In Silico Molecular Docking Analysis of Carrageenan Against Oral Pathogenic Proteins

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ABSTRACT:

INTRODUCTION:A class of naturally occurring linear sulfated polysaccharides known as carrageenans or carrageenins are taken out of red edible seaweeds. Because of their ability to stabilise, thicken, and gel, carrageenans are frequently employed in the food business.

MATERIALS AND METHOD:The 2D structures of carrageenan compounds were drawn using ChemDraw and energy minimized using Chem3D software .

RESULTS:Molecular docking scores and residual amino acid interactions of compound (3) against HmuY (heme-binding protein) of *Tannerella forsythia* (PDB ID – 6EU8), gingipain R protein of *Porphyromonas gingivalis* (PDB ID – 1CVR), and *Enterococcus faecalis* (PDB ID – 4M7U).

CONCLUSION: The present docking study suggests that carrageenan compounds may exhibit potential antimicrobial activity against oral pathogenic proteins. Further in vitro and in vivo studies are necessary to validate their therapeutic efficacy and safety.

Keywords: carrageenan ,molecule,*Tannerella forsythia*

INTRODUCTION:

Carrageenan is a naturally occurring sulfated polysaccharide extracted from red seaweeds such as *Chondrus crispus*, commonly known as Irish moss. It has been widely used for centuries in

traditional food preparations due to its excellent gelling, thickening, emulsifying, and stabilizing properties. (1)In modern food industries, carrageenan is commonly incorporated into dairy products, processed meat, infant formula, desserts, toothpaste, pharmaceutical formulations, and non-dairy milk products. Unlike gelatin, carrageenan is plant-derived and therefore suitable for vegetarian and vegan formulations.(1,2)

Chemically, carrageenans are linear polysaccharides composed of alternating units of β-D-galactopyranose linked by α-D-galactopyranose through 1,3 and 1,4 glycosidic bonds. Depending on the degree and position of sulfation, carrageenans are classified into kappa (κ), iota (ι), and lambda (λ) forms, each possessing different physicochemical properties. Kappa carrageenan forms strong rigid gels, iota carrageenan forms elastic gels, while lambda carrageenan acts mainly as a thickening agent due to its inability to gel. The biological activity and industrial applicability of carrageenan largely depend on these structural differences.(3)

Carrageenan has gained considerable attention not only because of its industrial importance but also because of its diverse biological and pharmacological activities. Previous studies have reported antiviral, anticoagulant, antioxidant, antitumor, immunomodulatory, and antimicrobial properties of sulfated polysaccharides isolated from marine algae.(4) Carrageenan exhibits potential therapeutic effects through regulation of oxidative stress pathways, modulation of inflammatory mediators, and interaction with cellular signaling mechanisms.(3,5)

In recent years, marine-derived bioactive compounds have attracted significant interest in dentistry and medical research because of the increasing prevalence of antimicrobial resistance among pathogenic microorganisms. (6)Oral pathogens such as *Porphyromonas gingivalis* and *Tannerella forsythia* play a major role in periodontal tissue destruction. *P. gingivalis* produces virulence factors such as gingipains, which are cysteine proteases involved in immune evasion, tissue invasion, and bacterial survival. Similarly, HmuY heme-binding protein in *T. forsythia* contributes to iron acquisition and pathogenicity. *Enterococcus faecalis*, another clinically important microorganism, is frequently associated with persistent endodontic infections and treatment failures due to its high resistance and biofilm-forming ability. Therefore, identifying natural compounds capable of interacting with these microbial proteins may contribute to the development of novel therapeutic agents.(7)

Molecular docking has become an important computational technique in drug discovery for predicting ligand–protein interactions and estimating binding affinity. It helps in identifying potential bioactive compounds by evaluating the interaction between ligands and target proteins at the molecular level.(8) Docking studies provide valuable insight into hydrogen bonding, hydrophobic interactions, and binding energies that influence biological activity. In the present study, carrageenan compounds were evaluated against selected bacterial target proteins including HmuY protein of *Tannerella forsythia* (PDB ID: 6EU8), gingipain R protein of *Porphyromonas gingivalis* (PDB ID: 1CVR), and *Enterococcus faecalis* protein (PDB ID: 4M7U) to assess their antimicrobial potential.(4,9)

Although carrageenan is approved as a food additive by regulatory agencies such as the U.S. Food and Drug Administration (FDA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA), its safety profile remains controversial. Several studies have reported conflicting findings regarding its effects on gastrointestinal health. Early toxicological studies suggested that degraded carrageenan may induce intestinal inflammation, ulceration, and gastrointestinal lesions in experimental models.(8,10)

Recent investigations have further highlighted the possible association between carrageenan consumption and inflammatory bowel diseases. Studies have reported that carrageenan may influence intestinal permeability, gut microbiota composition, and inflammatory signaling pathways associated with gastrointestinal disorders.(11) Experimental evidence also suggests that carrageenan may activate inflammatory pathways resulting in increased production of pro-inflammatory cytokines and disruption of epithelial barrier integrity. Furthermore, some studies indicate that carrageenan may alter gut microbiota composition and contribute to dysbiosis, particularly when consumed as part of ultra-processed foods.(9)

Despite these concerns, several researchers argue that food-grade carrageenan is safe when consumed within approved limits and that many adverse findings are associated with degraded forms or experimental conditions not representative of normal dietary exposure. Therefore, the biological effects of carrageenan continue to remain an active area of scientific investigation.(12)

Considering both the therapeutic potential and safety concerns associated with carrageenan, the present study was designed to evaluate its molecular interaction with important oral pathogenic proteins using molecular docking analysis. The findings may contribute to understanding the possible antimicrobial applications of carrageenan-derived compounds in oral and systemic infections while also highlighting the importance of evaluating their biological safety.(13)

MATERIALS AND METHODS

Ligand Preparation

The chemical structures of carrageenan compounds were prepared using the ChemOffice suite. The two-dimensional (2D) structures of the selected ligands were drawn using ChemDraw software. The generated structures were then converted into three-dimensional (3D) conformations using Chem3D software. Energy minimization of the ligands was carried out to obtain stable molecular conformations and to reduce steric hindrance and structural strain. The optimized ligand structures were saved in compatible file formats for molecular docking analysis.

Protein Preparation

The three-dimensional crystal structures of the target proteins were obtained from the Protein Data Bank (PDB). The selected target proteins included HmuY heme-binding protein of *Tannerella forsythia* (PDB ID: 6EU8), gingipain R protein of *Porphyromonas gingivalis* (PDB ID: 1CVR), and *Enterococcus faecalis* protein (PDB ID: 4M7U).

The downloaded protein structures were visualized and prepared using BIOVIA Discovery Studio. During protein preparation, water molecules, co-crystallized ligands, and unwanted heteroatoms were removed from the protein structures to avoid interference during docking analysis. The cleaned protein structures were then subjected to further preparation using MGL Tools, where polar hydrogens were added and Kollman charges were assigned to stabilize the

protein molecules. The prepared proteins were finally saved in PDBQT format for docking studies.

Molecular Docking Analysis

Molecular docking studies were performed to evaluate the interaction between carrageenan compounds and the selected target proteins. Docking was carried out by positioning the ligands within the active binding sites of the proteins to predict the binding affinity and interaction patterns.

The prepared ligand and protein files were imported into the docking software, and docking parameters were set based on the active site regions of the target proteins. The docking procedure generated multiple conformations for each ligand–protein complex, and the best docking pose was selected based on minimum binding energy and interaction stability.

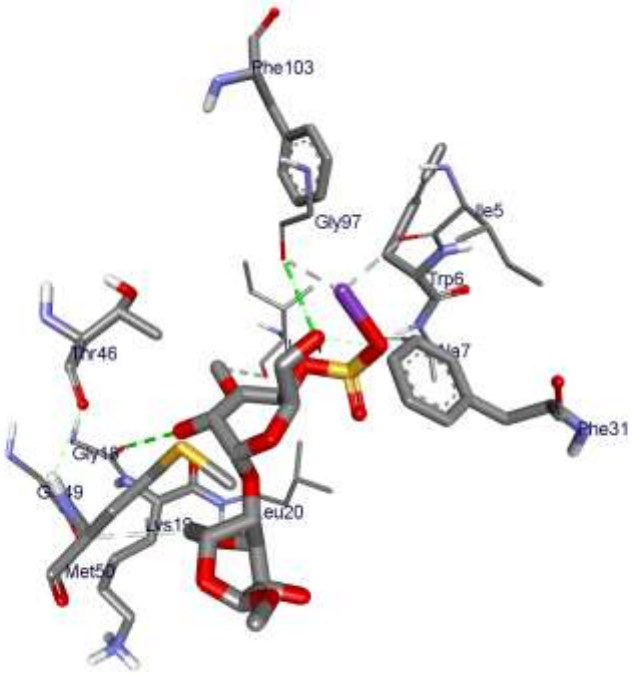
Analysis of Protein–Ligand Interactions

The docked complexes were analyzed using BIOVIA Discovery Studio to evaluate the molecular interactions between ligands and target proteins. The interaction analysis included identification of hydrogen bonds, hydrophobic interactions, van der Waals interactions, and electrostatic interactions formed between the ligands and amino acid residues present in the active site of the proteins.

The binding affinity scores and interacting amino acid residues obtained from docking analysis were recorded and compared to determine the potential antimicrobial activity of carrageenan compounds against periodontal and endodontic pathogens.

RESULTS:

4M7U with Compound 3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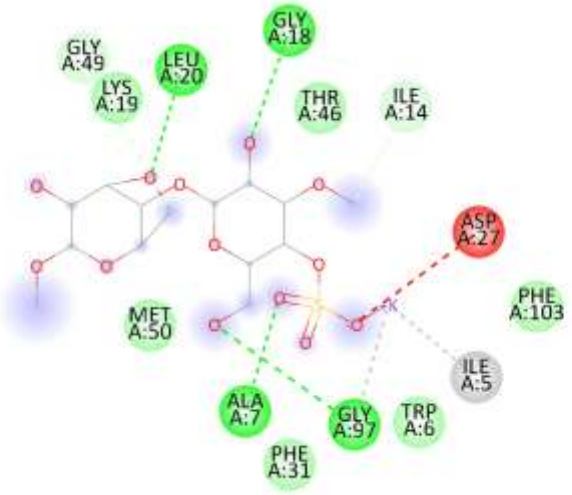
Thr46
Phe31
Leu20
Met50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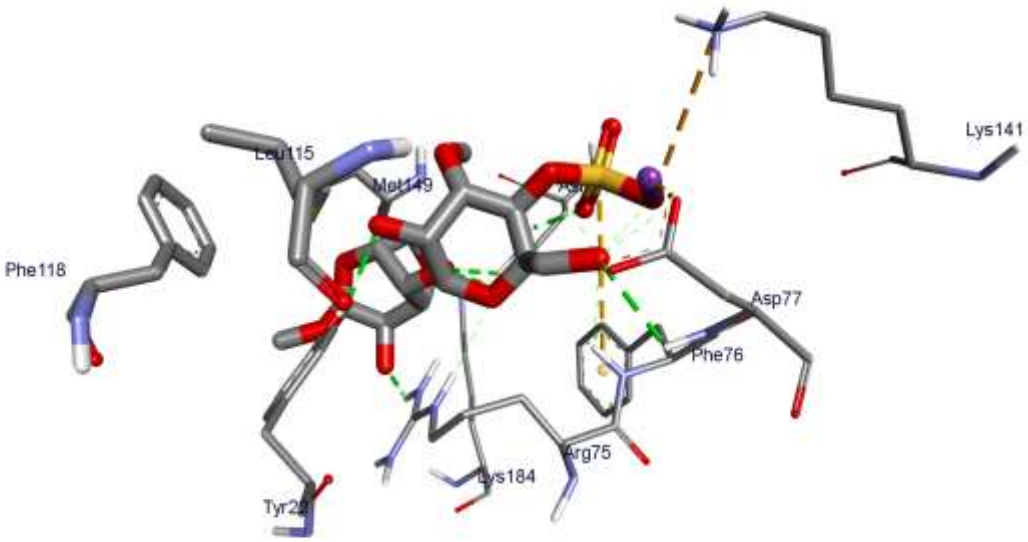
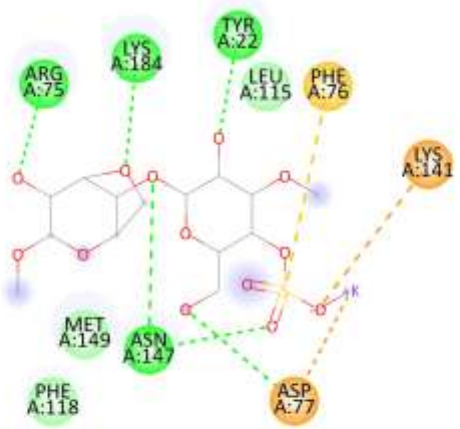
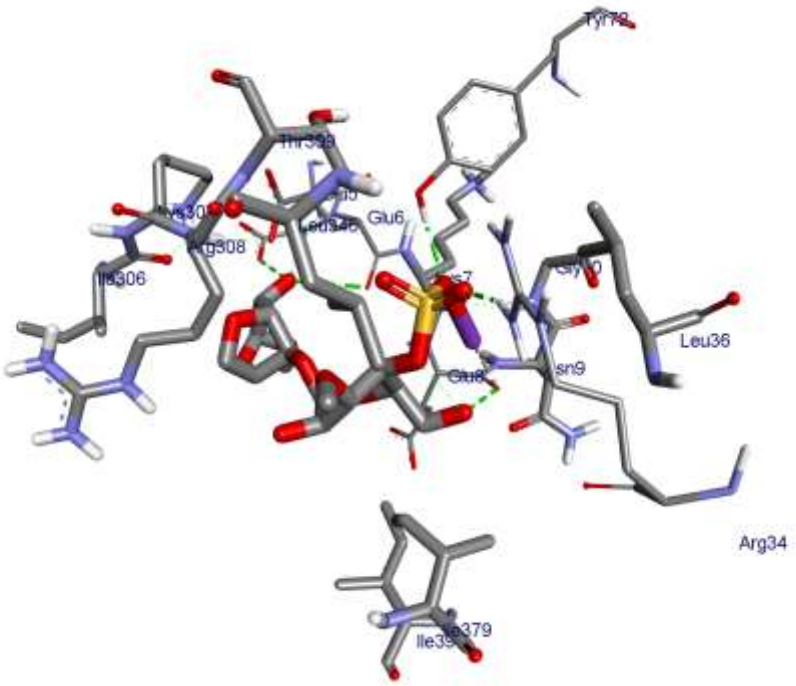
GLY A:49
LEU A:20
LYS A:19
THR A:46
ILE A:14
ASP A:27
PHE A:103
MET A:50
ILE A:5
ALA A:7
GLY A:97
TRP A:6
PHE A:31

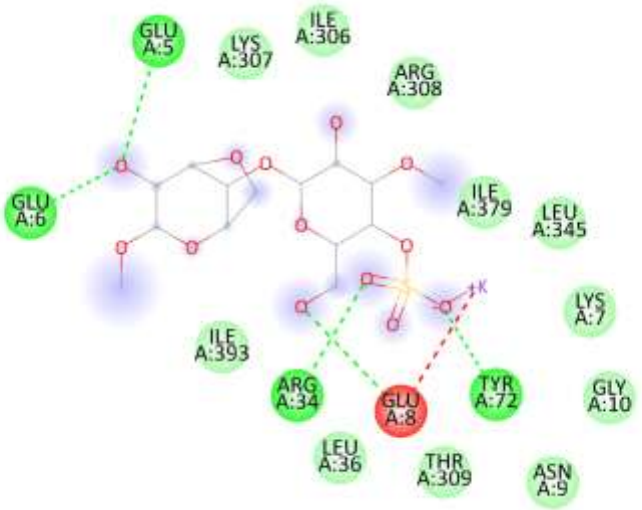
6EU8 with Compound 3





1CVR with Compound 3



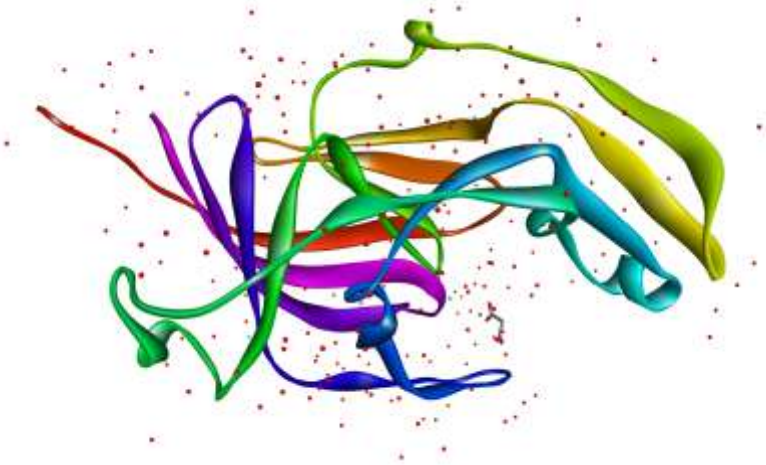


Molecular docking scores and residue interactions of compound (3) against HmuY (heme-binding protein) of *Tannerella forsythia* (PDB ID – 6EU8), gingipain R protein of *Porphyromonas gingivalis* (PDB ID – 1CVR), and *Enterococcus faecalis* (PDB ID – 4M7U).

Ligands	**Docking scores/Affinity (kcal/mol)**	**H-bond**	**Amino Acid Residual interactions**	
			Hydrophobic/Pi-Cation	**Van dar Waals**
6EU8	-6.2	Arg-75, Lys-184, Tyr-22, Asn-147	Phe-76, Lys-141, Asp-77	Leu-115, Met-149, Phe-118
1CVR	-7	Glu-5, Glu-6, Arg-34, Tyr-72	Glu-8	Lys-307, Ile-306, Arg-308, Ile-379, Leu-345, Lys-7, Gly-10, Asn-9, Thr-309, Leu-36, Ile-393
4M7U	-6.4	Leu-20, Gly-18, Ala-7, Gly-97	Gly-49, Ile-14, Asp-27, Ile-5	Met-50, Phe-31, Trp-6, Phe-103, Thr-46, Lys-19

Protein Preparation:

6UE8



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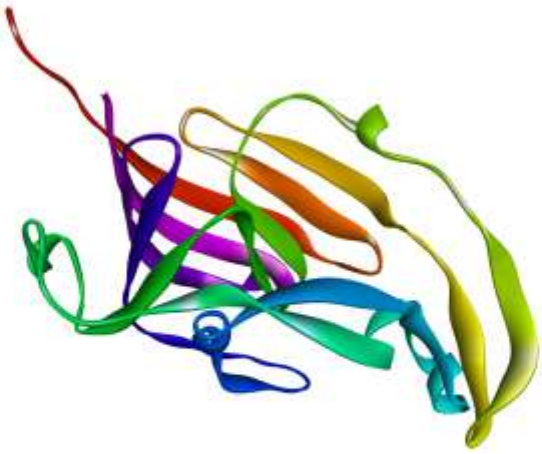
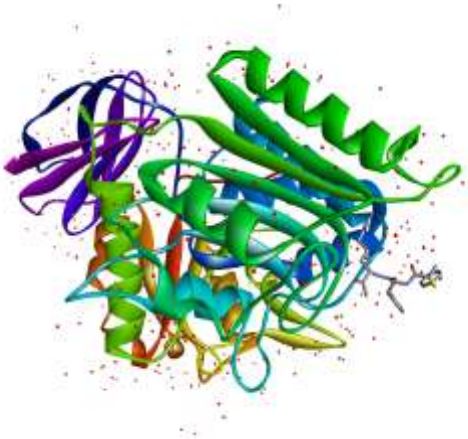


1CVR



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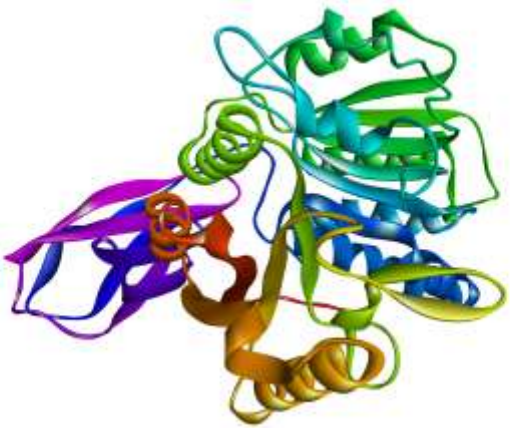
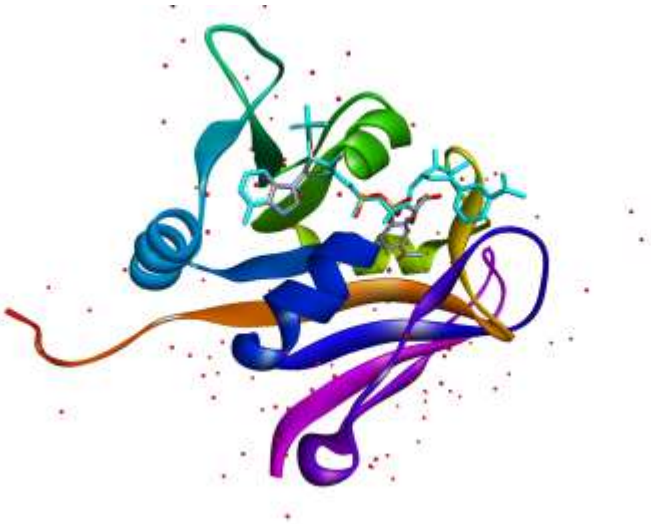
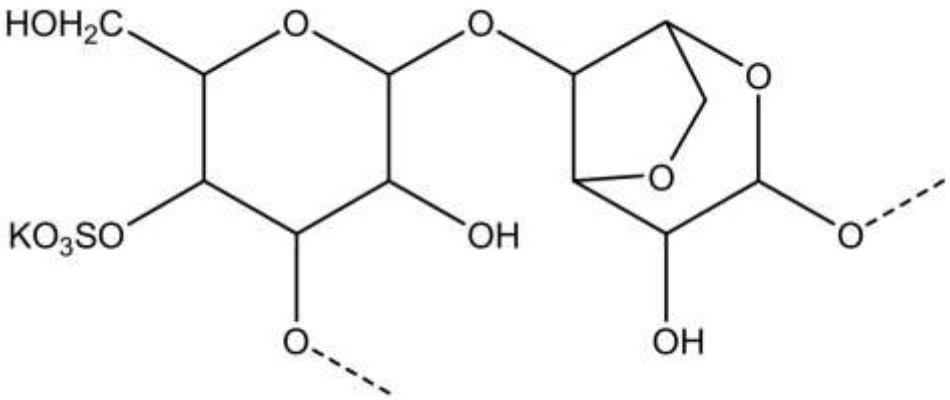


4M7U



Prepared 4M7U

Ligand Preparation:



DISCUSSION

Marine-derived polysaccharides have gained considerable attention in recent years because of their broad spectrum of biological activities and potential therapeutic applications. Carrageenan, a sulfated polysaccharide isolated from red seaweeds, has been extensively studied for its antiviral, antioxidant, anti-inflammatory, and antimicrobial properties. (2)In the present study, molecular docking analysis was carried out to evaluate the interaction of carrageenan compounds with important virulence-associated proteins of oral pathogenic microorganisms including *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*.(14)

The docking results demonstrated favorable binding affinities of the selected carrageenan compounds toward the target proteins, suggesting possible antimicrobial activity. Among the tested compounds, compound (3) exhibited comparatively stronger binding interactions with the active sites of the proteins. The observed interactions included hydrogen bonding, hydrophobic interactions, and van der Waals forces, which are important for stabilizing ligand–protein complexes. Strong binding affinity generally indicates a higher probability of inhibitory action against the biological activity of the target proteins.(3)

The interaction with HmuY heme-binding protein of *Tannerella forsythia* is particularly significant because this protein plays an essential role in heme acquisition and bacterial survival. Inhibition of this protein may interfere with iron uptake mechanisms, thereby reducing bacterial pathogenicity. Similarly, gingipain R protein of *Porphyromonas gingivalis* is a major virulence factor involved in periodontal tissue destruction, immune evasion, and degradation of host proteins.(5) The docking interaction of carrageenan compounds with gingipain R suggests a potential role in suppressing the virulence and progression of periodontal disease. In the case of *Enterococcus faecalis*, which is commonly associated with

persistent root canal infections and endodontic treatment failures, the observed interactions indicate possible antibacterial effects that may contribute to improved infection control.

The sulfated nature of carrageenan may contribute significantly to its biological activity. Sulfate groups are known to enhance electrostatic interactions with positively charged amino acid residues present in proteins, thereby improving binding stability. Previous studies have also suggested that sulfated polysaccharides can interfere with microbial adhesion, biofilm formation, and enzymatic activity, supporting the findings of the present investigation.(15)

Despite the promising docking results, certain limitations must be considered. Molecular docking is a computational prediction method and does not completely represent biological conditions within living systems. The interactions observed in silico require further validation through in vitro and in vivo experimental studies to confirm antimicrobial efficacy, toxicity, pharmacokinetics, and safety.(11) Additionally, although carrageenan demonstrates therapeutic potential, some studies have reported possible inflammatory and gastrointestinal effects associated with prolonged exposure or degraded forms of carrageenan. Therefore, careful evaluation of its safety profile remains essential before clinical application.

Overall, the present study highlights the potential role of carrageenan-derived compounds as promising bioactive agents against oral pathogenic microorganisms. The findings support further investigation into marine polysaccharides for the development of novel antimicrobial agents in dentistry and medicine.

CONCLUSION

The present molecular docking study demonstrated that carrageenan compounds exhibit favorable interactions with important virulence-associated proteins of *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*. Among the evaluated compounds, compound showed comparatively better binding affinity and stable interactions with the target proteins, suggesting potential antimicrobial activity against periodontal and endodontic pathogens.

The interaction of carrageenan compounds with proteins involved in bacterial survival, pathogenicity, and tissue destruction indicates their possible therapeutic significance in controlling oral infections. The presence of hydrogen bonds and hydrophobic interactions within the active sites of the proteins further supports the stability of the ligand–protein complexes.

Although the docking results are promising, further experimental studies including in vitro and in vivo analyses are necessary to validate the antimicrobial efficacy, pharmacological properties, and safety profile of carrageenan compounds. Overall, the findings of the present study suggest that carrageenan-derived compounds may serve as potential candidates for the development of novel antimicrobial agents targeting oral pathogenic microorganisms.

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