Chapter

Molecular Docking of Phytochemicals against *Streptococcus mutans* Virulence Targets: A Proteomic Insight into Drug Planning

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Abstract

Streptococcus mutans (*S. mutans*) is the most prevalent and most associated with dental caries. Here we aim to identify, through an *in silico* study, potential bioactive molecules against *S. mutans*. Twenty-four bioactive molecules with proven action against S. mutans were selected: 1-methoxyficifolinol; 5,7,2′,4′-tetrahydroxy-8-lavandulylflavanone (sophoraflavanone G); 6,8-diprenylgenistein; apigenin; artocarpesin; artocarpin; darbergioidin; dihydrobiochanin A; dihydrocajanin (5,2′,4′-trihydroxy-7-methoxyisoflavanone); erycristagallin; Erystagallin; ferreirin; fisetin; kaempferol; licoricidin; licorisoflavan A; licorisoflavan C; licorisoflavan E; luteolin (3′,4′,5,7-tetrahydroxyflavone); malvidin-3,5-diglucoside; myricetin; orientanol B; quercetin; and quercitrin. Moreover, we selected nine important target proteins for the virulence of this microorganism to perform as drug targets: antigen I/II (region V) (PDB: 1JMM); Antigen I/II (carbox-terminal region) (PDB: 3QE5); Spap (PDB: 3OPU); UA159sp signaling peptide (PDB: 2I2J); TCP3 signaling peptide (PDB: 2I2H); ATP-binding protein ComA (PDB: 3VX4); glucanosucrase (PDB: 3AIC); dextranase (PDB: 3VMO), and Hemolysin (PDB: 2RK5). Five molecules were revealed to be the best ligands for at least three target proteins, highlighting the following compounds: 11 (erystagallin),

10 (erycristagallin), 1 (methoxyficifonilol), 20 (malvidin-3,5-diglucoside), and 2 (sophoraflavanone G), which indicates a possible multi-target action of these compounds. Therefore, based on these findings, *in vitro* and *in vivo* tests should be performed to validate the effectiveness of these compounds in inhibiting *S. mutans* virulence factors. Furthermore, the promising results of these assays will allow the incorporation of these phytoconstituents in products for oral use for the control of tooth decay.

Keywords: dental caries, docking molecular, drug planning, phytochemicals, virulence

1. Introduction

The planning and development of new drugs require high-risk and high-cost investments [1]. This process can involve, for example, studying about 5000–10,000 compounds, a period of 7–12 years, and spending about $800 million for a single drug to be marketed [2]. Thus, alternatives that optimize the process and reduce these costs are considered promising [3].

Nevertheless, there are other issues to the success or failure of drugs that must be considered. The main factors responsible for the lack of success in the production of possible drugs during clinical trials are pharmacokinetic factors, such as absorption, distribution, metabolism, excretion, and toxicities [4].

With the evolution of biotechnology and bioinformatics, promising new approaches for drug planning and optimization have become possible [5]. To reduce costs, risks and have greater efficiency in the production process, the pharmaceutical industry has increasingly used *in silico* analysis, which enables the performance of various analytical tasks, such as the quantitative structure-activity relationship of a drug, definition of pharmacophores (region of a ligand molecule that is strongly bound to its receptors), and other forms of modeling [6].

The *in silico* approach to drug development assesses the properties and interactions of a given molecule using computational algorithms [7]. Research in the areas of "omics" (proteomics, genomics, and transcriptomics) has increased due to the use of computational analysis through spectrometry, crystallography, and magnetic resonance techniques, which allows detailed access to the structure of the molecule, thus enabling the planning of medications and also predict their effects [8]. Molecular docking, an *in silico* approach, has been widely used for the planning and development of new drugs [3].

In 1984, the Lock-Key model, proposed by Fisher, explained the theory of ligand-receptor interaction. The model suggested that the interaction between two corresponding structures (ligand and receptor) was due to geometric and energy affinity. In this model, both ligand and receptor were considered rigid structures. The Lock-Key model contributed to the understanding of the mechanism of action of drugs. Nonetheless, it does not explain the interactions in the environment or changes in the spatial conformation of the molecules. Considering these modifications is extremely important, as the conformation of structures can change before and after bonding. Consequently, modern molecular docking tools consider these factors.

Molecular docking assesses the interaction and recognition between macromolecules, in general proteins and ligands [9]. Besides, the algorithm can predict what would happen if these structures interacted in a microenvironment [10].

Prediction of these interactions allows for the creation of structure-based drug design, an advance in drug development as it allows screening of specific molecules for specific targets [11].

Therefore, computer-aided drug design (CADD) uses high-performance computational algorithms to design and optimize molecules to become new drugs. The use of CADD in drug development optimizes the development process, increasing success rate, decreasing laboratory and personnel costs, in addition to producing quick results [3].

Several drugs, currently available for use, have been discovered and improved with the aid of *in silico* tools, such as molecular dockings, zanamivir [12], imatinib [13], nelfinavir [14], and erdafitinib [15]. With the evolution of bioinformatics, biotechnology, and molecular biology, including the determination of protein structures by using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, it has become increasingly easier to use *in silico* tools to predict functioning drugs. Thus, in the last 20 years, more than 60 different molecular docking software were developed by universities and companies [16].

Molecular docking programs have different approaches and their characterization is according to incremental construction approaches, including shape-based algorithms, genetic algorithms, the Monte Carlo method, and systematic search techniques [17–20].

Despite the evidence of the effectiveness and advantage of using molecular docking for drug discovery, studies in this area are still incipient for oral diseases [5], which justifies the performance of new studies. Streptococcus mutans (*S. mutans*), a gram-positive, aciduric, and acidogenic bacterium, is the most prevalent in the dental biofilm [21] and the most studied [22]. This microorganism has relevant virulence factors that enable colonization of the tooth surface, including its high capacity to form biofilms, causing the development of carious lesions [23]. The dominant defense systems of S. mutans for biofilm formation and caries development are its ability to adhere to the surface of teeth and produce acids, associated with its resistance to this environment without suffering damage [24]. Thus, preventing the formation of this microbial complex is one of the most targeted strategies for caries control [23].

Additionally, natural products have been a promising source of positive molecules for drug development over the years [25]. Therefore, plants are a promising source of new chemical compounds (phytochemicals) with high biological potential. Phytochemicals are a class of organic compounds synthesized in small amounts from secondary plant metabolism and are related to plant defense, growth, reproduction, and adaptation, among others. Its main classes of compounds are terpenes, alkaloids, and phenolic compounds [26, 27].

In consequence, in this chapter, we performed, by molecular docking, a screening of molecules from plants that showed results of *in vitro* antimicrobial activity against *S. mutans*, to verify the possibility of interaction and inactivation of virulence factors of this bacteria.

2. Molecular docking between phytochemicals and S. mutans targets

2.1 Selection of the ligands

Ligands were selected from a literature search on phytoconstituents or plants with antimicrobial activity, *in vitro*, against *S. mutans*. The search was performed in the

Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed), using the following terms as keywords: S. mutans, natural products, and anti-cariogenic effects, without language specification or deadline. All articles that addressed the antimicrobial activity of phytoconstituents (isolated molecules) with action on S. mutans, or with activity related to the reduction of cariogenic dental biofilm, were considered relevant. After these filters, 24 articles remained that had defined chemical structures of molecules with an inhibitory effect against S. mutans. The molecules identified and selected for the study in these articles are shown in **Figure 1**.

2.2 Selection of protein targets in *S. mutans*

The first inclusion criterion was the selection of S. mutans target proteins with high relevance for the virulence of this microorganism [28]. The availability of the crystallographic structures resolved and available in the Protein Data Bank (PDB) was the second inclusion criterion. The protein targets (receptors), their functions, PDB identifiers, and grid box coordinates are presented in **Table 1**.

2.3 Molecular docking analysis

Molecular modeling was performed as described by Rodrigues et al. [29]. Using Hyperchem v. 8.0.3, the chemical structures of all compounds of interest (ligands) were drawn and their geometric structures were optimized using the MM+ force field. Subsequently, a new geometry optimization was performed based on the AM1 semi-empirical method (Austin Model 1). The optimized structures were subjected to conformational analysis using Spartan software for Windows 10.0. The Monte Carlo computational method with 1000 interactions, 100 optimization cycles, and 10 conformations with the lowest energy level was selected. The dihedral angles were evaluated by rotation according to the standard conditions (default) of the program, in which the number of simultaneous variations was 1–8, acyclic chains were subjected to rotations from 60 to 180°, and the torsion rings, to rotations from 30 to 120°. The conformations with the lowest minimum energies were selected and saved in .sdf format. Receivers (protein target) were obtained from the PDB. Receiver, PDB id, and selected three-dimensional coordinates for docking are described in **Table 1**. Docking simulations were performed in AutoDock 4.2 software. The preparation of receptors and ligands was performed using VEGA ZZ 3.0.1 and MOLEGRO Molecular Viewer 2.5 software. Initially, ligand and receptor structures were saved in .pqbqt format to be used in docking calculations. Then, PyRx 0.9 software was used to assist in the docking steps and the analysis of the results. The "grid maps", which represent the boxes with three-dimensional coordinates determined for each receiver, were calculated with AutoGrid. Each ligand was docked inside its "grid" with the Lamarckian algorithm implemented in the AutoDock software. The genetics-based algorithm ran 12 simulations per ligand with 2,500,000 energy ratings and a maximum number of 54,000 generations. The crossover rate was increased to 0.8, the gene mutation rate was 0.02, and the number of individuals in each population was 200. All other parameters were left with the default AutoDock settings. The results for each calculation were analyzed to obtain the affinity energy of docking score (Edock) in kcal/mol values for each ligand conformation in its respective complex; structure inaccuracies were ignored in the calculations. To verify the number and positions of hydrogen

bonds and non-covalent interactions between each ligand conformation and the catalytic residues of the receptors, the software PyMOL 1.4 and Molegro Molecular Viewer 2.5 were used.

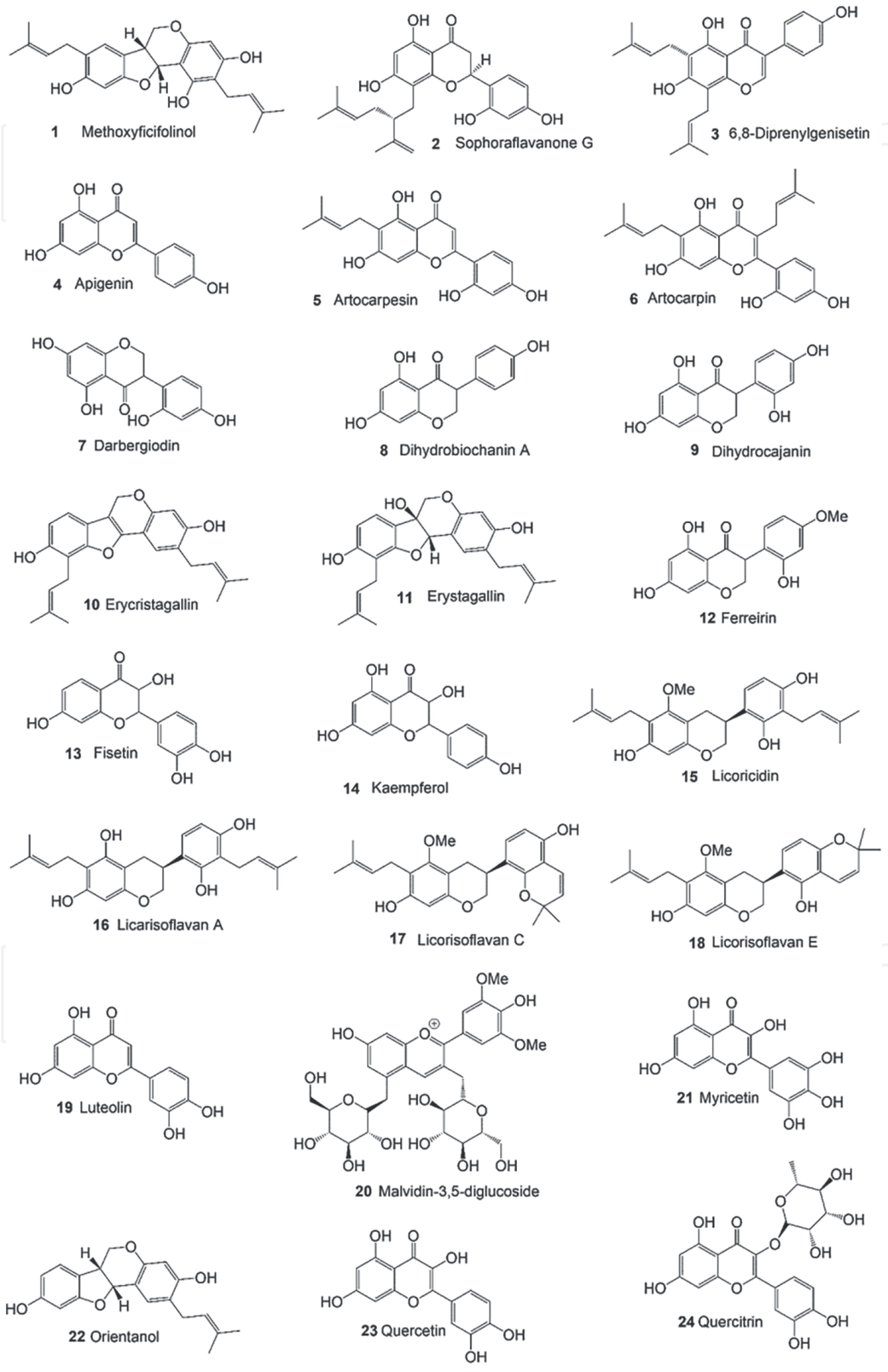

Figure 1.
Selected compounds for molecular docking in S. mutans *target proteins.*

Classification	Macromolecule (receiver)	PDB id	Coordinates			Ray (Å)
Function			X	Y	Z	
Adhesin	Antigen I/II (V-region)	1JMM	34.77	20.04	−7.82	20
Adhesin	Antigen I/II (carboxy-terminal)	3QE5	74.38	44.62	141.82	25
Adhesin	Spap	3OPU	−20.85	53.58	6.16	15
Signaling proteins *(quorum sensing)*	Signaling peptide UA159sp	2I2J	16.12	−1.42	3.73	15
Signaling proteins *(quorum sensing)*	Signaling peptideTCP3	2I2H	11.9	−3.45	0.99	15
Signaling proteins *(quorum sensing)*	ATP binding protein ComA	3VX4	35.31	35.17	13.77	15
Exoenzyme	Glucanosucrase	3AIC	192.19	44.63	197.26	15
Exoenzyme	Dextranase	3VMO	8.71	−13.02	−0.67	15
Exoenzyme	Hemolysin	2RK5	13.57	36.99	17.83	30

Table 1.
Names of macromolecules (receivers), identifier in the Protein Data Bank (PDB id), and selected three-dimensional coordinates for docking.

3. Molecular docking screening results

Molecular docking is an *in silico* methodology that makes it possible to simulate the orientation and conformations (poses) of a ligand near the active site of a target macromolecule, evaluating intermolecular forces, such as hydrophobicity, Van der Waals forces, hydrogen bonds, interactions electrostatic, and ionic bonds. Moreover, this methodology provides the energy of interaction (docking scores), the types of interaction, and the amino acid residues involved in the formation of the ligand-receptor complex [30].

The scores are used as a reference to rank the most stable poses of the ligand. Therefore, the lower the score value, the stronger and more stable the interaction with the selected target. The role and functioning of each of the nine selected S. mutans target proteins are briefly presented below, along with the presentation of the three best ligands for each of the proteins.

3.1 Adhesins

3.1.1 Region V of antigen I/II (PDB id: 1JMM)

The protein-antigen AgI/II is an adhesin present in the cell wall of S. mutans, which recognizes and binds to salivary glycoproteins on the tooth surface, enabling the formation of dental biofilm [31, 32]. Anti-AgI/II antibodies block the adhesion and colonization of S. mutans in the oral cavity [33, 34], justifying the interest in this adhesin in studies aimed at the development of an anticaries therapy [35].

AgI/II adhesin exhibits a functional supramolecular architecture on the cell surface [36], as well as an unusual tertiary structure, where a central variable domain (V-domain) appears like the tip of a formed stem by intertwined and flanked

regions rich in alanine and proline [37]. The carboxy-terminal domain (C-domain), connected to a small N-terminal domain that attaches to the cell wall through an anchoring region [38]. AgI/II binding sites for DMBT1 agglutinin are located in the V-domain and C-domain [39].

Docking identified as the best ligands for antigen I/II (V-region) PDB id: 1JMM were the compounds: maldivin-3,5-diglucoside (20) (Edock = −160.78 kJ/mol), licorisoflavan C (17) (Edock = −151.50 kJ/mol), and erystagallin (11) (Edock = −139.85 kJ/mol). Common steric interactions in the complexes formed between Ser818 and Ser697 residues and compounds 17 and 20 were observed. As well as between residue Trp818 and compounds 11 and 17 (**Figure 2**).

3.1.2 Antigen I/II (carboxy-terminal) (PDB id: 3QE5)

The carboxy-terminal domain of antigen I/II, as well as other proteins in this family, can bind salivary glycoproteins, extracellular matrix molecules, and ligands from other bacteria. This category of proteins is not exclusive to **S. mutans**. Homologous proteins subsist in other *Streptococci* [40].

The I/II antigen is highly conserved and may be associated with M protein in other streptococcal species. The carboxy-terminal region (with 800–1540 amino acid residues) includes proline-rich (P) repeats, conferring hydrophobicity, a transmembrane domain (with 1537–1556 amino acid residues), and an LPXTG motif required for anchorage to the cell wall catalyzed by sortase [32, 41].

The phytochemicals with the most promising linkages with the antigen I/II (carboxy-terminus) PDB id: 3QE5 were: erycristagallin (10) (Edock = −128.98 kJ/mol), sophoraflavanone G (2) (Edock = −105.77 kJ/mol), and erystagallin (11) (Edock = −105.16 kJ/mol). All compounds had in common hydrogen bonds with the Lys1120 residue and steric interactions with the Thr1118 residue, thus indicating that these amino acids are important for minimizing the binding energies and stabilizing the complexes (**Figure 3**).

3.1.3 Spap (PDB id: 3OPU)

The Spap protein, also called P1, is a multifunctional adhesin that mediates the sucrose-independent adhesion of bacteria to salivary film glycoproteins on the tooth

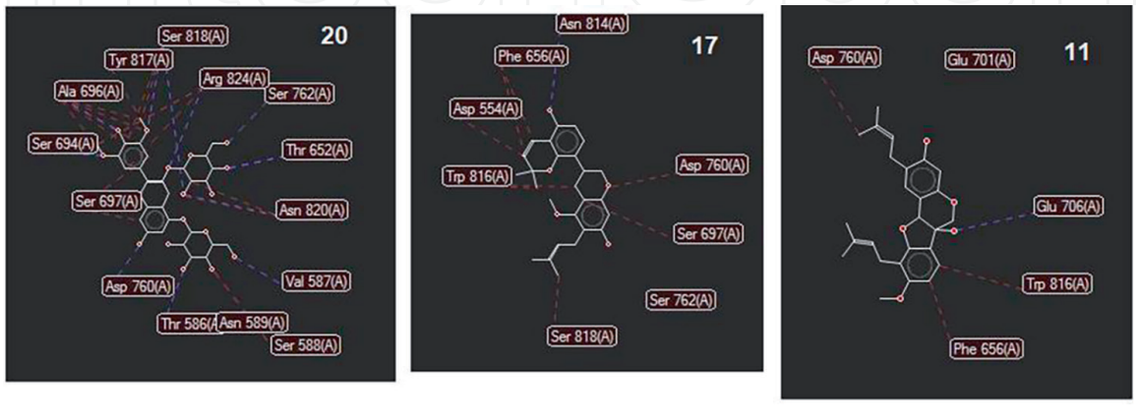

Figure 2.
Representations of the interactions between the three best ligands (compounds 20, 17, and 11) and the amino acid residues of the antigen I/II (V-region) PDB id: 1JMM. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

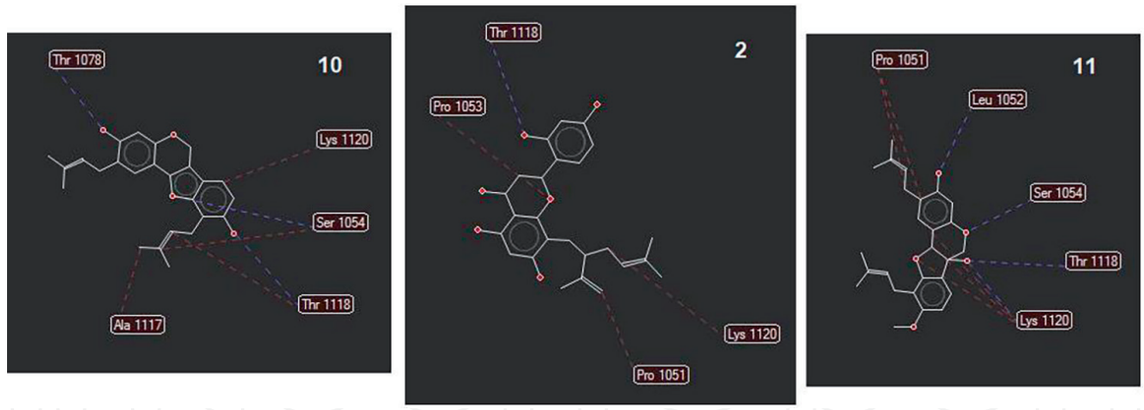

Figure 3.
Representations of the interactions between the three best ligands (compounds 10, 2, and 11) and the amino acid residues of antigen I/II (carboxy-terminal) PDB id: 3QE5. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

surface. Like other extracellular proteins, this adhesin can produce amyloid, which, in turn, is present in dental biofilms. Thus, this protein directly interferes with the facilitation and adhesion of cariogenic bacteria [21, 42].

The best interactions with Spap PDB id: 3OPU occurred with the compounds: sophoraflavanone G (2) (Edock = −136.98 kJ/mol), erystagallin (11) (Edock = −134.89 kJ/mol), and licorisoflavan (18) (Edock = −129.64 kJ/mol). The common interactions between these ligands and the active site of the protein, which contributed to the low values of the scores of these molecules, are the steric interactions with residues Lys1261 and Pro1210, and hydrogen bonds with residue Asp1208 (**Figure 4**).

3.2 Quorum sensing-associated signaling proteins

3.2.1 Signaling peptide UA159sp (PDB id: 2I2J)

The peptide-mediated quorum sensing in *S. mutans* is well conserved and regulates its genetic competence [28, 43, 44]. This signaling circuit plays regulatory roles in biofilm formation [43, 45, 46], stress response [47], and bacteriocin production, which are important virulence factors of this bacterium [43, 48].

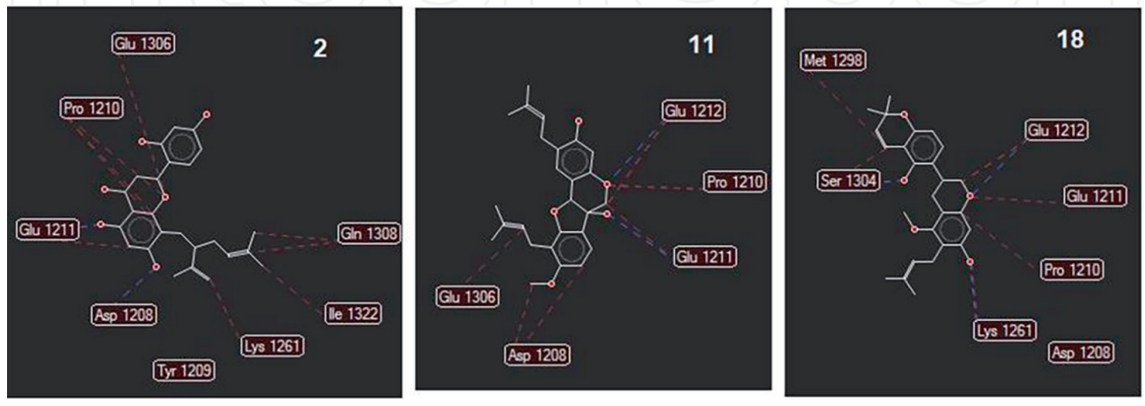

Figure 4.
Representations of the interactions between the three best ligands (compounds 2, 11, and 18) and the amino acid residues of Spap PDB id: 3OPU. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

Quorum-sensing allows bacterial communication, providing benefits during host colonization, defense against competitors, and adaptation to the environment [43, 49]. The chemical details of the signaling molecules of this system in **S. mutans** are known and only UA159sp has been identified as a signal peptide in wild-type **S. mutans** strain [44].

In a study, conducted by Syvitski et al. [50], peptides in which three or more residues were deleted from the C-terminal region of the signaling peptide UA159sp did not induce genetic competence and inhibited, by competition, the quorum sensing activated by UA159sp. Disruption of the amphipathic α-helix by replacing Phe-7, Phe-11, or Phe-15 residues with a hydrophilic residue resulted in a significant reduction or complete loss of peptide activity. In contrast to peptides truncated at the C-terminal region, these peptides with amino acid substitutions did not compete with UA159sp to activate quorum sensing, suggesting that disruption of the hydrophobic face of the α-helix structure results in a peptide that is not capable of binding to the receptor. Therefore, residues of the C-terminal region of the signaling peptide in the quorum-sensing system of streptococci are extremely important.

Quorum-sensing inhibitor drug design enables the development of more specific treatments for biofilm-dependent infectious diseases [51]. A benefit of using quorum sensing inhibitor drugs is that they are less susceptible to antimicrobial resistance than other antimicrobials, as they exert a lower selective pressure and do not directly kill bacterial cells [52].

Docking with the signaling peptide UA159sp PDB id: 2I2J identified as the best ligands the compounds: erystagallin (11) (Edock = −84.98 kJ/mol), erycristagallin (10) (Edock = −83.99 kJ/mol), and methoxyficifolinol (1) (Edock = −79.76 kJ/mol). In all ligands, the presence of hydrogen bonds with the Ser14 residue and steric interactions with the Ala18 residue is indicative of their importance for the stability of the interaction of these compounds with the active site (**Figure 5**).

3.2.2 Signaling peptide TPC3 (PDB id: 2I2H)

TPC3 peptide is a signal peptide synthesized by the mutant strain of **S. mutans** JH1005 that also can activate the quorum-sensing system.

For the signaling peptide TCP3 PDB id: 2I2H the best ligands were: erycristagallin (10) (Edock = −-99.74 kJ/mol), sophoraflavanone G (2) (Edock = −93.23 kJ/mol), and methoxyficifolinol (1) (Edock = −88.16 kJ/mol). As can be seen in **Figure 6**, hydrogen

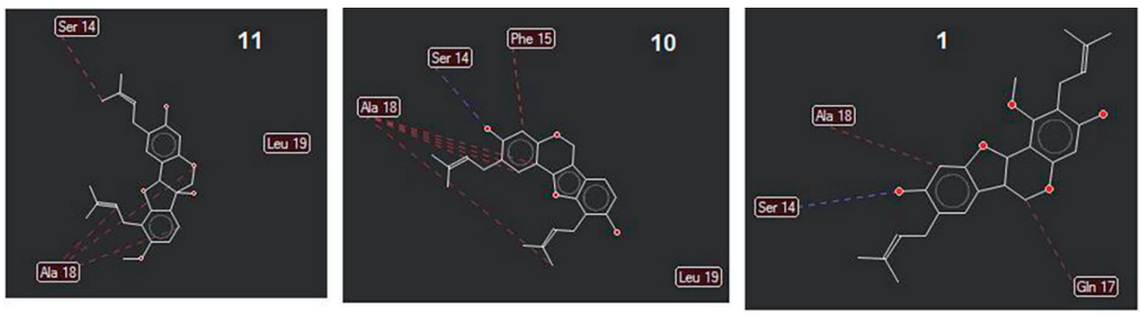

Figure 5.
Representations of the interactions between the three best ligands (compounds 11, 10, and 1) and the amino acid residues of the signal peptide UA159sp PDB id: 2I2J. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

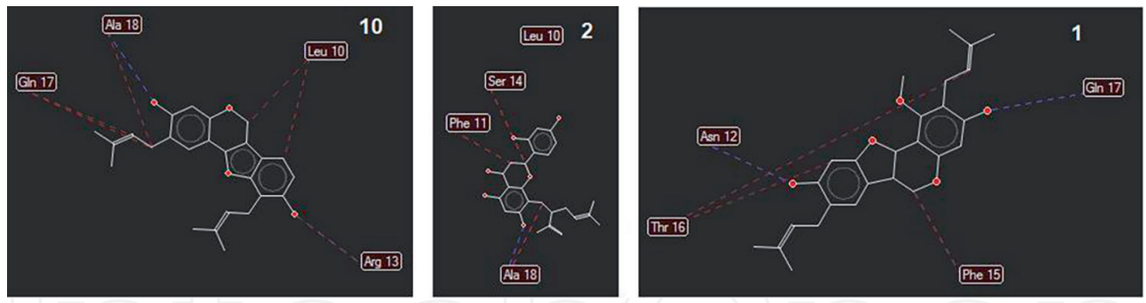

Figure 6.
Representations of the interactions between the three best ligands (compounds 10, 2, and 1) and the amino acid residues of the signal peptide TCP3 PDB id: 2I2H. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

bonds and steric interactions with Ala18 and Leu10 residues contributed to the energy reduction of these complexes, especially of the best ligands.

3.2.3 ATP binding protein ComA (PDB: 3VX4)

Quorum sensing is mediated by a signaling molecule autoinducer [53]. This system in some streptococcal species such as **S. mutans** is the ComABCDE pathway, in which inducing peptides are processed from the ComC precursor and exported to the extracellular space by ComA and ComB [43, 54]. ComA is a bifunctional ATP-binding cassette transporter comprising three domains: an N-terminal peptidase domain (PEP), a transmembrane domain, and a C-terminal nucleotide linker domain [55–57]. PEP is a peptidase belonging to the cysteine protease family [55, 58–60].

Docking with the ATP binding protein ComA PDB id: 3VX4 identified as the best ligands the compounds: licorisoflavan A (16) (Edock = −-132.56 kJ/mol), licoricidin (15) (Edock = −128.75 kJ/mol), and methoxyficifolinol (1) (Edock = −127.50 kJ/mol). When observing the interactions of the best ligands in the formed complexes, it was observed that hydrogen bonds with residues Thr568 and Ser563 and steric interactions with Lys567 are common, indicating that these interactions contributed to the reduction of the interaction energy and stabilization of the complexes (**Figure** 7).

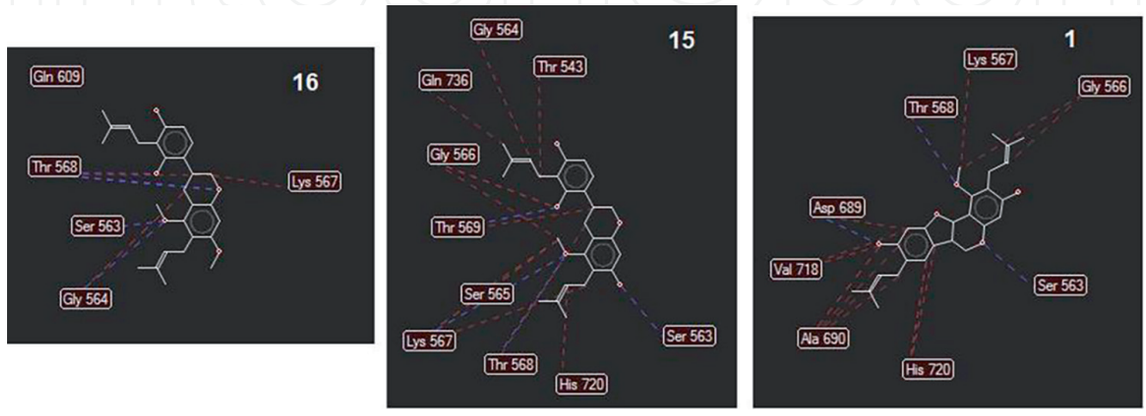

Figure 7.
Representations of the interactions between the three best ligands (compounds 16, 15, and 1) and the amino acid residues of the ATP binding protein ComA PDB id: 3VX4. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

3.3 Exoenzymes

3.3.1 Glucanosyucrase (PDB id: 3AIC)

Glucansucrases or glycosyltransferases (GTFs) are extracellular enzymes, produced by various bacteria, including **S. mutans**, that cleave sucrose into glucose and fructose and build sticky biofilm chains. The growth of the glucan chain was associated with adherence of one bacteria to another and the dental surface. Furthermore, modulate the diffusion of substances through the biofilm, which could occasionally serve as an extracellular energy reserve [61].

The glucanosucrase in *S. mutans* allows the metabolism of sucrose into lactic acid, which reduces the pH around the tooth, facilitating the dissolution of calcium phosphate from tooth enamel, which induces tooth decay [62]. These characteristics make the **S. mutans** glucanosucrase as one of the main and most studied targets for the development of new agents useful in the prevention of dental caries.

The best ligands that interacted with glucansucrase PDB id: 3AIC in the docking simulation were: erycrystagallin (10) (Edock = −145.72 kJ/mol), malvidin-3,5-diglucoside (20) (Edock = −138.84 kJ/mol), and erystagallin (11) (Edock = −136.44 kJ/mol). Hydrogen bonds with residues Asp480, Asp481, Asn537, and steric interactions with residues Leu433, Glu515, and Trp517 are common to the two best ligands and seem to be important for reducing the energy of formation of these complexes (**Figure 8**).

The docking study conducted out by Kim et al. [63] between rubusoside and **S. mutans** glucanosucrase (PDB id: 3AIC), identified residues Leu 433, Leu434, Ala478, Asp480, Glu515, Trp517, and Tyr916 as the main ones involved in the stabilization of the complex, and validated these residues as important anchoring sites for potential inhibitors of this enzyme.

Bhagavathy, Mahendiran, and Kanchana [64], performed molecular docking between seven phytochemical isolates of *Psidium guajava* and *S. mutans* glucanosucrase (PDB id: 3AIB) and demonstrated that the main residues involved in the formation of the complexes were Thr426, Ile427, Gln553, and Tyr978. These residues diverged from those identified in this study.

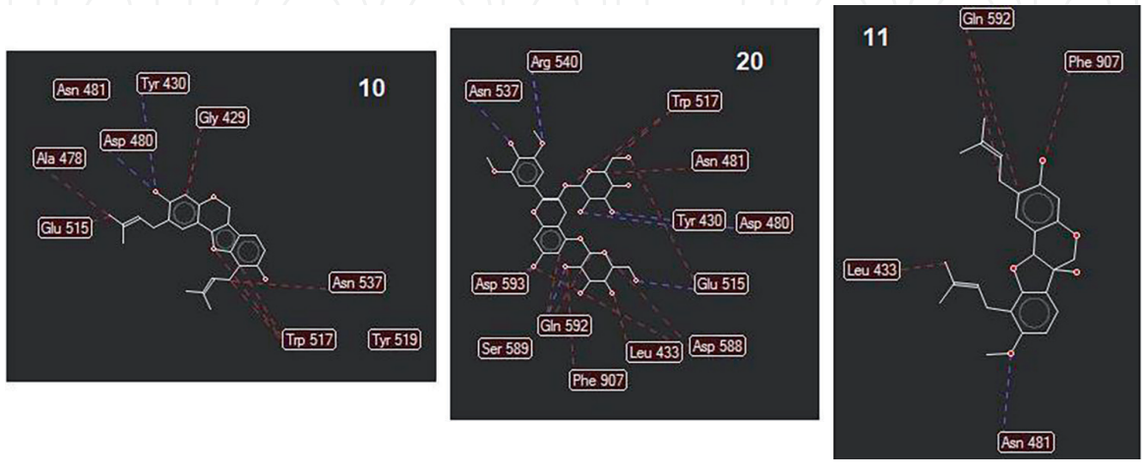

Figure 8.
Representations of the interactions between the three best ligands (compounds 10, 20, and 11) and the amino acid residues of the glucanoscarase PDB id: 3AIC. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

Opposing, Islam et al. [65] performed a molecular docking study between epigallocatechin gallate (EGCG) and the same **S. mutans** enzyme, glucanosucrase (PDB id: 3AIB). The results showed that the main interactions that stabilize the complex of the ligand (EGCG) with the enzyme occurred between the amino acid residues Glu515 and Trp517, which were the same residues identified in our work, reinforcing the importance of these residues for the stabilization of the complex.

3.3.2 Dextranase (PDB id: 3VMO)

S. mutans dextranase is an enzyme that hydrolyzes the α-1,6 bonds of dextran and produces isomalto-oligosaccharides of different sizes for metabolic use [66, 67]. This protein is composed of 850 aa residues with a molecular mass of 94.5 kDa, but it has multiple native and recombinant forms [68, 69]. According to the sequencing of several enzymes in this family, dextranases are divided into five regions: a signal peptide sequence (N-terminal with 24 aa), a variable N-terminal region (Ser25-Asn99), a conserved region (Gln100-Ala615), a glucan binding site (Leu616-Ile732), and a C-terminal variable region (Asn733-Asp850) [70, 71].

Some biochemical studies, based on the comparison of amino acid sequences with other glycosyltransferases, revealed that the Asp385 residue is essential for the catalytic reaction [72]. Besides, it was observed that Asp270 from cycloisomalto oligosaccharide glucanotransferases from *Bacillus circulans* T3040 [73] and Asp243 from endodextranase from *Thermotoga lettingae* TMO [74], corresponding to Asp385 from dextranase from **Streptococcus mutans**, were recognized as **S. mutans** residues catalytic.

Molecular docking performed with the dextranase PDB id: 3VMO identified as the best ligands: licorisoflavan A (16) (Edock = −138.02 kJ/mol), malvidin-3,5-diglucoside (20) (Edock = −136.94 kJ μg/mol), and licoricidin (15) (Edock = −129.73 kJ/mol). Compounds 15 and 16 showed steric interactions in common with residues Tyr257 and Ala559 and showed steric interactions and hydrogen bonds with the key residue Asp385 which has already been identified as essential for catalytic reaction. Diglucoside 20, on the other hand, had a lower energy conformation distinct from compounds 15 and 16 and interacted with other amino acid residues in the active site of the enzyme (**Figure 9**).

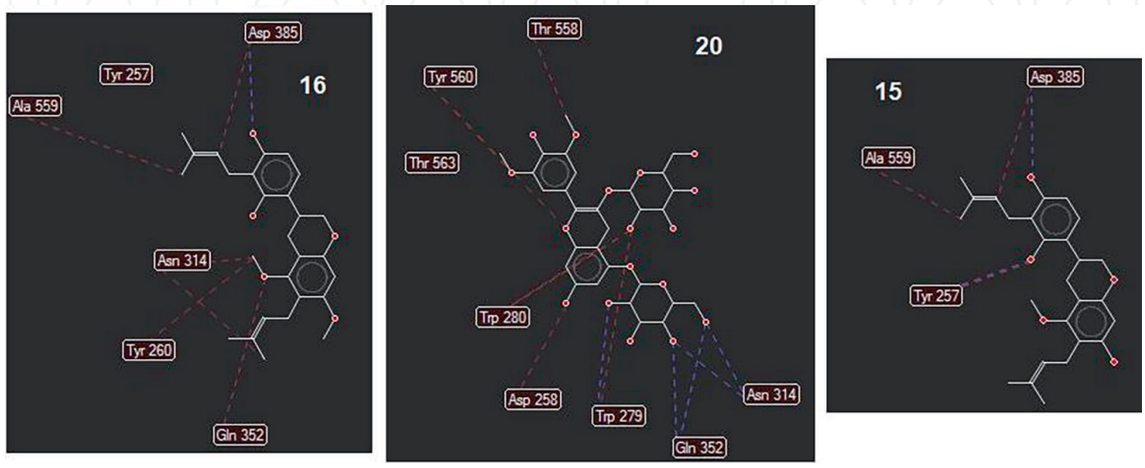

Figure 9.
Representations of the interactions between the three best ligands (compounds 16, 20, and 15) and the amino acid residues of dextranase PDB id: 3VMO. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

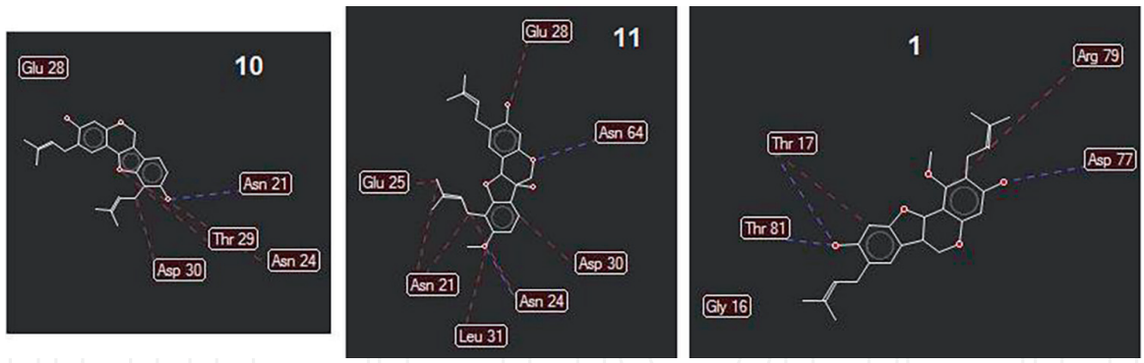

Figure 10.
Representations of the interactions between the three best ligands (compounds 10, 11, and 1) and the amino acid residues of hemolysin PDB id: 2RK5. Blue dashed lines represent hydrogen bonds and red dashed lines represent steric interactions.

3.3.3 Hemolysin (PDB id: 2RK5)

Hemolysins are exotoxins capable of promoting erythrocyte lysis. They are toxins produced by some species of streptococci [75] and contribute to the virulence process of **S. mutans** [76]. In **S. mutans**, alpha- and gamma-hemolytic strains are described [77], as well as beta-hemolytic [78].

Docking with hemolysin PDB id: 3VMO identified as the best ligands the compounds: erycristagallin (10) (Edock = −112.64 kJ/mol), erystagallin (11) (Edock = −104.10 kJ/mol), and methoxyficifolinol (1) (Edock = −100.63 kJ/mol). The steric interactions and hydrogen bonds with Asn21, Asn24, and Asp30 residues were common for compounds 10 and 11, and seem to be important for the stabilization of the complexes. Compound 1, despite belonging to the same chemical class as compounds 10 and 11, showed a more stable conformation in another position of the active site, consequently, is stabilized by interactions with different amino acid residues, but which contributed less to the stabilization of the complex (**Figure 10**).

4. Concluding remarks

In the research phase for phytochemicals with activity against *S. mutans*, carried out in the present study, no specific research was carried out for the classes of phytoconstituents. However, surprisingly, all isolated and identified chemical structures (24 compounds) belonged to the class of phenolic compounds, more specifically the class of flavonoids (2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 24) and isoflavonoid derivatives (1, 10, 11, and 22).

Five phytocompounds evaluated were elected as one of the three best ligands for at least three target proteins, highlighting the following compounds: 11 (erystagallin) (highlighted for 6 targets), 10 (erycristagallin) (highlighted for 5 targets), 1 (methoxyficifonilol) (highlighted for 4 targets), 20 (malvidin-3,5-diglucoside), and 2 (sophoraflavanone G), which provided indications of a possible and desirable multitarget action of these compounds.

Based on these findings, these selected compounds should have theirs *in vitro* and *in vivo* activities evaluated, to validate the efficiency of these compounds in inhibiting the virulence factors of planktonic *S. mutans* and in biofilms. The positive results in these tests will allow the incorporation of these phytoconstituents in toothpaste, mouthwashes, among others, and could be an effective alternative for the control of tooth decay.

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Conflict of interest

The authors declare that they have no conflict of interest with this manuscript.

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