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Treating Periodontitis by Inhibiting Sialic Acid Binding Protein Present in Fusobacterium Nucleatum Using Herbal Compounds - An Insilico Study.

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INTRODUCTION:

Periodontitis is a common oral disease affecting the periodontium in the oral cavity. Periodontium is defined as those tissues supporting and investing the tooth, comprises root cementum, periodontal ligament, bone lining the tooth socket (alveolar bone) and that part of the gingival facing the tooth (dentogingival junction).[1] The infection and inflammation of periodontium is called as periodontitis. This results in a gradual loss of alveolar bone. If periodontitis is left untreated, it may also lead to the loss of teeth.

The major cause of periodontitis is the formation of a bacterial plaque. It is sticky and is found over the surface of the tooth (biofilm). In addition to pathogenic microorganisms in the biofilm, genetic and environmental factors, especially tobacco use, contribute to the cause of this disease. Genetic, dermatological, haematological, granulomatous, immunosuppressive, and neoplastic disorders can also have periodontal manifestations.[2] *Fusobacterium nucleatum* is one among the important species of bacteria, present in the biofilm, causing periodontitis.

Fusobacterium nucleatum is a gram negative bacteria present in the oral cavity.. It is spindle shaped (fusiform rods) and of varying lengths. This bacterium has the ability to adhere to a wide range of both gram positive and gram negative bacteria present in the plaque. F.*nucleatum* is an anaerobic bacteria which grows in an environment with only upto 6% oxygen saturation. F.nucleatum uses the production of toxic metabolites. These toxic components have the ability to arrest the proliferation of the normal nearby cells of the periodontium. Hence leading to loss of the alveolar bone followed by the loss of teeth.[3]

Sialic acids are a family of related nine-carbon sugar acids that play important roles in both prokaryotes and eukaryotes. Sialic acid binding proteins are incorporated or decorated onto the lipooligosaccharides as terminal sugars in multiple bacteria including F.nucleatum to evade the host immune system. F.nucleatum scavenge sialic acids from their host and use them for molecular mimicry. The first step of this process is the transport of sialic acid to the cytoplasm, which often takes place using a tripartite ATP-independent transport system consisting of a periplasmic binding protein and a membrane transporter.

They cover their alien identity by decorating their outer layer with various forms of a linear monosaccharide that is also found on the surface of most animal cells including humans. SA is one among these linear monosaccharides. By adding this nine-carbon sugar acid to the outermost end of certain glycolipids that coat the bacterial surface, the pathogen can primarily derail the activation of the host's most primitive immune response: the complement system.[4]

Given the incidence of periodontitis, increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. While several agents are commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives.[5] The oils of many plant species like Australian lee tree, peppermint and sage were proved to be most potent essential oils against oral bacteria whereas thymol and eugenol were potent essential oils components.[6] This study is about finding a potent herbal compound that can inhibit SA binding protein present in *Fusobacterium nucleatum* and thus aid drug designers in designing a drug to cure periodontitis.

MATERIALS AND METHODS:

Herbal compounds:

Phenolic compounds (a group of secondary metabolites) are widely distributed in plants and have shown to possess antimicrobial properties [7]. A literature study was made on the herbal compounds with antibacterial activity. Among the various antibacterial compounds the following eight compounds were selected for docking : vanillic acid, caffeic acid, protocatechuic acid, syringic acid, p-coumaric acid, oleuropein, quercetin [8] and rutin [9]. Rutin has been found to enhance the antibacterial activities of flavonoids [9].

Retrieving data for docking:

To proceed with docking, we need complete information about the structure of these compounds. The 3D conformer for all these eight compounds were downloaded from **pubchem.** These downloaded compounds were converted from sdf to pdb format by using the software "Discovery studio". The structure of sialic acid binding protein from F.*nucleatum* was downloaded from **Protein Data Bank (PDB).** This protein can be retrieved using PDB ID -

4MNP. The downloaded protein will exist in pdb format and hence it does not require any conversion. The protein consists of only one chain i.e. Chain A with 312 nucleotides. It has one unique ligand. The name of the ligand is **5-N-ACETYL-BETA-D-NEURAMINIC ACID** i.e. **BETA-SIALIC ACID** with the formula $C_9 H_{19} NO_9$. It has only one chain-chain A. This downloaded protein is again opened using the software. Discovery studio for a 3 dimensional view of protein and binding site of the ligand.

Docking:

Docking is a process in which we can find the best fit position or conformation of a ligand in the active site or a binding site of a protein. The herbal compounds that can inhibit the protein are called as ligands. The surface of any protein is not smooth and has many elevations and depressions. These depressions are known as pockets. The pockets with maximum volume is known as the binding site. The binding site is where the ligand binds to the protein.Docking is used in receptor based drug designing. SA binding protein and the eight ligands were docked using the software iGEMDOCK . The software is runed and the downloaded protein is browsed in the dialogue box "Prepare binding site" and set "Define binding site type" as "By bounded ligand". The other categories in the dialogue box is left as default. All the eight ligands are added in "Prepare compounds" dialogue box. The other parameters are left as default and docking is done by clicking on "Start docking".

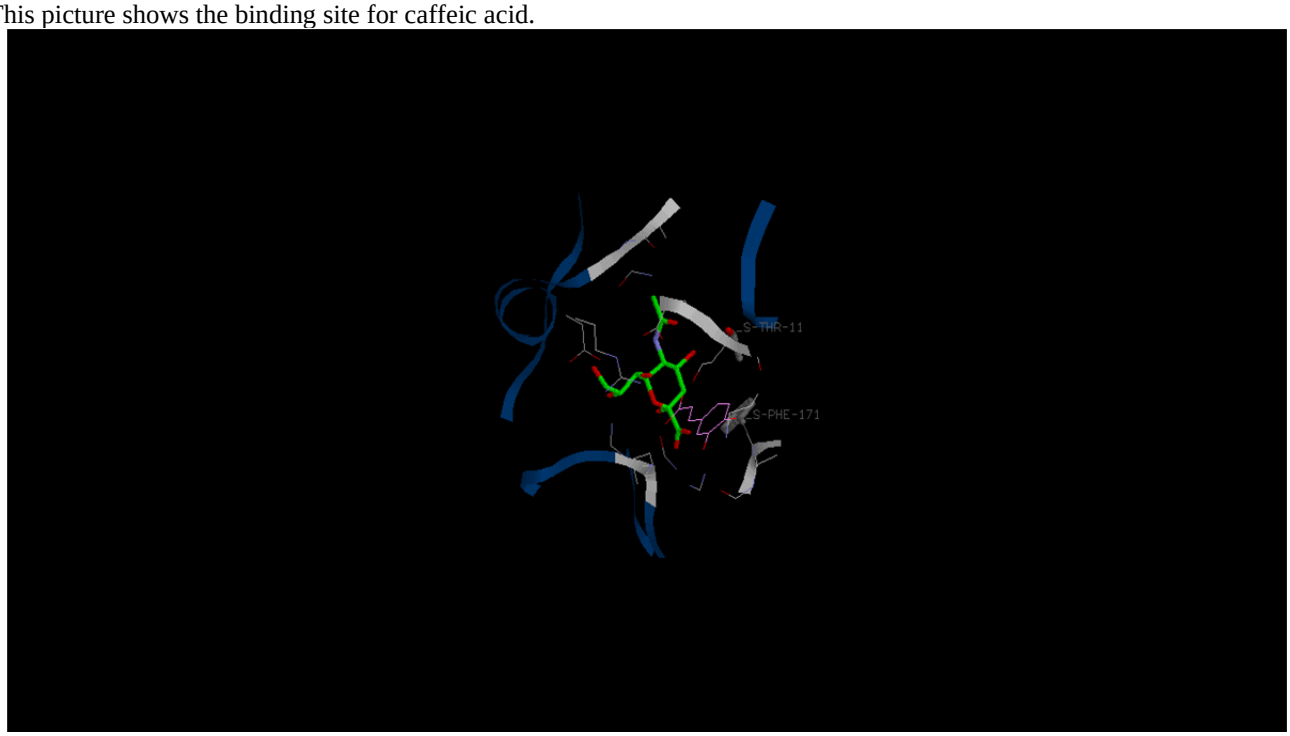
RESULTS AND DISCUSSION:

A good inhibitor is one that can bind to the protein with minimal interaction energy. **Caffeic acid** came out as the best inhibitor of SA binding protein present in F.*nucleatum* with a total energy of -65.3496. The interaction takes place using Vanderwaals forces of attraction. The interaction profile of all the eight ligands is as follows:

#C:/Users/new/Desktop/iGEMDOCKv2.1/iGEMDOCKv2.1/output/extracted_cav/cav4mnp (1)_SLB.pdb with C:/

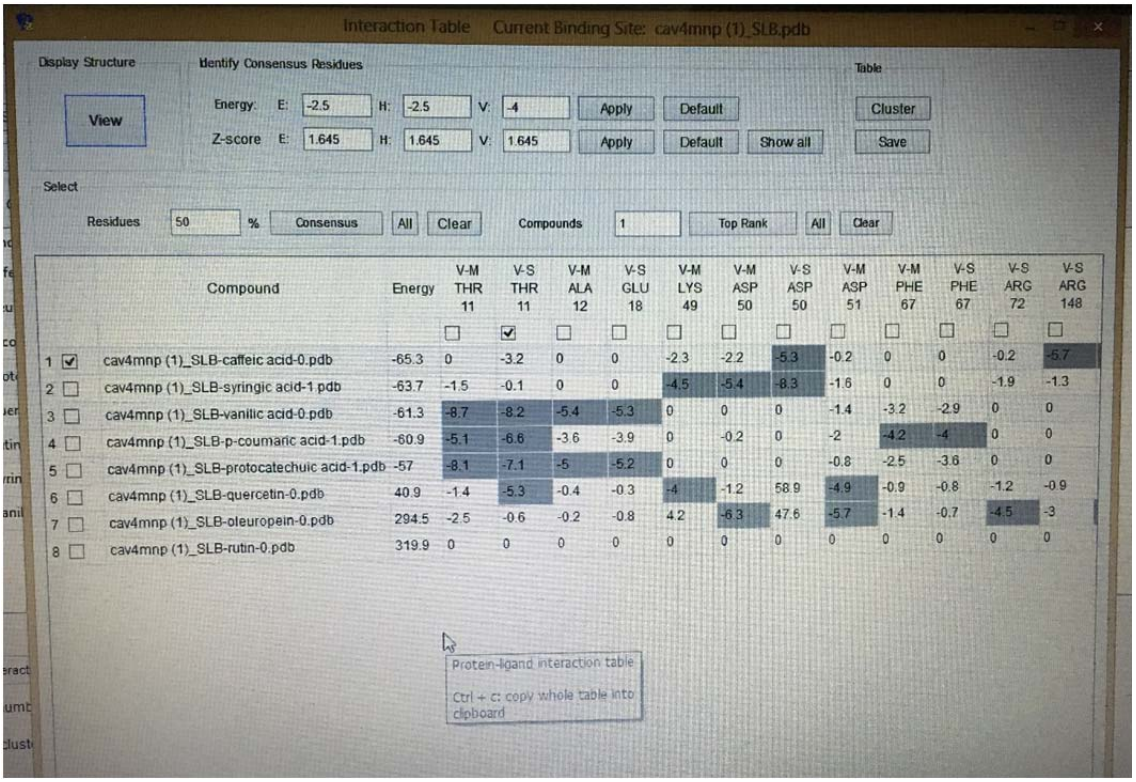
#Ligand	TotalEner	VDW	HBond	Elec	AverConPair
cav4mnp (1)_SLB-caffeic acid-0.pdb	-65.3496	-65.3496	0	0	27.9231
cav4mnp (1)_SLB-oleuropein-0.pdb	294.473	294.473	0	0	24.7632
cav4mnp (1)_SLB-p-coumaric acid-1.pdb	-60.9417	-60.9417	0	0	30.9167
cav4mnp (1)_SLB-protocatechuic acid-1.pdb	-56.9946	-56.9946	0	0	29.7273
cav4mnp (1)_SLB-quercetin-0.pdb	40.9031	40.9031	0	0	25.8182
cav4mnp (1)_SLB-rutin-0.pdb	319.949	319.949	0	0	19.7442
cav4mnp (1)_SLB-syringic acid-1.pdb	-63.74	-63.74	0	0	29.2857
cav4mnp (1)_SLB-vanilic acid-0.pdb	-61.2905	-61.2905	0	0	30.4167

This picture shows the binding site for caffeic acid.



All the compounds, except rutin, were binded at the SLB ligand binding site. Rutin requires the high energy for binding compared to the other seven compounds. Hence, rutin cannot be the ideal compound for inhibiting SA binding protein of F.*nucleatum.*

The interaction analysis of the compounds with the protein is shown. The boxes shaded in grey show the best interactions of the compounds at the binding site.



	Compound	Energy	V-M THR 11	V-S THR 11	V-M ALA 12	V-S GLU 18	V-M LYS 49	V-M ASP 50	V-S ASP 50	V-M ASP 51	V-M PHE 67	V-S PHE 67	V-S ARG 72	V-S ARG 148
1	cav4mnp (1)_SLB-caffeic acid-0.pdb	-65.3	0	-3.2	0	0	-2.3	-2.2	-5.3	-0.2	0	0	-0.2	-5.7
2	cav4mnp (1)_SLB-syringic acid-1.pdb	-63.7	-1.5	-0.1	0	0	-4.5	-5.4	-8.3	-1.6	0	0	-1.9	-1.3
3	cav4mnp (1)_SLB-vanilic acid-0.pdb	-61.3	-8.7	-8.2	-5.4	-5.3	0	0	0	-1.4	-3.2	-2.9	0	0
4	cav4mnp (1)_SLB-p-coumaric acid-1.pdb	-60.9	-5.1	-6.6	-3.6	-3.9	0	-0.2	0	-2	-4.2	-4	0	0
5	cav4mnp (1)_SLB-protocatechuic acid-1.pdb	-57	-8.1	-7.1	-5	-5.2	0	0	0	-0.8	-2.5	-3.6	0	0
6	cav4mnp (1)_SLB-quercetin-0.pdb	40.9	-1.4	-5.3	-0.4	-0.3	-4	-1.2	58.9	-4.9	-0.9	-0.8	-1.2	-0.9
7	cav4mnp (1)_SLB-oleuropein-0.pdb	294.5	-2.5	-0.6	-0.2	-0.8	4.2	-6.3	47.6	-5.7	-1.4	-0.7	-4.5	-3
8	cav4mnp (1)_SLB-rutin-0.pdb	319.9	0	0	0	0	0	0	0	0	0	0	0	0

CONCLUSION:

Molecular Docking helps to reduce time, effort and money required for a wet lab research in finding the best inhibitor in a group of compounds. This study concludes that caffeic acid can be a potent inhibitor of SA binding protein present in *Fusobacterium nucleatum.* The future prospects of this research is to help researchers in proceeding with the next step i.e. finding the favourable conditions and the amount of caffeic acid required to inhibit SA binding protein present in *Fusobacterium nucleatum.* This also helps drug designers to design a drug containing caffeic acid and thus treating periodontitis.

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