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In silico Identification of Potential Inhibitor Targeting *Streptococcus mutans* and Lactobacillus Acidophilus for the Treatment of Dental Caries

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Dental caries is one of the most common chronic diseases, and it is caused by the acid fermentation of bacteria that have become attached to the teeth. *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*) anchor surface proteins to the cell wall and form a biofilm to aid adhesion to the tooth surface. Some natural plant products, particularly several flavonoids, are effective inhibitors. However, given the scarcity of inhibitors and the emergence of drug resistance, the development of new inhibitors is critical. The high-throughput virtual screening approach was used in this study to identify new potential inhibitor of against *S. mutans* and *L. acidophilus* by using ligand (Ellagic acid).
Aim: To evaluate the drug interaction ligand (Ellagic acid) and protein [A3VP1 of AgI/II] of *Streptococcus mutans* (PDB ID: 3IPK), glucan-1,6 - alpha-glucosidase from *Lactobacillus acidophilus* NCFM (PDB ID: 4AIE).

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Materials and Methodology: The pdb format of two selected proteins was retrieved from the RCSB protein database. Then inhibitors were docked with protein (A3VP1 of AgI/II) and glucan-1,6-alpha-glucosidase to identify the potent inhibitor. An evaluation criterion was based on the binding affinities by using AutoDock.
Results: The binding energy of Ellagic acid - *Streptococcus mutans* docked complex-10.63 kcal/mol and with Ellagic acid – *Lactobacillus acidophilus* docked complex was -7.30 kcal/mol.
Conclusion: In this study, Showed that lesser binding energy better is the binding of the ligand and protein. These findings can provide a new strategy for dental caries disease therapy by using Ellagic acid as a inhibitor against *Streptococcus mutans and Lactobacillus acidophilus*

Keywords: Dental caries; Lactobacillus acidophilus; molecular docking; potential inhibitors; Streptococcus mutans.

1. INTRODUCTION

Tooth decay is one of the most severe public health concerns. Dental caries is the most prevalent illness as a result of lifestyle changes. Since bacteria cause dental caries (tooth decay), there has been a continuing interest in creating vaccines or passive vaccination regimens for management or prevention [1]. Although dental caries is not deadly, and caries is now thought to be primarily preventable in developed nations with a healthy diet and excellent oral hygiene, there are still substantial difficulties with paediatric illness, particularly among impoverished communities. As a result, caries is one of the most frequent infectious illnesses in the world. As a result, research on vaccine formulations including peptide components derived from the surface proteins of *Streptococcus mutans*, a key agent linked with tooth caries [2]. *Streptococcus mutans* acid resistant strains, such as *Lactobacillus acidophilus*, are important pathogens. The primary characteristics of *S.mutans* able to form the colony of an existing biofilm, which is harmful to oral disorders.

S. mutans adherence inside dental plaque can be mediated by both sucrose-independent and sucrose-dependent mechanisms. In sucrose-independent adhesion, salivary components inside the acquired enamel pellicle may begin the attachment process, whereas sucrose is primarily responsible for colonising the tooth surface in sucrose-dependent adhesion. Antigen I/II surface protein present on most oral streptococci substantially predisposes *S. mutans* to sucrose-independent adherence [3]. Proteins in the antigen I/II family have comparable structural properties based on amino acid domains, but their usefulness in binding salivary agglutinins, salivary pellicle components, and other plaque bacteria varies [4,5]. The interaction between antigen I/II and salivary components is primarily mediated by the alanine-rich and proline-rich domains [6–10].

The acid tolerance of *S. mutans* is primarily mediated by an F1F0-ATPase proton pump and involves adaptation with an accompanying change in gene and protein expression. Evidence suggests that acid tolerance may be aided by synthesising water insoluble glucan and biofilm formation [11,12].

Lactobacillus acidophilus (LB) is another bacteria that substantially influences *S. mutans*-induced biofilm in the oral cavity. LB bacteria are commensal microorganisms that colonise the human mouth cavity, among other places. Because it ferments carbohydrates into acidic compounds, which lower the pH in the oral cavity and encourage biofilm development, LB is closely linked to the development of dental caries in dentine [13]. Low pH and antibacterial compounds, such as hydrogen peroxide or bacteriocins generated by LB microorganisms, on the other hand, encourage the cleansing of the oral cavity from microorganisms that are non-adaptive to such environmental circumstances, such as Porphyromonas. The specific etiologic factor in dental caries is *Lactobacillus acidophilus*. They claim that this microorganism grows luxuriantly in the presence of active lesions, with it constantly found on the teeth and in the saliva, whereas in the absence of active lesions, it is either completely absent or present in relatively small numbers.

Furthermore, given recent advances in computer technology and the rapid accumulation of structural, chemical, and biological data on an ever-increasing number of therapeutic targets, it is easy to see how the use of *in silico* approaches such as chemoinformatics, molecular modelling, and artificial intelligence

(AI) has grown significantly in recent decades [13–18]. Indeed, *in silico* methods currently allow for the virtual screening of millions of compounds in a reasonable amount of time, lowering the initial expenses of hit discovery and increasing the likelihood of identifying the required therapeutic candidates.

To be sure, *in silico* moves toward now empowering the virtual screening of millions of mixtures in a reasonable time, accordingly decreasing the underlying expenses of hit distinguishing proof and further developing possibilities of tracking down the ideal medication applicants. As of now, a few atomic demonstrating procedures are accessible to work with drug disclosure errands, a large portion of them being grouped into structure-based and ligand-put together approaches. Structure-based techniques depend on the data obtained from the 3D construction of an objective of interest. They permit positioning data sets of particles as indicated by ligands primary and electronic complementarity to a given objective [19]. In this unique situation, sub-atomic docking is among one of the most well known and fruitful construction situated *in silico* techniques, which assist with foreseeing the associations between particles and natural targets [19]. For the most part, this interaction is achieved by first anticipating the sub-atomic direction inside a receptor and afterwards assessing their complementarity using a scoring capacity [19].

Since its first appearance during the 1970s, docking has ended up being a significant apparatus to see how synthetic mixtures collaborate their sub-atomic targets and for drug revelation and improvement. Indeed, the number of studies announcing: (I) the utilisation of atomic docking to distinguish underlying determinants fundamental for effective ligand-receptor restricting, and (ii) the improvement of more precise docking techniques have vigorously expanded since its first appearance [19–31]. Among the first and additional fascinating investigations on the utilisation of docking in drug revelation and science is Kuntz et al. in the mid-1980s [28]. According to Kuntz ID, stated that computational strategy empowering the investigation of mathematically achievable ligand-receptor arrangements for the known heme-myoglobin/metmyoglobin and thyroxine/prealbumin structures [28]. This ignited up with the present study to evaluate the the drug interaction ligand (Ellagic acid) and protein [A3VP1 of AgI/II] of *Streptococcus mutans* (PDB ID: 3IPK), glucan-1,6 - alpha-glucosidase from *Lactobacillus acidophilus* NCFM (PDB ID: 4AIE).

2. METHODOLOGY

2.1 Preparation of Ligand

The two dimensional structure of the selected ligand Ellagic acid was retrieved from ChemDraw Professional 16.0 software (Fig. 1), and it was saved in mol format. The optimised 3D Ellagic acid structure was generated through the energy minimisation process using Chem3D 16.0 software, and it was saved in pdb format (Figs. 2 and 3).

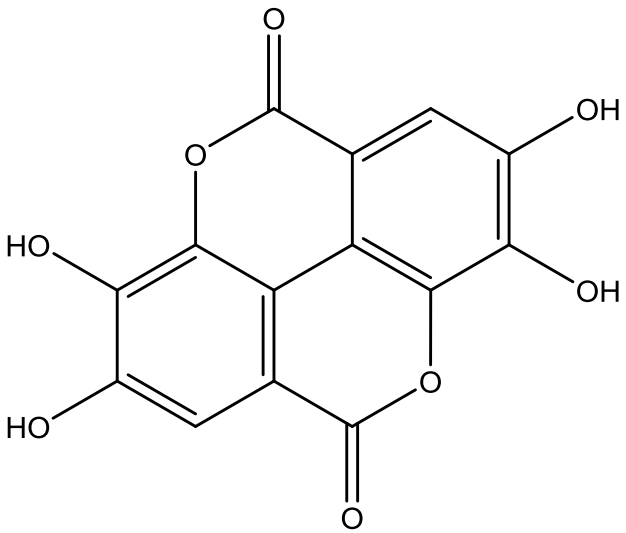

Fig. 1. 2D structure of the selected ligand Ellagic acid

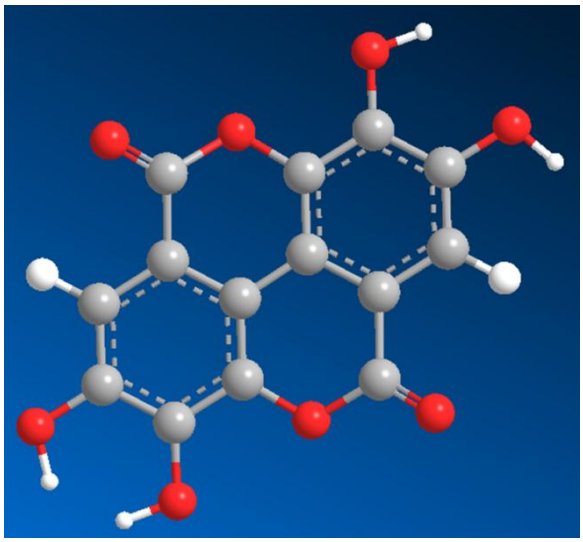

Fig. 2. Chem 3D structure of Ellagic acid – Before optimisation

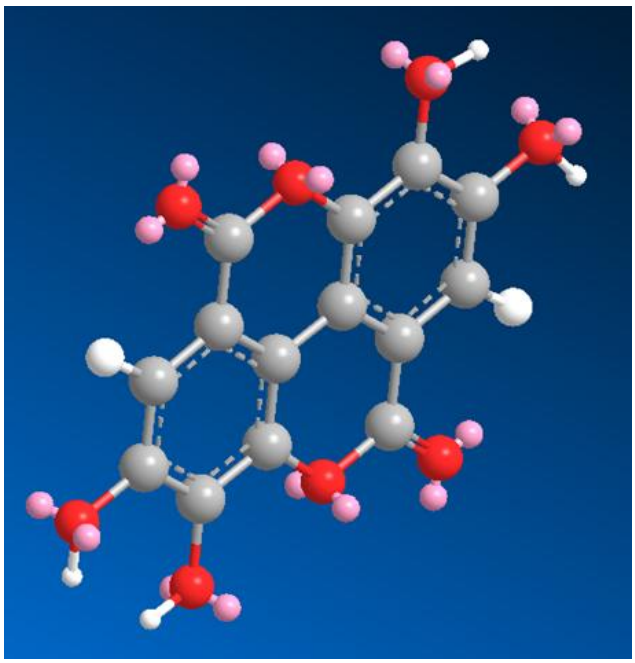

Fig. 3. Chem 3D structure of Ellagic acid – After optimisation

2.2 Preparation of Target Proteins

The selected target proteins were (1) Crystal structure of A3VP1 of AgI/II of *Streptococcus mutans* (PDB ID: 3IPK) classified under cell adhesion, and its structure was determined by X-ray diffraction method having a resolution of 2.04 Aº, and (2) Structure of glucan-1,6-alpha-glucosidase from *Lactobacillus acidophilus* NCFM (PDB ID: 4AIE) classified under and its structure was determined by X-ray diffraction method having a resolution of 2.05 Aº.

The pdb format of two selected target proteins was retrieved from the RCSB Protein Data Bank database. The optimisation of the target proteins was performed by eliminating the heteroatoms and water molecules.

2.3 Molecular Docking Study

The optimised ligand Ellagic acid was docked with the crystal structure of A3VP1 of AgI/II of *Streptococcus mutans* (PDB ID: 3IPK) and structure of glucan-1,6-alpha-glucosidase from

Lactobacillus acidophilus NCFM (PDB ID: 4AIE) respectively using AutoDock 4.2.6 software. The flexible docking study was performed using the pdbqt format of Ellagic acid, rigid macromolecule and predicted active, flexible residues.

Concerning the EMBL-EBI PDBsum Generate tool, the active residues were predicted to be modelled as flexible residues.

(a) PDB ID: 3IPK – SER 697, ASP 760, SER 761, SER 762, TRP 816, ARG 824
(b) PBD ID: 4AIE – TYR 330, ASN 498, GLU 499, GLU 500, PRO 529

The grid box was built to cover all predicted flexible active residues of the respective target protein. The grid parameters followed in molecular docking analysis are depicted in Figs. 4 and 5.

Centre of ligand molecule: -0.003, -0.000, -0.012

Lamarckian genetic algorithm (LGA) with the maximum of 2.5 million energy evaluations was used to explore the molecular docking analysis. The molecular docking parameters followed for flexible docking of ligand Ellagic acid towards selected two target proteins in Fig. 6.

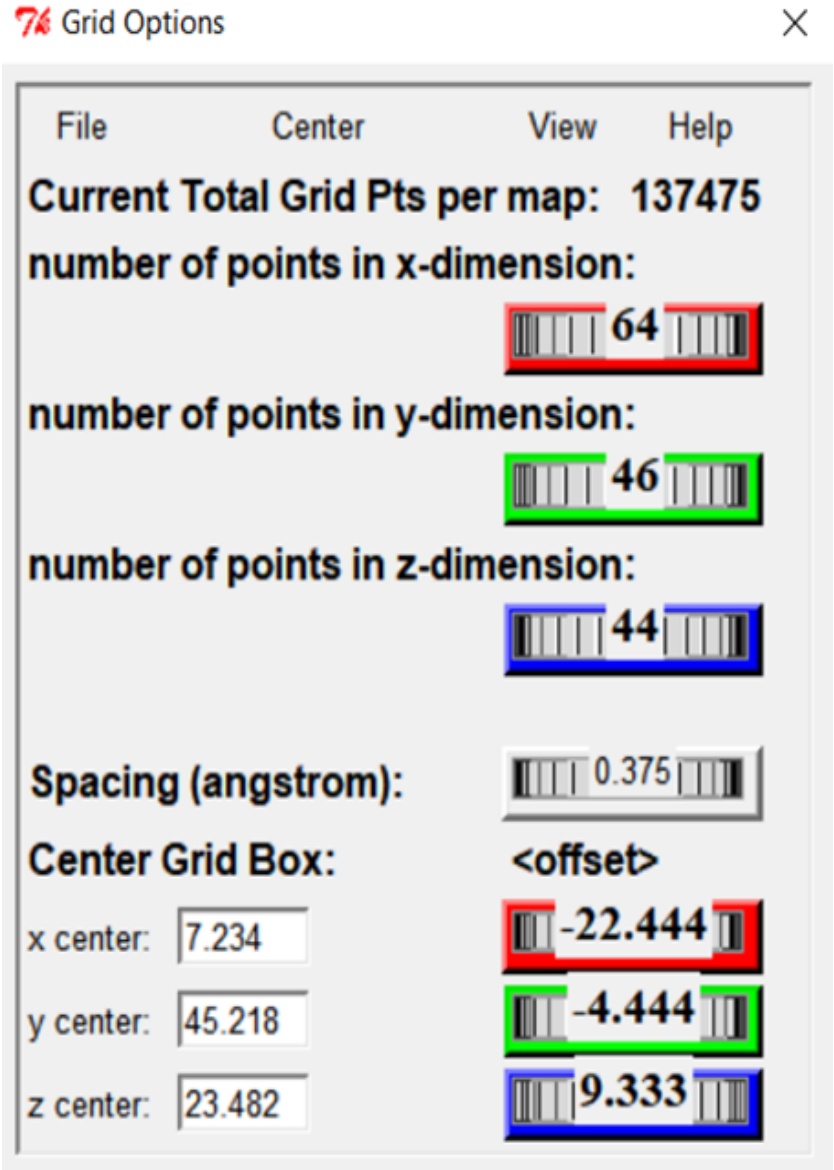

Fig. 4. Grid parameters followed in the molecular docking of Ellagic acid with *Streptococcus mutans* (PDB ID: 3IPK)

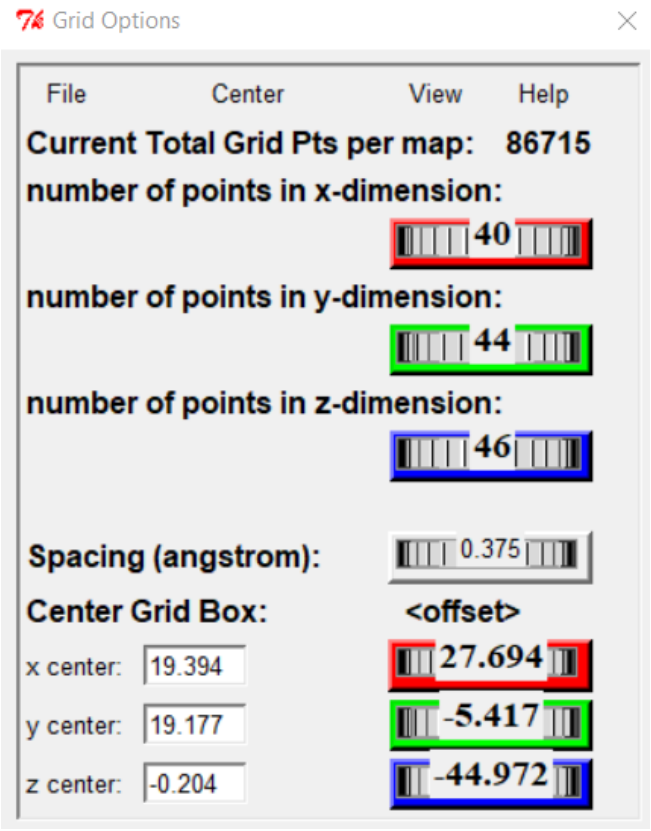

Fig. 5. Grid parameters followed in the molecular docking of Ellagic acid with *Lactobacillus acidophilus* (PDB ID: 4AIE)

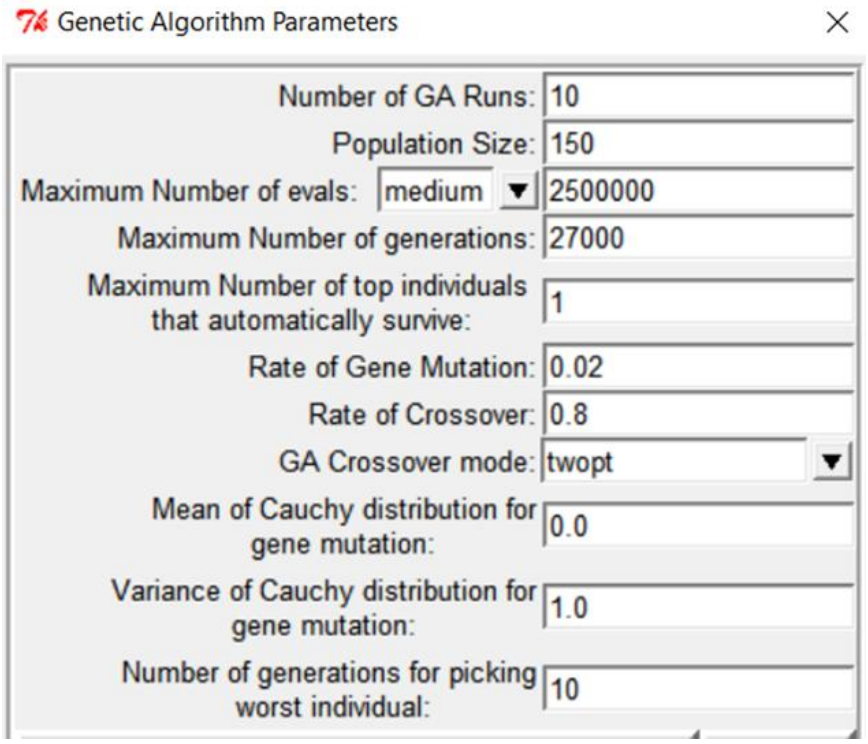

Fig. 6. Docking parameters followed for both the molecular docking analysis

3. RESULTS

The binding energy for the topmost confirmation of the respective docked complex was predicted using AutoDock 4.2.6 software. The 2D and 3D protein-ligand binding interactions for the topmost confirmation of the respective docked complex were interpreted using BIOVIA Discovery Studio Visualizer 2021 Client software

1. Figs. 7 and 8 showed the Docking results of binding energy Ellagic acid with *streptococcus mutans* was -10.63 kcal/mol.
2. Fig. 9 showed the 3D docking pose of the Ellagic acid – *Streptococcus mutans* (PDB ID: 3IPK) docked complex.
3. Fig. 10: 2D representation of binding interactions of the Ellagic acid – *Streptococcus mutans* (PDB ID: 3IPK) docked complex
4. Figs. 11 and 12 showed most potential inhibitors exhibited more favourable binding free energy with Ellagic acid – *Lactobacillus acidophilus* docked complex was -7.30 kcal/mol.
5. Fig. 13 3D docking pose of the Ellagic acid – *Lactobacillus acidophilus* (PDB ID: 4AIE) docked complex
6. Fig. 14 showed that 2D representation of binding interactions of the Ellagic acid – *Lactobacillus acidophilus* (PDB ID: 4AIE) docked complex.

The lower the binding energy (the more negative the free binding energy results in stronger complexes), the better the ligand-protein binding

DOCKING RESULTS OF ELLAGIC ACID WITH *Streptococcus mutans* (PDB ID: 3IPK)

binding_energy=-10.63
ligand_efficiency=-0.48
inhib_constant=16.22
inhib_constant_units=nM
intermol_energy=-11.82
vdw_hb_desolv_energy=-5.33
electrostatic_energy=0.1
moving_ligand_fixed_receptor=-6.59
moving_ligand_moving_receptor=-5.23
total_internal=-9.27
ligand_internal=-2.07
torsional_energy=1.19
unbound_energy=-9.27
filename=dock.dlg
cIRMS=0.0
refRMS=49.29
rseed1=None
rseed2=None

Fig. 7. Docking results of Ellagic acid with *Streptococcus mutans* (PDB ID: 3IPK)

```
Estimated Free Energy of Binding    =  -10.63 kcal/mol  [=(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, Ki   =   16.22 nM (nanomolar)  [Temperature = 298.15 K]

(1) Final Intermolecular Energy     =  -11.82 kcal/mol
    Moving Ligand-Fixed Receptor    =   -6.59 kcal/mol
       vdW + Hbond + desolv Energy  =   -6.51 kcal/mol
       Electrostatic Energy         =   -0.08 kcal/mol
    Moving Ligand-Moving Receptor   =   -5.23 kcal/mol
       vdW + Hbond + desolv Energy  =   -5.33 kcal/mol
       Electrostatic Energy         =   +0.10 kcal/mol
(2) Final Total Internal Energy     =   -9.27 kcal/mol
    Internal Energy Ligand          =   -2.07 kcal/mol
    Internal Moving-Fixed Receptor  =   -5.27 kcal/mol
    Internal Moving-Moving Receptor =   -1.93 kcal/mol
(3) Torsional Free Energy           =   +1.19 kcal/mol
(4) Unbound System's Energy  [=(2)] =   -9.27 kcal/mol
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Fig. 8. Binding energy calculation for the Ellagic acid – *Streptococcus mutans* docked complex

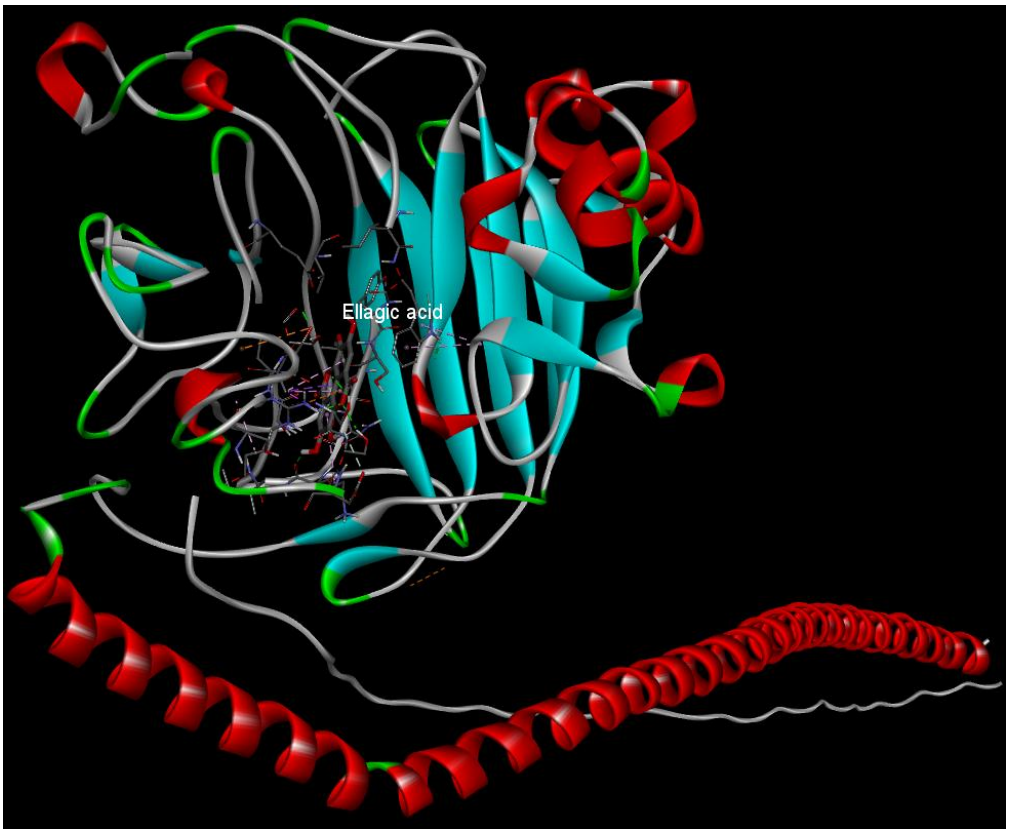

Fig. 9. 3D docking pose of the Ellagic acid – *Streptococcus mutans* (PDB ID: 3IPK) docked complex using BIOVIA Discovery Studio Visualizer software

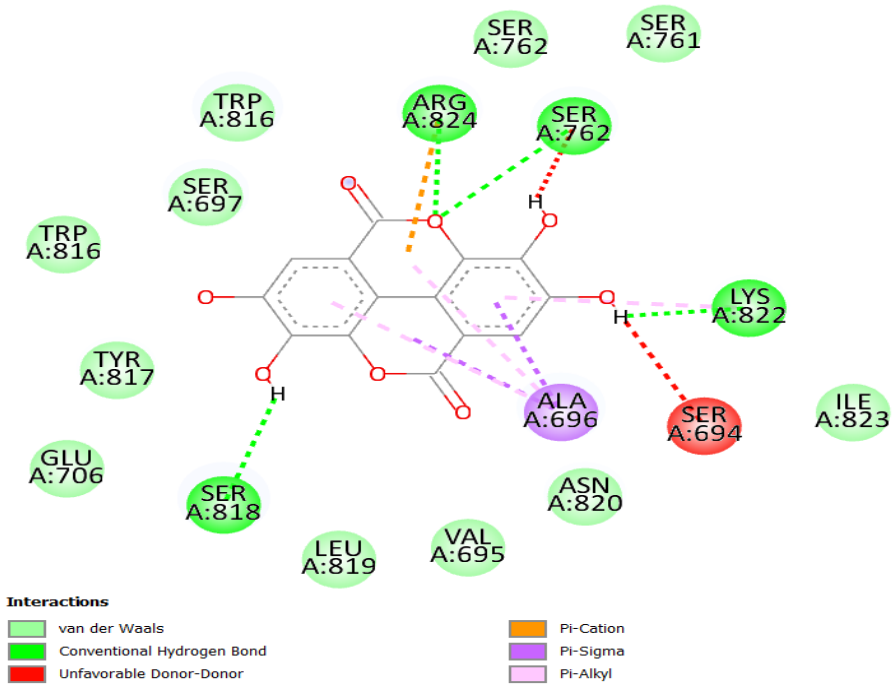

Fig. 10. 2D representation of binding interactions of the Ellagic acid – *Streptococcus mutans* (PDB ID: 3IPK) docked complex using BIOVIA Discovery Studio Visualizer software

DOCKING RESULTS OF ELLAGIC ACID WITH *Lactobacillus acidophilus* (PDB ID: 4AIE)

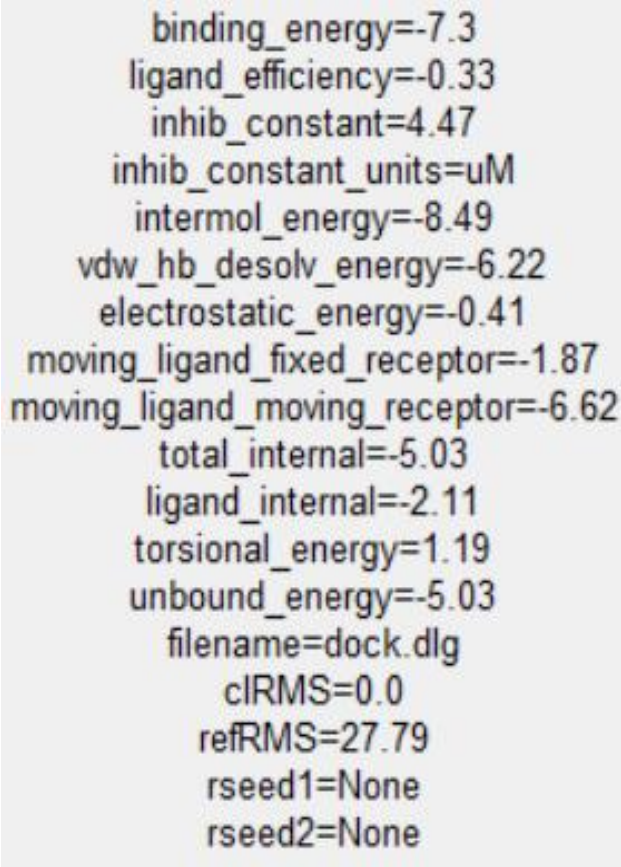

Fig. 11. Docking results of Ellagic acid with *Lactobacillus acidophilus* (PDB ID: 4AIE)

```
Estimated Free Energy of Binding    =   -7.30 kcal/mol  [=(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, Ki   =    4.47 uM (micromolar)  [Temperature = 298.15 K]

(1) Final Intermolecular Energy     =   -8.49 kcal/mol
    Moving Ligand-Fixed Receptor    =   -1.87 kcal/mol
       vdW + Hbond + desolv Energy  =   -1.91 kcal/mol
       Electrostatic Energy         =   +0.05 kcal/mol
    Moving Ligand-Moving Receptor   =   -6.62 kcal/mol
       vdW + Hbond + desolv Energy  =   -6.22 kcal/mol
       Electrostatic Energy         =   -0.41 kcal/mol
(2) Final Total Internal Energy     =   -5.03 kcal/mol
    Internal Energy Ligand          =   -2.11 kcal/mol
    Internal Moving-Fixed Receptor  =   -1.84 kcal/mol
    Internal Moving-Moving Receptor =   -1.08 kcal/mol
(3) Torsional Free Energy           =   +1.19 kcal/mol
(4) Unbound System's Energy  [=(2)] =   -5.03 kcal/mol
```

Fig. 12. Binding energy calculation for the Ellagic acid – *Lactobacillus acidophilus* docked complex

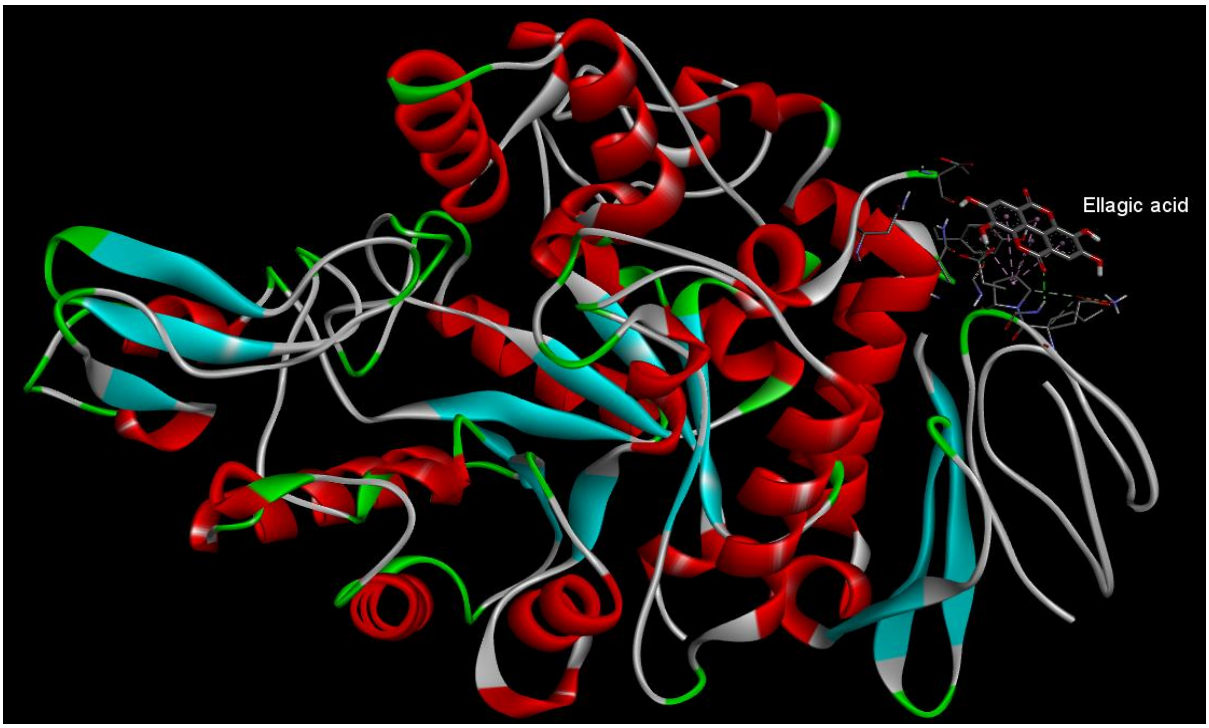

Fig. 13. 3D docking pose of the Ellagic acid – *Lactobacillus acidophilus* (PDB ID: 4AIE) docked complex using BIOVIA Discovery Studio Visualizer software

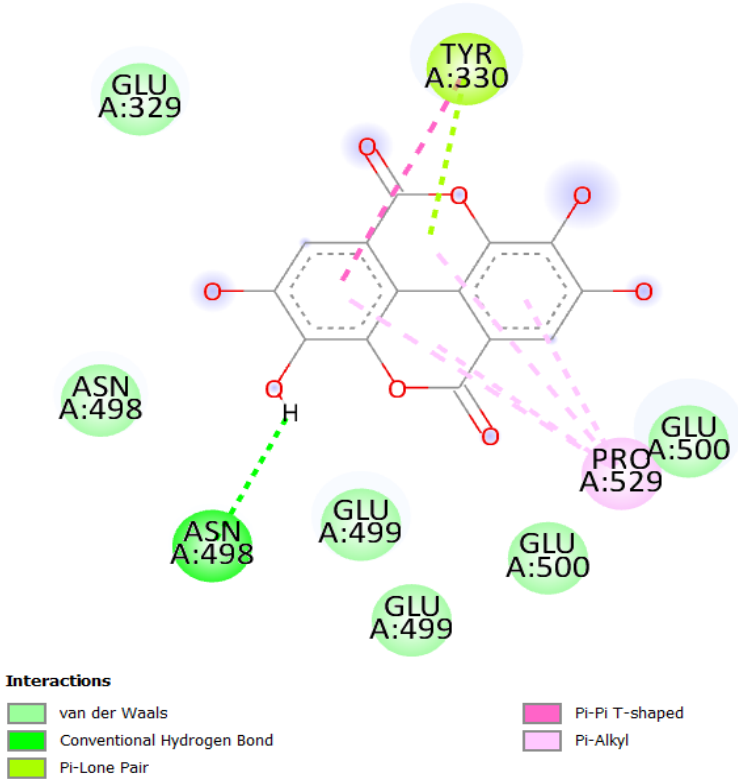

Fig. 14. 2D representation of binding interactions of the Ellagic acid – *Lactobacillus acidophilus* (PDB ID: 4AIE) docked complex using BIOVIA Discovery Studio Visualizer software

4. DISCUSSION

Dental caries is a chronic disease that affects a large percentage of the global population. Microflora in teeth exhibits subtle variations due to poor oral hygiene and dietary habits [32,33]. *S. mutans*, one of the most common dental caries pathogens, adheres to the tooth surface via cell wall surface proteins and dental plaque, producing pathogenic acid metabolites through carbohydrate fermentation and causing demineralisation of Soft tissues [32,34,35].

Recent research has shown that some oral bacteria produce alkali to reduce mouth acidity and thus prevent cavities [36]. Streptococcal AgI/II proteins can interact with other microorganisms known to populate the oral cavity, leading to the production of polymicrobial biofilms. These interactions frequently stabilise the colonisation of microorganisms linked to the development of periodontitis or tooth caries. The Ag I/II protein family, represented by SpaP, SspA, or SspB, has been found on *S. mutans* surface and the surfaces of other bacteria such as Streptococcus pyogenes Streptococcus agalactiae, and Streptococcus suis [2]. Six different areas are found in the genetic sequences encoding Ag I/II. The A area, which is high in alanine, and the P region, which is rich in proline, are the most significant of these. Region V, positioned between them, contains the majority of the distinct sequences found in various strains. The A and V sections encode sticky epitopes found on the surface of bacterial cells (referred to as adhesive types) that are important for bacterial cell affinity to salivary glycoproteins [37].

Moreover, inhibiting A3VP1 of AgI/II and glucan-1,6-alpha-glucosidase activity may become an effective therapy for dental caries. In this study, we tested compounds against A3VP1 of AgI/II and glucan-1,6-alpha-glucosidase that catalysed the binding of surface proteins to the cell wall. To calculate the precision of our screening system, heteroatoms and water molecules. The Grid score is based on the anchor and-grow algorithm, which involves docking a flexible ligand to a rigid receptor. Here we used the genetic algorithm parameter. Aside from Gtfs, enzymes such as mutanase and -1,6-glucosidase influence the production and structure of glucans. As a result, the structure of glucans in the biofilm matrix is variable, and water-insoluble polysaccharides predominate in matured dental plaque. To our best knowledge, this is the first study to include molecular information—docket about ellagic acid and binding proteins against *S. mutans* and *L. acidophilus*.

In general, van der Waals energy contributed the most to total binding free energy. In our study, results state that having lesser binding energy.

5. CONCLUSION

Docking and scoring have evolved dramatically in recent years. It has evolved into a valuable tool in the drug discovery process. The purpose of this study was to investigate the viability of docking approaches for our target Ellagic acid and to identify the compound. The predictive power of docking and scoring functions was compared. Our findings indicate that the docking programmes investigated here do a reasonable job of docking and significantly aid the drug discovery process.

Furthermore, the analysis of the docked ligands with the protein revealed some significant molecular interactions. The ligand docking results revealed that the binding pocket contains amino acid residues (a) PDB ID: 3IPK – SER 697, ASP 760, SER 761, SER 762, TRP 816, ARG 824 (b) PBD ID: 4AIE – TYR 330, ASN 498, GLU 499, GLU 500, PRO 529. ARG 824, SER 762, LYS 822, SER 818 (PDB ID: 3IPK)were the most powerful hydrogen bond forming amino acid residues. At the same time, PDB ID: 4AIE ASN 498 were the most significant hydrogen bond forming amino acid residues. Finally, we discovered a potent Ellagic acid that will be beneficial in developing new, less toxic, and highly effective drugs for the treatment of prevention and treatment for dental caries**.**

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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