv\ GB + \Delta GBind\ vdw)$

2.3. Molecular Dynamics (MD) Simulation

MD simulation was performed using a DESMOND. The molecular system was built with protein-ligand complex immersed in an orthorhombic box of SPC solvent model. The solvated system was neutralized using counter ions and physiological NaCl salt concentration of 0.15 M. OPLS4 force field was utilized. The simulation was 100 ns using NPT assemble class at a temperature of 300 K and atmospheric pressure of 1.013 bar.

3. Results

3.1. Molecular docking and analysis

Mfa1 fimbria consists of shaft, mfa1 and three tip proteins (Mfa3-5). The MFa1 consists of two β-sandwich domains and a long loop that connects the first β strand with the second referred to as β1β2-loop with arginine residue exposed. This arginine acts as a recognition site for RgpA/B an Indigenous protease that plays a role in cleaving the loop which further enables removal of the first β strand resulting in the formation of mature protein with an empty position for β-strand that's donated from other fimbrial proteins leading to formation of polymeric fimbria. Mfa1 also contains Trp residue at position 554 which marks the starting of flexible c-terminal firmly anchored [13]. The putative site predicted use resulted in Dscore (Drugabillty Score) and SiteScore of 1.044 and 1.04 suggesting that following site can be used for drug targeting. The molecular docking of Leaf metabolites of *Aegle Marmelos* to site resulted in ligands exhibiting diverse binding free energies ranging from -10.88 to -67Kcal/mol. The Top 10 ligands and their Binding Free Energies are given in Table 1.

2'-beta-D-glucopyranosyloxy-marmesine (IMPHY005166) is a glycosylated derivative of marmesin, which belongs to the class of furanocoumarins. These compounds are recognized for their role in the biosynthesis of furanocoumarins and are known for their anticancer, antioxidant, and antimicrobial activities [14,15]. The Docking of 2'-beta-d-glucopyranosyloxy-marmesine resulted in highest binding free energy of -67.84. At the active site of Mfa1 it was found to be stabilized by the 6 hydrogen bonds with amino acid residues Glu66, Gly69, Lys70, Gly 194, Glu198 (Figure1A). In addition to hydrogen bonds, the ligand also established hydrophobic interactions with the amino acid residues Trp67 and Thr195, as well as a salt bridge interaction with Lys70. These interactions contribute to the increased binding free energies of the ligand. However steric clashes were also observed with amino acid residues Glu66, Glu198 and Val352 which may impact its stability and efficacy. Similarly, Rutin (IMPHY015047) also known as quercetin-3-rutinoside, belongs to flavonoids is well known for its diverse bioactivities. Its found to have strong antioxidant potential helping in neutralizing of free radicles [16,17. Rutin, is also known to possess Vaso protective and anti-inflammatory effects [18]. The rutin was found to bind to Mfa1 with binding free energies of -65.95Kcal/mol. At the active site rutin was found to be stabilized by the stabilized by 16 hydrogen bonds with amino acid residues such as Asn181, Thr184, Lys191, Lys192, Gly 194, Glu327, Asp328, Thr 339, Asn358, lys363, Phe364 (Figure 1B). In Addition to hydrogen bonds two hydrophobic interaction were found with amino acid residue 327. Similarly, few steric clashes

Table 1. Binding Free Energies of leaf Bioactive compounds against Mfa1.

Bioactive Compound	ΔGbind (Kcal/mol)
IMPHY005166	-67.84
IMPHY015047	-65.95
IMPHY006258	-61.14
IMPHY013674	-55.73
IMPHY012464	-49.42
IMPHY014836	-49.42
IMPHY016552	-49.37
IMPHY014838	-48.63
IMPHY015072	-48.14
IMPHY015022	-47.36

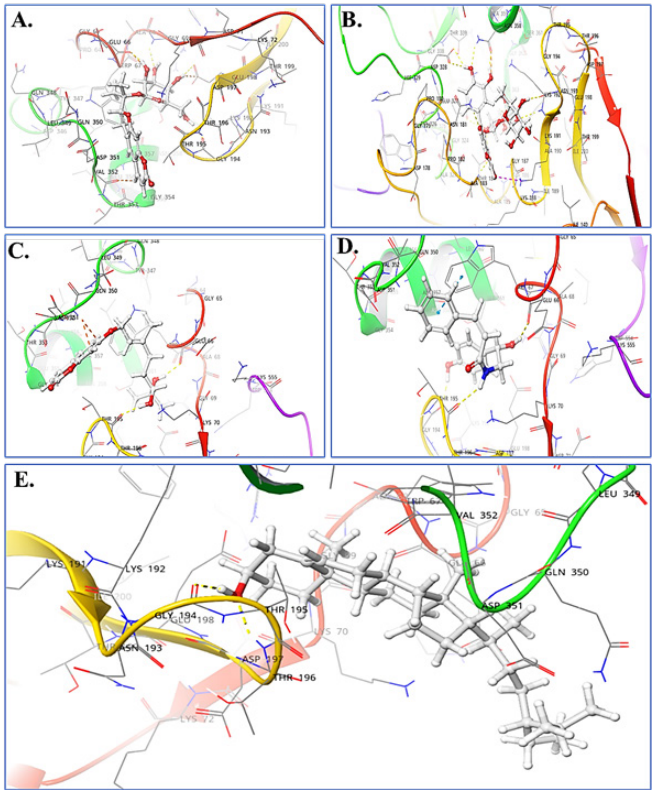

Fig. 1. (A) 2'-beta-d-glucopyranosyloxy-marmesine at the Active site of Mfa1. (B) Rutin at the active site of Mfa1 (C) Marmin at the active site of Mfa1 (D) N-[2-Hydroxy-2-(4-hydroxyphenyl)ethyl] cinnamide at the active site of Mfa1 (E) Clionastero at the active site of Mfa1.

with Asn358 and Phe 364 were also observed. Marmin (IMPHY006258), belong to class of coumarin compounds known for its smooth muscle relaxation by inhibiting histamine receptors, thus preventing the release of histamine from mast cells [19]. Which further leads to its anti-ulcer and anti-allergic activities [19]. At the active site of Mfa1 marmin was found to bind with free binding energies of -61.14Kcal/mol and was found to be stabilized by hydrophobic interaction compared to hydrogen bond.with amino acid residues Glu66, Trp67, Lys70,Glu198, Val352(Figure 1C).In addition to dominant hydrophobic interaction four hydrogen bonds were also observed with amino acid residues Glu66,Lys 70, Thr169 and Asp351. Single steric clash was observed with Asp351. The dominance of hydrophobic interaction over the hydrogen bonds may show decreased stability overtime.

N-[2-Hydroxy-2-(4-hydroxyphenyl)ethyl] cinnamide (IMPHY013674) is a cinnamide derivative recognized for its antimicrobial properties, which disrupt microbial membranes and essential microbial enzymes. Additionally, it exhibits anti-cancer activities, antioxidant effects, and neuroprotective benefits [20-23].

At the active site of Mfa1, similar to marmin, it was found to be stabilized by five hydrogen bonds with the amino acid residues Glu66, Gly69, Lys70, Gly194, and Thr195, contributing to its binding free energies (Figure 1D). Additionally, hydrophobic interaction with amino acid residues Trp67, Glu198, and Val352 along with π-stacking interaction with the amino acid residue Trp67 was observed.

Clionasterol (IMPHY012464) is a terpenoid known for its diverse biological activities, including inhibition of the classical pathway, antioxidant properties, anti-inflammatory effects, and antimicrobial activities [24-26]. At the active site of Mfa1, clionasterol was found to be stabilized majorly by hydrophobic interactions with amino acid residues Glu66, Trp67, Thr195, Glu198, and Gln350 (Figure 1E). In addition to hydrophobic interaction, clionasterol was found to be stabilized by three hydrogen bonds with amino acid residues Gly194, Thr196, and Asp197.

To further elucidate the stability and understand the conformational changes that occur on binding of ligands and to get a better insight into the Ligand – protein complexes the top three poses were further subjected to molecular dynamic simulations

3.2 Molecular dynamic simulations

The structure stability of docked posed of 2'-beta-d-glucopyranosyloxy-marmesine, Rutin, and Marmin was subjected to 100 molecular dynamic studies. Structural parameters such as RMSD, RMSF, Rg and Intermolecular Hydrogen Bonds were studied.

3.3 Root mean square deviation (RMSD) analysis

The obtained MD simulation results were analyzed using Cα and ligand RMSD values. RMSD values indicate the stability of the complex. The comparison of 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1, Rutin-Mfa1 and Marmin-Mfa1 RMSD revealed that the deviation observed in the Cα backbone atom were quite similar and the deviation values were less than 2 Angstrom towards the end of simulation (last 20ns) overall the average RMSD values throughout the simulations were found to be less than 2.5Angstrom which are well within the acceptable range27. However, at the binding pocket, the ligand RMSD in case of 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1 and Marmin-Mfa1 were found to be higher than any acceptable range. In marmin-Mfa1 complex reached maximum Ligand RMSD value greater than 10Angstrom. The ligand was found to be stable during the initial 50ns with few increased deviations around 20ns and 40ns.

However, post 50ns the ligand deviation kept increasing till the end of simulation. Similarly, in the 2'-beta-D-glucopyranosyloxy-marmesine-Mfa1 complex, the ligand's RMSD was notably higher from the start of the simulations, with values ranging from 30 to 64 angstroms during the initial 40 ns. After this period, the deviation stabilized, averaging around 30 angstroms. Both the complex is highly unstable and unlikely to be targeted. However, in Rutin-MFa1 complex, the average ligand RMSD value was less than 2.5 throughout the simulation. Therefore, it can be considered that Rutin can form stable complex and potentially show better in vitro efficacy (Figure 2 A, B and C).

3.4. Root mean square fluctuations

Root mean square fluctuation (RMSF) measures the flexibility of the protein backbone throughout the simulation. A comparison between the 2'-beta-D-glucopyranosyloxy-marmesine-Mfa1 complex and the Marmin-Mfa1 complex revealed that both protein systems exhibited similar behavior, with no significant changes in the flexibility of the protein backbone upon ligand binding (Figure 3 A, B and C).

3.5. Radius of gyration (Rg)

Rg is known to indicate the compactness and impact

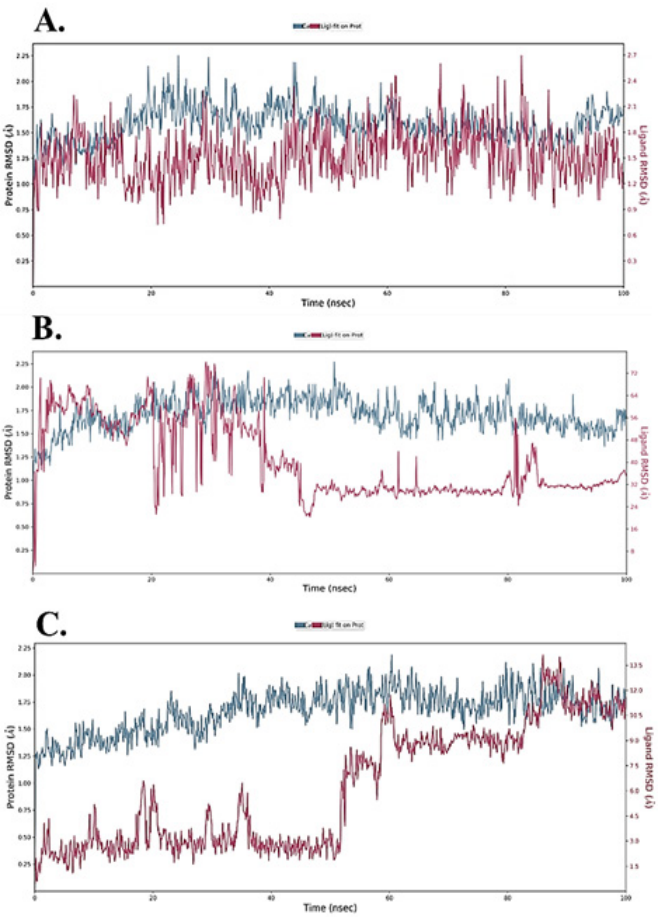

Fig. 2. ((A) RMSD graph of Rutin-Mfa1 complex. (B) Protein-Ligand RMSD of 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1 Complex. (C) Protein-Ligand RMSD of Marmin-Mfa1.

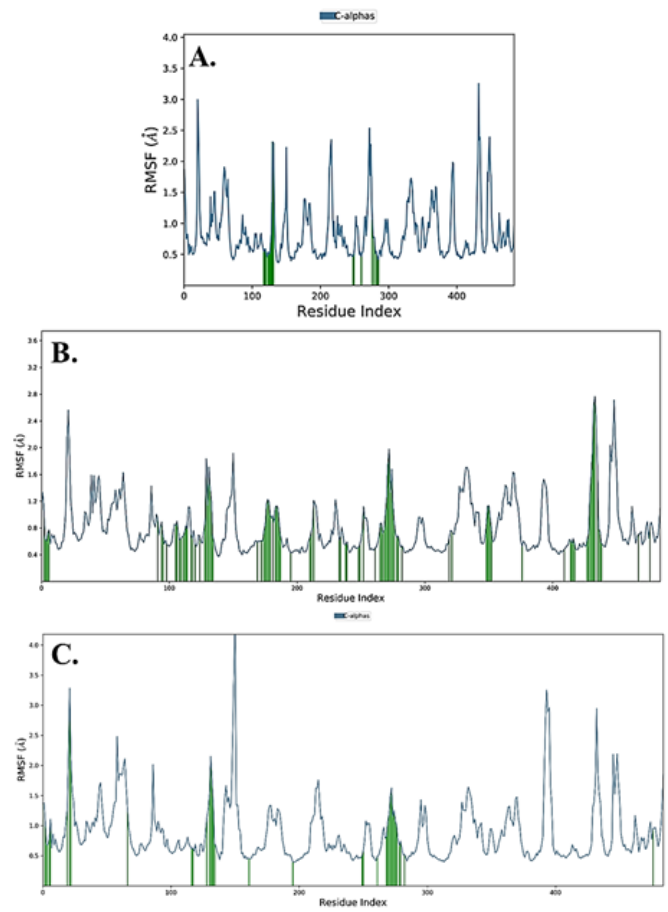

Fig. 3. (A) RMSF graph of C-alpha atoms of Mfa1-rutin complex. (B) Protein RMSF of 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1 Complex. (C) Protein RMSF Marmin-MFA1 complex.

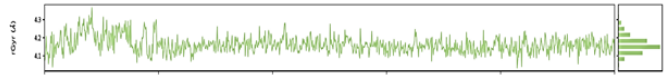

Fig. 4. Radius of Gyration of Rutin-Mfa1 complex.

of ligand binding on protein folding during simulations. The comparison between the complex indicates that Rg value in the 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1 maintained Rg value of around 4 Angstrom for initial 20ns however slight increases were found post 20ns. In the case of Rutin-Mfa1 complex the Rg Values showed slight increase initially and converged after 20ns to 4.1Angrstom at the later part of the simulation (Figure 4). Where as incase of Marmin-Mfa1 complex the Rg values kept on fluctuating till 50ns post which convergence was observed although the Rg values were found higher than any other complex.

3.6. Intermolecular hydrogen bonds across simulation

Hydrogen bond interactions and formations between the ligand and the active site of the protein are essential for understanding the binding affinity of these molecules. The analysis revealed that both the 2'-beta-D-glucopyranosyloxy-marmesine-Mfa1 and Marmin-Mfa1 complexes were unable to maintain a high number of contacts with their respective ligands, with a total of fewer than four contacts during most of the simulation. In contrast, the Rutin-Mfa1 complex exhibited a higher number of contacts, maintaining around five contacts throughout the simulation (Figure 5 A and B).

The overall Molecular dynamic analysis suggests that Rutn-Mfa1 complex exhibits strong binding affinity towards the Mfa1 and therefore may also show superior in-vitro activity. Due to its strong binding, stability towards the Mfa1. The inappropriate use of existing therapies not only leads to resistance but also increases the likelihood of adverse effects and costs. Therefore, there is a strong motivation to explore new therapeutic targets and interventions. While rutin has demonstrated effective efficacy in vitro against endodontic infections, the development of new molecules targeting Mfa1 should continue to be pursued.

4. Discussion

The current study presents a novel approach to targeting Mfa1 of *Porphyromonas gingivalis* using phytochemicals to address endodontic infections. This is a significant study as Mfa1 plays a crucial role in bacterial adhesion and biofilm formation, which remains the key factor responsible for its pathogenicity. The novelty of the work lies in the identification and investigation of using bioactive molecules of leaf extract of *Aegle marmelos*, particularly Rutin, as a potential inhibitor for Mfa1, which to date has not been specifically targeted by any existing therapeutic agents [4]. The work also aligns with the growing interest in using phytochemicals to develop new antimicrobial strategies, especially in context of targeting and evading antibiotic resistance [28].

The findings of the study are supported by existing

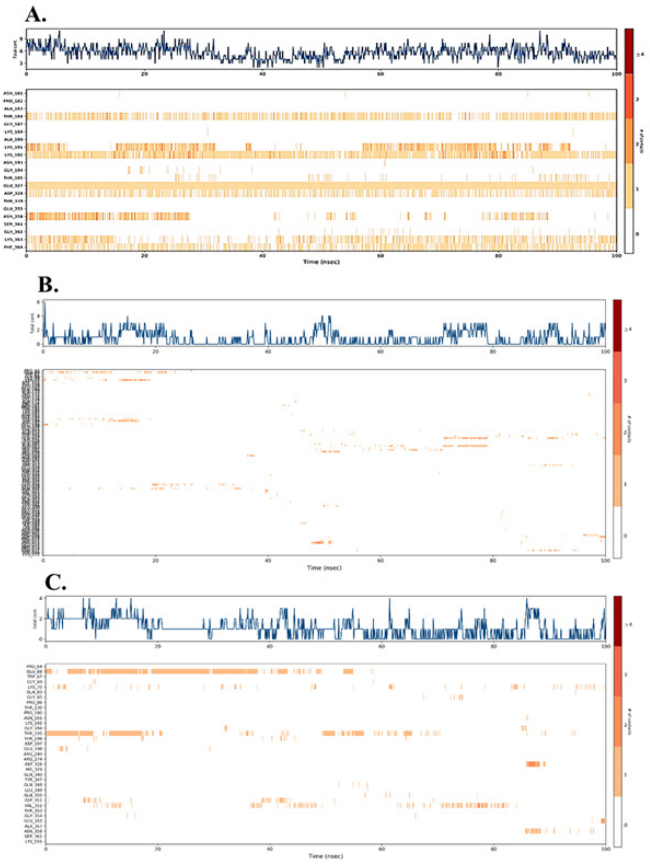

Fig. 5. (A) Hydrogen Bonds contact timeline of Rutin-Mfa1 complex. (B) Hydrogen Contact timeline 2'-beta-d-glucopyranosyloxy-marmesine-Mfa1 Complex. (C) Hydrogen bond Contact Timeline Marmin-Mfa1complex.

literature that highlights the crucial role played by Mfa1 fimbriae in adhesion, colonization and biofilm formation of *P.gingivalis* 4,29. Previous research has elucidated on structural and functional aspects of Mfa1, and its role in the bacterium's pathogenicity and its potential to act as a therapeutic target 5. However, there have been drugs specifically targeting Mfa1, which underscores the novelty and importance of this study in filling the gap. Furthermore, *Aegle marmelos* is well documented for its known anti-microbial and anti-inflammatory properties, which further supports our hypothesis of potential efficacy of its metabolites in combating oral pathogens [30, 31].

While in-silico results are promising, the study acknowledges the need for further in-vitro and in vivo experimentational validation. This would confirm the efficacy of Rutin or compounds sharing similarity to its backbone in inhibiting Mfa1-mediated adhesion and biofilm formation. Future research should also explore the potential synergistic effect of these natural compounds in synergy with existing treatments to enhance their therapeutic efficacy. By addressing this area, this study not only contributes to understanding endodontic infection but also opens new avenues for development of alternative therapies that can help mitigate challenges posed due to antibiotic resistance.

5. Conclusion

The study highlights the potential of *Aegle marmelos* leaf metabolites, especially rutin as a potential candidate for targeting Mfa1 fibiriae in *P. gingivalis*, offering a new avenue for the treatment of endodontic infections. Addressing the limitation and pursuing further research, could contribute to overcoming the challenges posed by antibiotic resistance and improve the outcome in dental health and hygiene.

Acknowledgments

The authors gratefully acknowledge the funding of the Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, through Project Number: GSSRD-24.

Consent for publication

The authors agree the final manuscript for publication.

Ethics approval and consent to participate

The present research did not involve the use of humans or animals.

Conflicts of interest

The authors declare no conflicts of interest.

Supplementary materials

The supporting information regarding results is shown in supplementary data with legends.

Author's contribution

N.B: Conceptualization, supervision, original drafting and funding. N.E.M.J: Methodology and Primary drafting, and editing, Data validation and formal. M.B: Analysis, Investigation and Data Curation., H.S.O and A.S.A: Writing review of literature and editing, H.A and S.M.J: Visualization and supervision. R.Q.W and A.A.A: Project administration. H.A: Funding acquisition. HA and SAH: Writing review of literature and editing.

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