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Research Article

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Determination of three dimensional structure of dentilisin, a virulence factor of *Treponema denticola* by homology modelling and identification of inhibitors for dentilisin

Sambandam Cecilia[1], Sivabakya T. K[2]. and Chitraa R. Chandran[2]

[1]*Department of Microbiology, Tagore Dental College and Hospital, Rathinamangalam, Chennai, India*
[2]*Tagore Dental College and Hospital, Rathinamangalam, Chennai, India*

ABSTRACT

Hence the dentilisin produced by Treponema denticola is considered to be one of the important virulence factors involved in chronic periodontitis. Hence in the present study, an attempt has been done to screen important polyphenolic compounds that can effectively bind the dentilisin thereby these compounds can be considered as potential drug candidates in the control of chronic periodontitis. The three dimensional structure of dentilisin was predicted by homology modelling. The structures of 25 polyphenolic compounds are obtained from PubChem database. The OPEN BABEL software was used to convert sdf format to pdb format. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. Based on the binding energy the four polyphenolic compounds were shortlisted for the further study. Four polyphenolic compounds viz., ellagic acid, flavoxate, myricetin and sylybin are found to excellent in binding dentilisin on accurate docking. These four compounds thus can have the ability to inhibit dentilisin.

Keywords: *Treponema denticola*, dentilisin, chronic periodontitis, homology modelling, molecular docking

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INTRODUCTION

Periodontitis is a chronic immunoinflammatory infectious disease leading to the destruction of periodontal ligament and adjacent supportive alveolar bone induced by pathogenic biofilms containing numerous periodontal pathogens. Among the periodontal pathogens, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* are commonly co-isolated in subgingival biofilm samples from adult periodontitis lesions [1–4].

Several studies report the co-existence of *P. gingivalis* and *T. denticola* in close association with chronic periodontitis lesions [5-7]. T. denticola is reported to coaggregate with P. gingivalis [8] after which they localized closely together in matured subgingival plaque [9]. This coaggregation seems to contribute to the pathogenesis of periodontal diseases [10]. It has been found that the coaggregation is due to binding of *Porphyromonas gingivalis* fimbriae to *Treponema denticola* dentilisin [11]. Hence the dentilisin is considered to be one of the important virulence factors involved in chronic periodontitis and can be a suitable drug target in the management of it.

The polyphenolic compounds are one of the common phytochemicals found in many plants and has been reported to have many biological functions [12]. Most of these compounds are produced by the plants to protect themselves and the researches have found that these compounds have many antimicrobial properties [13].

Hence in the present study, an attempt has been done to screen important polyphenolic compounds that can effectively bind the dentilisin thereby these compounds can be considered as potential drug candidates in the control of chronic periodontitis. For the docking study, the three dimensional structure of dentilisin is essential. As the three

dimensional structure is not available, in the present study the homology modelling has been used to design the same.

EXPERIMENTAL SECTION

Prediction of three dimensional structures

The three dimensional structure of dentilisin was predicted by homology modelling. It is the method to determine 3D structure of protein with the help of 3D structure of homologous proteins. Softwares used were Modeller 9.11 and Easy modeller 2.0 GUI. First the primary structure of dentilisin protein was retrieved from UniProtKB database (www.uniprot.org/help/uniprotkb). The primary structure in FASTA format was submitted in BLASTp (*blast.ncbi.nlm.nih.gov/*) to find the homologous proteins. The proteins of low e value were selected. The 3D structures of the homologous proteins that were selected were retrieved from RCSB database (*www.rcsb.org/*). The 3D structure of homologous proteins was submitted along with the primary structure of dentilisin protein to Modeller software through GUI Easy Modeller. The predicted 3D structure is then validated with the Ramachandran plot. It is also further validated in ProQ online tool (*www.sbc.su.se/~bjornw/ProQ).*

Active site prediction

The possible binding sites of dentilisin protein are searched using binding site prediction online tool Q site finder. The binding sites which are more flexible are selected for this study.

Generation and optimization of Ligand

The structures of polyphenolic compounds are obtained from PubChem database (*pubchem.ncbi.nlm.nih.gov/*). The compounds obtained were in sdf format. The OPEN BABEL software (www.vcclab.org/lab/babel/start.html) was used to convert sdf format to pdb format. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. A population size of 150 was set with 70 generation and one solution for quick docking. Based on the binding energy the polyphenolic compounds were shortlisted for the further study. The selected compounds were then analyzed for drug- relevant properties based on "Lipinski's rule of five". Other drug like properties were analysed using OSIRIS Property Explorer (http://www.organicchemistry.org/prog/peo/) and Mol soft, the drug-likeness and molecular property explorer (http://www.molsoft.com/mprop/). On the basis of binding affinity and drug like properties, all these compounds were taken for further molecular docking study.

Protein-ligand docking

The selected compounds were subjected accurate docking (very slow docking) by setting population size of 800 with 80 generation and 10 solutions. After the completion of the docking the post docking analysis is performed to find the docking pose and its energy values.

RESULTS AND DISCUSSION

Three dimensional structure prediction and validation

The primary structure of dentilisin protein was retrieved from UniprotKB database. Its uniprot code is P96091. The dentilisin protein is made of 722 aminoacid residues. The residues from 1 to 18 is signal peptide. The primary structure of dentilisin in FASTA format is shown in Figure 1.

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>tr|P96091|P96091_TREDN Serine protease (Dentilisin) from Treponema
denticola OS=Treponema denticola GN=prtP PE=3 SV=1
MKKKILFFLIAIALIVSSCNFGMNTAVIGNKDNGTVLNGYTGGSGNSSSIELPNGANYKP
DDKDIVDGYFIVKTKDGFDKTLFEKKGFIVEGNISLTDTGFTYWYLNKKGDNKKNLLRAA
SIEGVLSAEHDYKVVEPDGSKAPNQNDSPVDPSNAGTYGLTDGNYLDDPEANNADYGLSI
TDALRAYKEIGYGDKTVVAGIIDTGINMKHKDFKDENGESIVLYAKSCATDGTGGTYIGD
GNPFTEIPIGLNWDKGAHGTHCSGTIAARGNNNIGIAGVAWKNTKLISYQSLGVEGSGRT
WSVYGAMADLTKIVKILRKPKANRSVAENNALPSYLQNTDFQITQETVPVNMSLGGSYGS
EFAFAVLTNAVKHNILPVIAMGNEGRYTAAYPAAFPGVLAVGATNGKDKKVHFSNSGAWI
SVSAPGDGIKSCGIHGEDDYETMSGTSMATPFVTGTIAYLLSFDNAHKITPIQMKALLEK
TADKVDGATDFTDSLGHGEVNVYKAAKAIKDGNIPAPNDIYTEAEVTVTVKNNDGHSNDI
VPCKITLVDEETHAPLAYVAGTGSNPPAFKGLIKGRSYSVYGAFLGSSSNQTFTAQDVDN
PITIQFNKKIVWVSTVPNLHYNSGDDDTDTRIMVYKADASGNLDLNTAQSIVDYDQDLLD
TTCFEAETGAKYYVLITGFMKGGVFQGGNYALKIDRTPLNSNGCKYRRYGKKSQQCFNER
QP
```

Figure 1: Primary structure of dentilisin in FASTA format

The 3D structure of dentilisin was successfully predicted by homology modelling. The predicted 3D structure of dentilisin protein is given in Figure 2. Its 3D structure is viewed as PDB file with Rasmol structure colour scheme. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white.

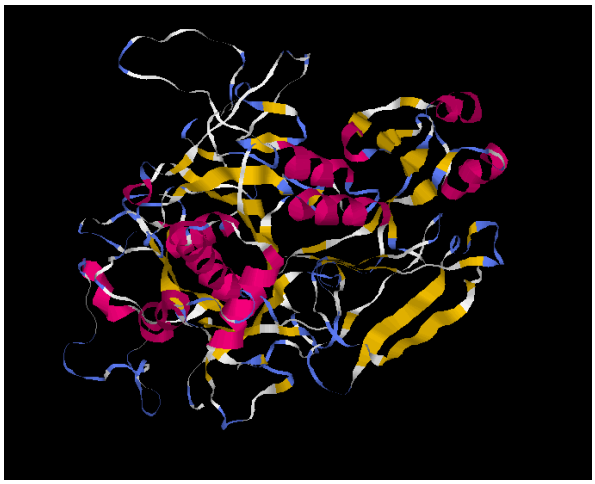

Figure 2: Three dimensional structure of dentilisin obtained by homology modelling

The Ramachandran plot was generated from the predicted 3D structure of dentilisn protein to validate it. The Ramachandran plot is shown in Figure 3. From the figure is seen that most of the residues clustered tightly in the most-favoured regions with very few outliers showing that the predicted structure is good.

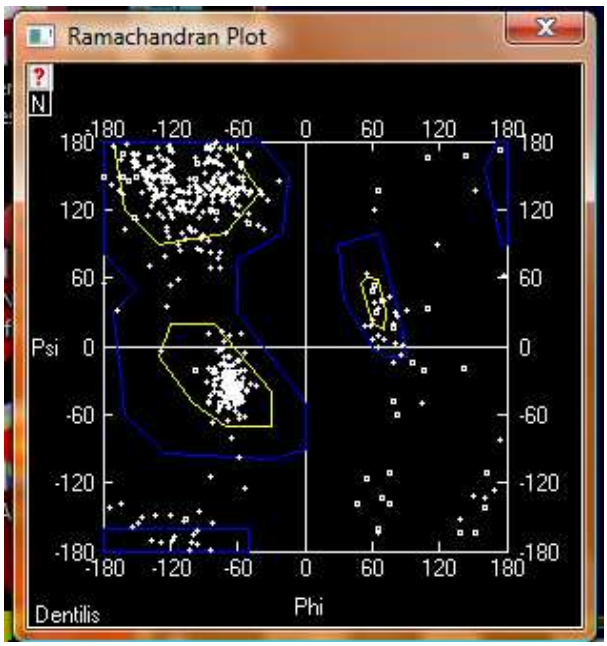

Figure 3: Ramachandran plot of predicted dentilisin structure

The predicted structure was further validated by ProQ online tool. The predicted LGscore and Maxsub are **3.505** and **0.175** respectively. The values obtained shows that the predicted structure is very good model.

Preparation of ligands

A total of 25 polyphenolic compounds were from pubchem database in sdf format. It was converted to pdb format using OPEN BABEL software. All the 25 ligands were then subjected to virtual rapid screening with iGEMDOCK software and four compounds were found to have good fit with a low binding energy. The structures and their names of the four compounds were shown in the Figure 4. The selected four compounds were then studied for its drug relevant properties.

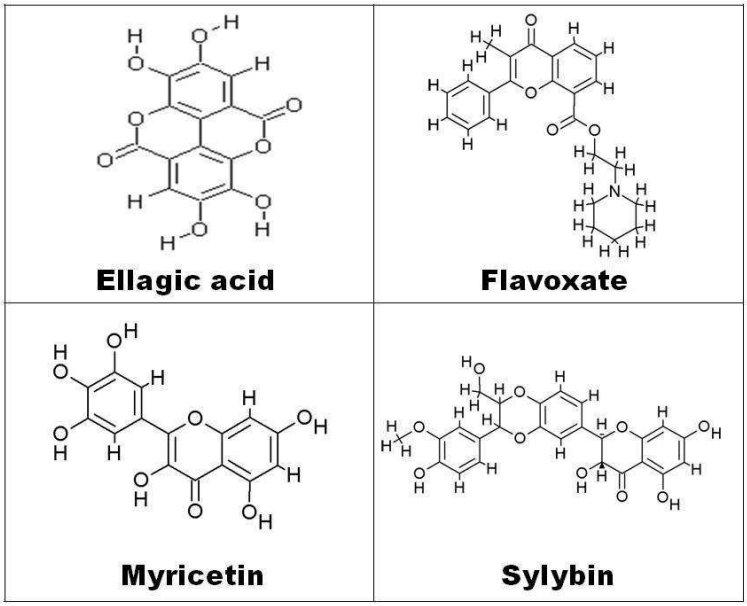

Figure 4: The structures and the names of the four compounds selected

The Table 1 depicts the values related to the Lipinski's rule of Five. From the table it is evident that all the four selected compounds obey the rule.

Table 1: The Lipinski's properties of the selected four polyphenolic compounds

S. No.	Polyphenolic compounds	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	Ellagic acid	302.01	1.366	4	8
2.	Flavoxate	427.16	3.489	0	5
3.	Myricetin	318.14	2.182	5	8
4.	Sylybin	482.12	0.815	5	10

Molecular docking

After the confirmation of drug likeliness, the four compounds were then subjected to further molecular docking with iGEMDOCK subjecting to accurate docking (very slow docking) by setting population size of 800 is set with 80 generation and 10 solutions. The results were projected in the Table 2.

Table 2: The results of iGEMDOCK showing binding energies of four polyphenolic compounds

S.No.	Polyphenolic compounds	Total binding Energy (kcal/mol)	Vander Waals force	H Bond	Electrostatic bond
1.	Ellagic acid	-99.35	-80.47	-18.87	0
2.	Flavoxate	-120.39	-117.87	-2.5	0
3.	Myricetin	-120.47	-110.18	-10.29	0
4.	Sylybin	-126.87	-101.55	-25.32	0

From the table it is clear that all four compounds have low binding energy showing its possibility being competitive inhibitors for dentilisin protein. The docking pose of all four compounds are shown in Figure 5.

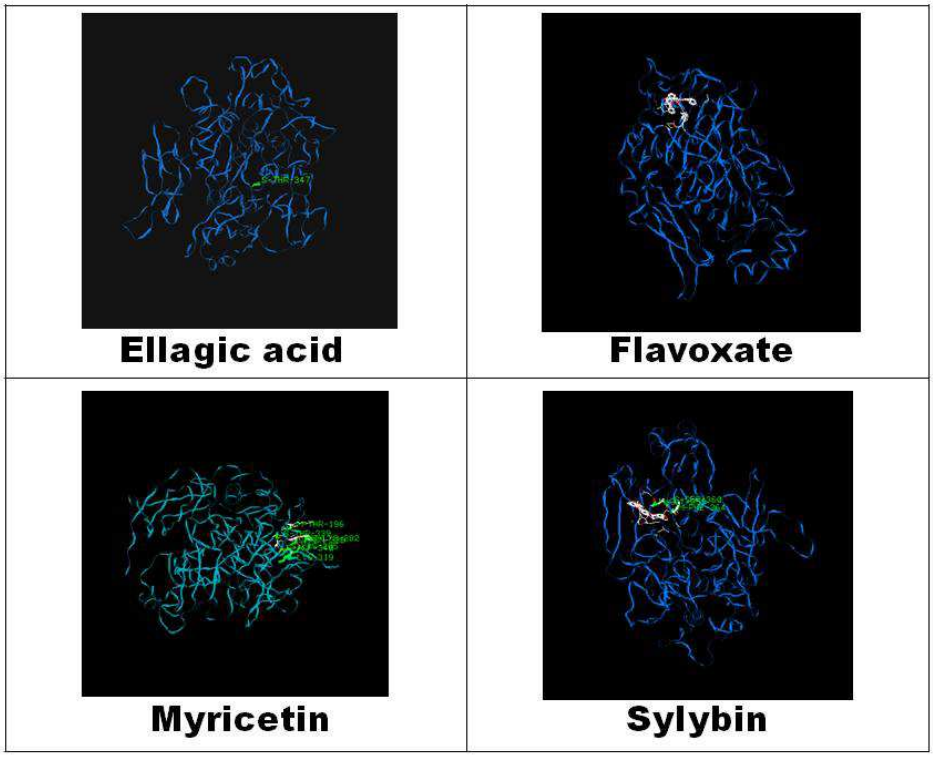

Figure 5: Docking pose of four polyphenolic compounds

CONCLUSION

The dentilisin is considered to be one of the important virulence factors involved in chronic periodontitis. Hence the inhibitors of the dentilisin protein can be an effective drug in the prevention and control of chronic periodontitis caused by *Treponema denticola*. In the present study 25 polyphenolic compounds were studied for its ability to inhibit the dentilisin protein by molecular docking method. Four polyphenolic compounds viz., ellagic acid, flavoxate, myricetin and sylybin are found to be excellent in binding dentilisin. These four compounds thus can have the ability to inhibit dentilisin.

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