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Original Article

Comparison of the Effect of Fluoride 0.2% and a Combined Mouthwash (Flavonoid Compounds and Fluoride 0.2%) Against *Streptococcus mutans* and *Lactobacillus acidophilus*: *In Silico* and *In Vitro* Study

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Abstract

Background: Tooth decay is one of the most common health problems in the world. Nowadays, finding new compounds to prevent tooth decay is more necessary than ever. The purpose of this study was to compare the effects of fluoride 0.2% and mouthwash combined with flavonoid compounds against bacterial samples.

Methods: The crystal structures of glucansucrase from *Streptococcus mutans* and glucan-1, 6-alpha-glucosidase from *Lactobacillus acidophilus* were obtained from the Protein Data Bank. By using AutoDockTools (1.5.7), ligands and protein interactions were calculated and ready for AutoDock vina. The agar well diffusion and the minimum inhibitory concentration/minimum bactericidal concentration (MIC/MBC) methods were used to investigate the inhibitory effect of mouthwashes, and the results were obtained with SPSS software.

Results: Compounds eriocitrin and galangin showed the highest amount of H-bonds with amino acids against glucansucrase. In addition, catechin, eriocitrin, and isorhamnetin compounds demonstrated the highest amount of H-bonds with amino acids against glucan-1, 6-alpha-glucosidase. *In vitro* results revealed that groups a (Fluoride 0.2%+eriocitrin against *S. mutans*) and d (Fluoride 0.2%+eriocitrin against *L. acidophilus*) represented the most effect among all compounds, respectively (Inhibition zone=26±0.5 mm, MIC=250 µg/mL, MBC=500 µg/mL and inhibition zone=31±0.5 mm, MIC=125 µg/mL, MBC=250 µg/mL).

Conclusion: Fluoride 0.2% with eriocitrin was more effective in both methods (*In silico* and *in vitro*) compared to fluoride 0.2% due to its good inhibitory effect at different concentrations against *S. mutans* and *L. acidophilus*.

Keywords: Flavonoids, *Streptococcus mutans*, *Lactobacillus acidophilus*, Mouthwashes, Molecular docking simulation

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Background

Dental caries is one of the most common chronic and preventable diseases in the world (1). In general, caries is considered to be the result of the interaction between caries-causing oral flora and fermentable carbohydrates on tooth surfaces over time (2). According to several epidemiological studies, *Streptococcus mutans* is related to dental caries, and it seems that this gram-positive bacterium plays the main role in the initiation of caries (3). *S. mutans* affects tooth enamel by fermenting sucrose and producing lactic acid. In addition, this bacterium uses sucrose to make dental plaque. Dental plaque is made of dextran, which is a type of polysaccharide (4). *S. mutans* produces three types of glucosyltransferases (GTF-I, GTF-SI, and GTF-S). The glucosyltransferase gene (gtfB) is encoded by GTF-I, is involved in the initial stages of glucan synthesis from sucrose, and is insoluble in water. This gene is one of the most important factors in the virulence of this bacterium (5-7). Glucan also plays a role in the formation of dental plaque and the firm adhesion of microorganisms to the tooth surface, resulting in the accumulation of acid and the beginning of decalcification on the surface of the enamel (8). Further, using advanced molecular techniques, several studies have shown that lactobacilli are located in the advanced areas of carious lesions, and probably these bacteria are related to dental caries (9). The reason for

surface decay is the effect of acidic products resulting from bacterial fermentation, following the digestion of the protein matrix (matrix) by bacteria that cause tooth decay (10). Flavonoids are a group of naturally occurring polyphenolic compounds characterized by the flavan core and are one of the most common classes of compounds in fruits, vegetables, and plant-derived beverages (11). More than 8000 compounds with flavonoid structures have been identified. Together with carotenoids, they are responsible for the vibrant colors of fruits and vegetables. Some of the best-known flavonoids are quercetin and kaempferol. In plants, these compounds protect against UV rays, pathogens, and herbivores (12). Flavonoids are considered health-promoting and disease-preventing food supplements. Epidemiological, clinical, and animal studies show that flavonoids have antibacterial (13), antifungal (14), anticancer (15), and antioxidant (16) effects. According to the contents, this study focused on an *in vitro* and *in silico* comparison of the effects of fluoride 0.2% and a combination mouthwash (Flavonoid compounds and fluoride 0.2%) against *S. mutans* and *L. acidophilus* for use in mouthwashes and toothpastes.

Methods

In Silico: The crystal structure of glucansucrase from *S. mutans* (3AIE) and the structure of glucan-1, 6-alpha-glucosidase from *L. acidophilus* (4AIE) were received from https://www.rcsb.org (Protein Data Bank). The structures of flavonoids as ligands were received from https://pubchem.ncbi.nlm.nih.gov (PubChem).

In Vitro: All flavonoid compounds had been purchased from Sigma-Aldrich Company (Germany). The bacterial strains (*S. mutans* ATCC 35668 and *L. acidophilus* ATCC 4356) were organized by the Iranian Industrial Microorganisms Collection Center (Lyophilized). Microbiological assessments had been accomplished with the use of a Memmert- INC153T2T3 incubator.

In Silico

Ligand Preparation

The 2-dimensional (2D) structures of flavonoids (Table 1) as ligands (No. 1-10) were retrieved from ChemDraw Ultra 12.0.2.1076 (Figure 1) and saved in .pdb format. Use ChemBio3D Ultra 12.0 for the optimization of the ligands (17).

Table 1. Structures Information of Flavonoid Compounds

No.	Name	PubChem CID	Molecular Formula	Molecular Weight (g/mol)
1	Acacetin	5280442	$C_{16}H_{12}O_5$	284.26
2	Apigenin	5280443	$C_{15}H_{10}O_5$	270.240
3	Caffeic acid	689043	$C_9H_8O_4$	180.16
4	Catechin	9064	$C_{15}H_{14}O_6$	290.271
5	Chrysin	5281607	$C_{15}H_{10}O_4$	254.241
6	Daidzein	5281708	$C_{15}H_{10}O_4$	254.23
7	Eriocitrin	83489	$C_{27}H_{32}O_{15}$	596.538
8	Fisetin	5281614	$C_{15}H_{10}O_6$	286.2363
9	Galangin	5281616	$C_{15}H_{10}O_5$	270.240
10	Isorhamnetin	5281654	$C_{16}H_{12}O_7$	316.26

Protein Preparation

The 2D structure of glucansucrase from Streptococcus mutans (PDB ID: 3AIE) and glucan-1, 6-alpha-glucosidase from Lactobacillus acidophilus (PDB ID: 4AIE) were obtained from the Protein Data Bank (https://www.rcsb.org/) with a resolution of 3.00 Å (Å: Angstrom) and in .pdb format. Subsequently, using Discovery Studio Client 4.5 software, the ligands and water molecules were separated from the main structures. Finally, the structures were prepared by adding polar hydrogens using AutoDockTools-1.5.6 software and saved in .pdbqt format.

Molecular Docking

Using the AutoDockTools (1.5.7) program, all polar hydrogen atoms have been introduced to the protein structure, and the partial masses of the ligands have been calculated and introduced with the Discovery Studio 4.5 Client program. The grid box for 3AIE was 118 × 118 × 118 dimensional, and dimensions have been additionally taken into consideration as X-center = 62.833, Y-center = 55.306, and Z-center = 3.139. Furthermore, the grid box for 4AIE was 94 × 94 × 94 dimensional, and dimensions have been additionally taken into account as X-center = 9.002, Y-center = 26.444, and Z-center = 27.248. The overall findings obtained through molecular docking were examined using AutoDockTools-1.5.6 software (18). To analyze the results in greater detail, the ligand-receptor complex corresponding to the conformation with the highest binding energy was prepared and assessed for each compounds using Discovery Studio Client 4.5 software (2D and 3D).

In Vitro

The bacteria that were prepared as lyophilized were inoculated in the broth medium. Brain heart infusion (BHI) agar/broth and Man–Rogosa–Sharpe (MRS) agar/broth were used for *S. mutans* and *L. acidophilus*., respectively. Next, the bacterial samples were kept for 48 hours in an anaerobic jar at 37 °C. Then, the bacteria suspension was prepared according to McFarland's 0.5 standard.

Agar Well Diffusion Method

To perform this experiment, wells of 5 mm in diameter were created by a sterile pipette in culture media containing cultured bacterial suspension. Then, the wells were filled with samples (*S. mutans* wells: Fluoride 0.2% (control), fluoride 0.2% + eriocitrin (a), Fluoride 0.2% + galangin (b). *L. acidophilus* wells: Fluoride 0.2% (control), fluoride 0.2% + catechin (c), Fluoride 0.2% + eriocitrin (d), Fluoride 0.2% + isorhamnetin (e)). Next, the plates were put inside the incubator for 24 hours at 37 °C. It is worth noting that

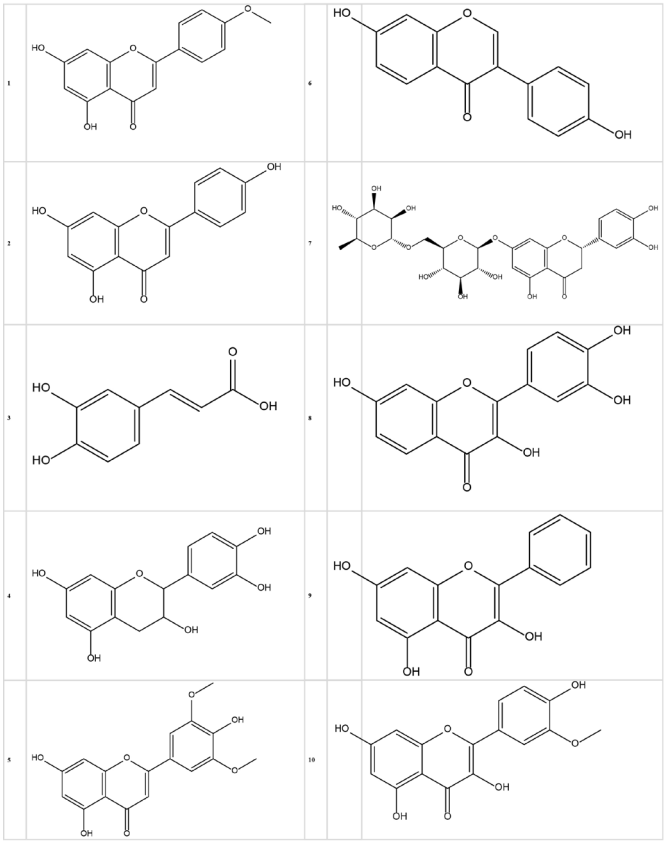

Figure 1. 2D Structures of Flavonoid Compounds (1-10). *Note*. 2D: 2-dimensional

all the steps were performed near the flame and in a sterile environment (19). This experiment was repeated three times, and their mean was reported in the results.

Broth Dilution Method

To determine the minimum inhibitory concentration (MIC), a series of 9 tubes were used to test the different dilutions of each compound. It is noteworthy that the control sample was diluted in 9 separate tubes. The initial concentration of each compound was 2 mg/mL (2000 µg/mL), which was obtained by inserting 1 mL of the compounds into the first tube containing 1 mL of culture medium at a concentration of 1 mg/mL (1000 µg/mL). Different dilutions were obtained from tubes No. 1 (2 mg/mL) to No. 9 (0.007 mg/mL). To this end, 1 mL of the compounds in the first tube with a concentration of 2 mg/mL was diluted with 1 mL of culture medium in the second tube (BHI broth and MRS broth were utilized for *S. mutans* and *L. acidophilus*, respectively). In this way, 1 mL was removed from the first tube and added to the second tube, containing 1 mL. This was continued up to tube No. 9, then 1 mL was removed from the last tube and ejected, which eventually resulted in the half-dilution of the previous tube. Then, 50 µL of microbial suspension containing 1.5×108 bacteria were transferred to the tubes. All the test tubes were incubated (for 24 hours at 37℃). After incubation, the tubes were tested for turbidity because of the bacterial growth. All tubes with no bacterial growth were sampled and cultured to determine the minimum bactericidal concentration (MBC) of the compounds. For this purpose, the tubes showing no bacterial growth were cultured on the culture medium (BHI agar and MRS agar were used for *S. mutans* and *L. acidophilus*, respectively). After incubation for 24 hours, the cultured plates were controlled for microbial growth. The lowest concentration of compounds in the relevant plates, exhibiting bacterial growth failure, was considered the MBC of those compounds (20).

Results

In Silico

All affinities of flavonoid compounds (1-10) against bacterial strains were calculated, the results of which are provided in Table 2. The affinity of all compounds was reported between -5.2 and -9.2 kcal/mol. Among these, compounds 7 (eriocitrin) and 9 (galangin) demonstrated the highest amount of H-bonds with amino acids against 3AIE; further, these compounds created the highest amount of variety and number of hydrogen bonds with amino acids such as asparagine, glutamine, aspartic acid, and glutamic acid with compound 7 (eriocitrin) and amino acids serine, tryptophan, and threonine with compound 9 (galangin). Compounds 4 (catechin), 7 (eriocitrin), and 10 (isorhamnetin) showed the highest amount of H-bonds with amino acids against 4AIE; moreover, these compounds created the highest amount

Table 2. Chem 3D and AutoDockVina Results of Compounds (1-10)

Compounds	Total Energy (KCal/Mol)	Affinity ΔG_{bind} (KCal/Mol)		H-Bonds		P_i-P_i	
		S. Mutans (3AIE)	*L. acidophilus* (4AIE)	*S. Mutans* (3AIE)	*L. acidophilus* (4AIE)	*S. Mutans* (3AIE)	*L. acidophilus* (4AIE)
Acacetin	21.4567	-7.4	-8.4	PHE866 GLN864	ARG401 ARG212 ASP198	ALA865 MET614 LYS618	ASP316 TYR62 PHE162 TRP242 VAL199
Apigenin	14.4725	-7.3	-8.7	ASN481 ASP477	ARG212 GLU201	ASP480 LEU433 ALA478	ASP316 ARG401 GLU240 TRP242
Caffeic acid	20.9818	-5.2	-6.5	LEU395 SER397	SER3 SER261	VAL1074	PRO255
Catechin	-3.9469	-7.6	-8.9	ASP593 ASP477	**SER253 GLU259 ASP234 LYS232 HIS258**	GLN592 TYR916 ASP588	PRO255
Chrysin	15.6501	-6.7	-8.5	GLN592	-	TYR916 ASP909 ASP588	LYS279 TRP242
Daidzein	27.1388	-6.5	-7.4	GLN864	GLN273	ASP666 LYS618 MET614	GLU240 TRP242
Eriocitrin	31.4286	-8.6	-9.2	**ASN625 GLN864 ASP666 GLU622**	**ASN361 ASP327 ASN326 LEU285 TYR530 PRO284 SER322**	LYS618	VAL398
Fisetin	23.3827	-7.4	-6.9	-	ARG401 ASP198	TRP517	TRP242 VAL199 ASP316
Galangin	22.9427	-8.7	-7.0	**SER1083 THR298 TRP299**	-	ALA246 TRP300	TRP242 GLU240 ASP316 ARG401 TYR62 ASP198
Isorhamnetin	27.6031	-7.4	-8.6	ASP909 GLU515	**ARG212 ASN242 ASP316 ARG401**	TRP517 ASP588 HIS587 TYR916 LEU433	PHE162 TRP242

Note. SER: Serine; VAL: Valine; TYR: Tyrosine; TRP: Tryptophan; PHE: Phenylalanine; HIS: Histidine; ARG: Arginine; ASN: Asparagine; GLN: Glutamine; PRO: Proline; ASP: Aspartic acid; THR: Threonine; LEU: Leucine; GLU: Glutamic acid; LYS: Lysine; GLY: Glycine. *S. Mutans*: *Streptococcus mutans*; *L. acidophilus*: *Lactobacillus acidophilus; SD: Three dimensional.*

of variety and number of hydrogen bonds with amino acids such as serine, glutamic acid, aspartic acid, lysine, and histidine with compound 4 (catechin), as well as amino acids aspartic acid, asparagine, leucine, tyrosine, proline, and serine with compound 7 (eriocitrin), along with amino acids arginine, asparagine, and aspartic acid with compound 10 (isorhamnetin). According to Figure 2, compound 7 (eriocitrin) has the ability to bind and inhibit both structures with an affinity of -8.6 for 3AIE and -9.2 for 4AIE. This compound against *S. mutans* (3AIE) can inhibit the structure by creating hydrogen bonds with amino acids glutamine (864 H), asparagine (625 H), aspartic acid (666 H), and glutamic acid (622 H). Additionally, against *L. acidophilus* (4AIE), it can inhibit the structure by creating hydrogen bonds with amino acids such as aspartic acid (327 H), asparagine (361 H and 326 H), leucine (285 H), tyrosine (530 H), proline (284 H), and serine (322 H).

In Vitro: Herein, we have evaluated and compared the antibacterial activity of flavonoid compounds that performed best in the previous section (*In silico*). According to Figure 3, which deals with the inhibition zone of compounds against target bacteria, eriocitrin + fluoride 0.2% represented the best performance compared to all compounds. Based on the data in Table 3, it seems that the compound eriocitrin has a wide range of antimicrobial activities. Groups a (Fluoride 0.2% + Eriocitrin against *S. mutans*) and d (Fluoride 0.2% + Eriocitrin against *L.*

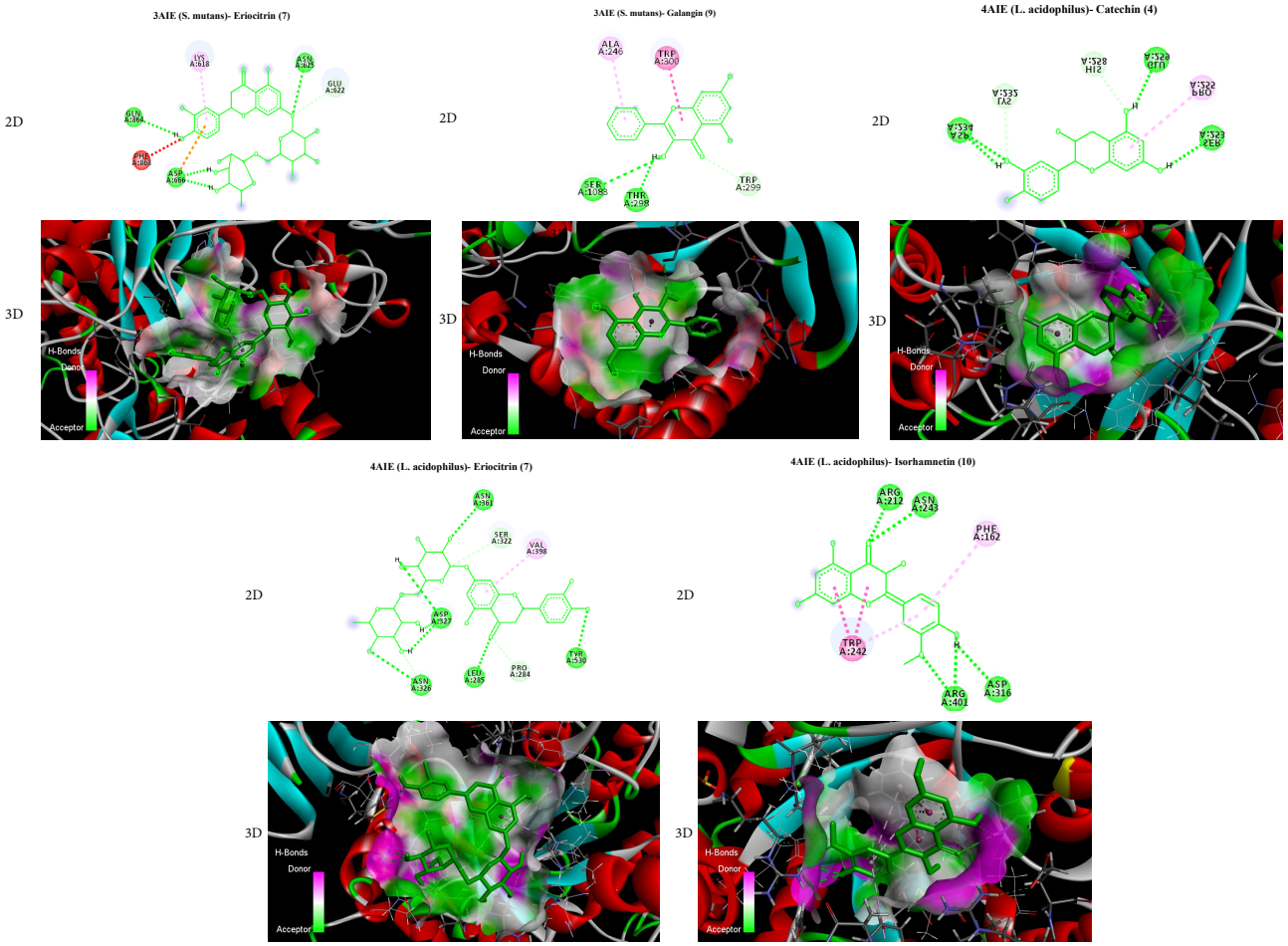

Figure 2. AutoDock Vina Results of Catechin, Eriocitrin, and Isorhamnetin in the Binding Site of Glucan-1,6-alpha-glucosidase From *L. acidophilus*, as Well as Eriocitrin and Galangin in the Binding Site of Glucansucrase From *S. mutans*. *Note*. Bonds are shown by means of Discovery Studio software (2D and 3D). *2D: 2-dimensional*

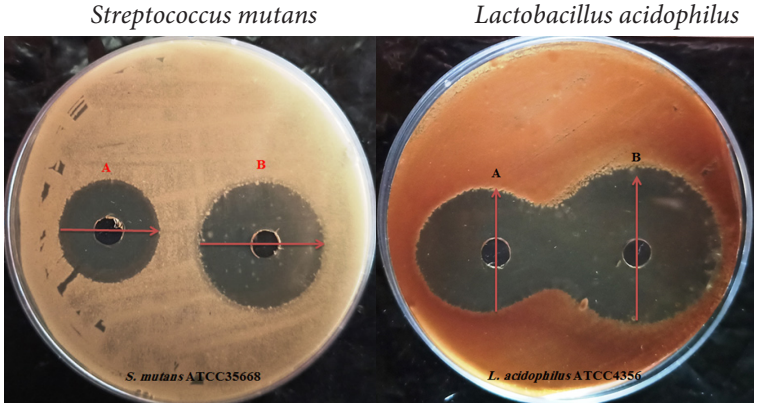

Figure 3. Antibacterial Results of Flavonoid Compounds: [*Streptococcus mutans*: A: Control (Fluoride), B: Eriocitrin + Fluoride - *Lactobacillus acidophilus*: A: Control (Fluoride), B: Eriocitrin + Fluoride]

acidophilus) were most effective between all compounds, respectively, (IZ = 26 ± 0.5 mm, MIC = 250 µg/mL, MBC = 500 µg/mL and IZ = 31 ± 0.5 mm, MIC = 125 µg/mL, MBC = 250 µg/mL).

Discussion

Tooth decay is still considered one of the most serious public health problems, and every year, it imposes a great financial burden on healthcare services all over the world, especially in developing countries (21). One of the most important ways to reduce costs is to prevent rotting. Currently, the most effective way to prevent tooth decay, in addition to reducing the intake of sugary substances, is to use mechanical methods such as brushing and flossing (22). However, other methods and chemical antimicrobial agents such as mouthwashes have been used due to the

Table 3. Antibacterial Properties of Flavonoid Compounds

Groups		*S. Mutans*	*L. acidophilus*
a (Fluoride + Eriocitrin)	IZ	26 ± 0.5 mm	-
	MIC	250	-
	MBC	500	-
b (Fluoride + Galangin)	IZ	20 ± 0.5 mm	-
	MIC	500	-
	MBC	≥1000	-
c (Fluoride + Catechin)	IZ	-	25 ± 0.5 mm
	MIC	-	250
	MBC	-	500
d (Fluoride + Eriocitrin)	IZ	-	31 ± 0.5 mm
	MIC	-	125
	MBC	-	250
e (Fluoride + Isorhamnetin)	IZ	-	22 ± 0.5 mm
	MIC	-	500
	MBC	-	≥1000
Fluoride (Control)	IZ	20 ± 0.5 mm	27 ± 0.5 mm
	MIC	500	250
	MBC	≥1000	500

Note. IZ (Millimetre-mm): Inhibition zone; MIC (µg.mL-1): Minimum inhibitory concentration; MBC (µg.mL-1): Minimum bactericidal concentration. (*S. mutans* wells: Fluoride (Control), Fluoride + Eriocitrin (a), Fluoride + Galangin (b). *L. acidophilus* wells: Fluoride (Control), Fluoride + Catechin (c), Fluoride + Eriocitrin (d), Fluoride + Isorhamnetin (e)); *S. Mutans*: *Streptococcus mutans*; *L. acidophilus*: *Lactobacillus acidophilus*.

insufficiency of mechanical methods alone to control plaque and prevent periodontal diseases and tooth decay, on the one hand, and the complex bacterial etiology of periodontal diseases and tooth decay, on the other hand (23). Mouthwashes contain effective preventive substances and play a significant role in improving oral health. From a dental point of view, the ideal mouthwash should have properties such as non-discoloration of teeth and mucous irritation, no toxic effects, and a good taste. However, a mouthwash that has all the mentioned properties has not yet entered the market, and researchers are still attempting to find a mouthwash that has the maximum of these properties (24). Oral streptococci are an important part of the collection of dental plaques, and one of the most important members of this collection is *S. mutans*, which has been associated with caries in several epidemiological studies and is believed to play the main role in the initiation of caries (25). These bacteria make up to 60% of the natural flora of the surfaces inside the mouth, which can cause the formation of biofilm on the surface of the tooth enamel by producing non-soluble viscous polymer materials such as glucan and levan. The primary mechanism of binding *S. mutans* is the formation of glucan homopolymers from sucrose by glucosyltransferase. Glucosyltransferase catalyzes two main reactions, which include the breakdown of sucrose into glucose and fructose (sucrazy activity) and the transfer of glucose units at the 6-C-3/C position to produce glucan (transferase activity) (26). The present study was conducted with the aim of investigating the effect of combined mouthwash (Flavonoid compounds and fluoride 0.2%) and fluoride 0.2% on the growth of *S. mutans* and *L. acidophilus*. Examining the *in silico* results showed that most of the flavonoid compounds, which were selected in this research, had the ability to inhibit the bacterial samples in a range of -5.2 to -9.2 kcal/mol. The binding energy determines the degree of binding between the compounds and the active sites of the 3AIE and 4AIE. Whatever this number is more negative, binding between the compound and the 3AIE and 4AIE is stronger. In this research, the flavonoid compound eriocitrin was reported to have an effective and stronger interaction than others. It created the highest hydrogen bonds with amino acids such as asparagine, glutamine, aspartic acid, and glutamic acid by glucansucrase from *S. mutans*, as well as hydrogen bonds with amino acids such as aspartic acid, asparagine, leucine, tyrosine, proline, and serine by glucan-1, 6-alpha-glucosidase from *L. acidophilus*. Many studies have been reported on the effects of various flavonoid classes in *in silico* and *in vitro* conditions. In this regard, Bao et al sought to identify potential inhibitors targeting *S. mutans* sortase A. They reported that several similar compounds composed of benzofuran, thiadiazole, and pyrrole, which exhibited good affinities and appropriate pharmacokinetic parameters, were potential inhibitors to impede the catalysis of SrtA (27). Likewise, Babaeekhou and Ghane *in silico* studied the antimicrobial activity of ginger on cariogenic bacteria and stated that some ginger compounds with high affinity to investigated enzymes can be considered candidate compounds for anti-caries drug development studies (28). Eriocitrin is a flavanone-7-O-glycoside between the flavanone eriodictyol and the disaccharide rutinose. It is commonly found in lemons and other citrus fruits. Similarly, Nisar et al evaluated the pharmacological effects of eriocitrin and concluded that eriocitrin has potent biological actions due to its strong antioxidant, antitumor, anti-allergic, antidiabetic, and anti-inflammatory activities. Eriocitrin is more potent in suppressing oxidative stress in diabetes mellitus and other chronic diseases incurred by excessive oxidative stress. During metabolism, eriocitrin is metabolized by the gut microbiota and a chain of molecules such as eriodictyol, methy-eriodictyol, 3,4-dihydroxyhydrocinnamic acid (DHCA), and much more conjugated molecules (29). *In vitro* results showed that eriocitrin + fluoride 0.2% has the best performance in comparison to all compounds (IZ = 26 ± 0.5 mm, MIC = 250 µg/mL, MBC = 500 µg/mL against *S. mutans* and IZ = 31 ± 0.5 mm, MIC = 125 µg/mL, MBC = 250 µg/mL against *L. acidophilus*). In addition, Miyake and Hiramitsu examined antimicrobial substances against oral bacteria from lemon peel and found that 8-geranyloxypsolaren, 5-geranyloxypsolaren, and 5-geranyloxy-7-methoxycoumarin exhibited high antibacterial activities (30). Moreover, Zajkani et al compared the effect of fluoride 0.2% and a combination mouthwash (xylitol and fluoride) on *S. mutans* and

L. acidophilus growths and reported that the fluoride mouthrinse at different concentrations, because of having a good inhibitory effect on *S. mutans* and *L. acidophilus* in both methods, was more effective compared with Fuchs mouthrinse (31). Adamczak et al investigated the antibacterial activities of some flavonoids and organic acids, as well as 13 common flavonoids (flavones, flavonols, and flavanones) and 6 organic acids (aliphatic and aromatic acids). They reported that all tested compounds showed antimicrobial properties, but their biological activity was moderate or relatively low. Bacterial growth was most strongly inhibited by salicylic acid (MIC = 250–500 µg/mL). These compounds were generally more active against gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, than gram-positive ones, including *Enterococcus faecalis* and *Staphylococcus aureus* (32).

Conclusion

According to the results of this study, eriocitrin can be used as a new mouthwash, along with fluoride 0.2%.

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Authors' Contribution

Conceptualization: Yasin SarveAhrabi, Behin Omidi.
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Methodology: Yasin SarveAhrabi, Behin Omidi, Sarina Nejati Khoei.
Resources: Yasin SarveAhrabi, Behin Omidi.
Supervision: Yasin SarveAhrabi, Behin Omidi.
Writing–original draft: Yasin SarveAhrabi.
Writing–review & editing: Yasin SarveAhrabi, Behin Omidi.

Competing Interests

The authors declare that they have no conflict of interests.

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References

1. Al-Nasser L, Lamster IB. Prevention and management of periodontal diseases and dental caries in the older adults. Periodontol 2000. 2020;84(1):69-83. doi: 10.1111/prd.12338.
2. Wade WG. Resilience of the oral microbiome. Periodontol 2000. 2021;86(1):113-22. doi: 10.1111/prd.12365.
3. Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K. Contribution of severe dental caries induced by *Streptococcus mutans* to the pathogenicity of infective endocarditis. Infect Immun. 2020;88(7):e00897-19. doi: 10.1128/iai.00897-19.
4. Pahumunto N, Piwat S, Chanvitan S, Ongwande W, Uraipan S, Teanpaisan R. Fermented milk containing a potential probiotic *Lactobacillus rhamnosus* SD11 with maltitol reduces *Streptococcus mutans*: a double-blind, randomized, controlled study. J Dent Sci. 2020;15(4):403-10. doi: 10.1016/j.jds.2020.03.003.
5. Yu J, Yan F, Lu Q, Liu R. Interaction between sorghum procyanidin tetramers and the catalytic region of glucosyltransferases-I from *Streptococcus mutans* UA159. Food Res Int. 2018;112:152-9. doi: 10.1016/j.foodres.2018.06.027.
6. Hoshino T, Fujiwara T. The findings of glucosyltransferase enzymes derived from oral streptococci. Jpn Dent Sci Rev. 2022;58:328-35. doi: 10.1016/j.jdsr.2022.10.003.
7. Abo Bakr RA, Tawfick MM, Mostafa ZA, Abdulall AK. Virulence traits-based behavior of *Streptococcus mutans* bacteria from dental plaque and dental caries conditions. Microb Biosyst. 2021;6(1):75-85. doi: 10.21608/mb.2021.100068.1044.
8. Wu J, Fan Y, Wang X, Jiang X, Zou J, Huang R. Effects of the natural compound, oxyresveratrol, on the growth of *Streptococcus mutans*, and on biofilm formation, acid production, and virulence gene expression. Eur J Oral Sci. 2020;128(1):18-26. doi: 10.1111/eos.12667.
9. Schulz-Weidner N, Weigel M, Turujlija F, Komma K, Mengel JP, Schlenz MA, et al. Microbiome analysis of carious lesions in pre-school children with early childhood caries and congenital heart disease. Microorganisms. 2021;9(9)1904. doi: 10.3390/microorganisms9091904.
10. Asgari I, Soltani S, Sadeghi SM. Effects of iron products on decay, tooth microhardness, and dental discoloration: a systematic review. Arch Pharm Pract. 2020;11(1):60-82.
11. Karak P. Biological activities of flavonoids: an overview. Int J Pharm Sci Res. 2019;10(4):1567-74. doi: 10.13040/ijpsr.0975-8232.10(4).1567-74.
12. Reddy AV, Moniruzzaman M, Madhavi V, Jaafar J. Recent improvements in the extraction, cleanup and quantification of bioactive flavonoids. Stud Nat Prod Chem. 2020;66:197-223. doi: 10.1016/b978-0-12-817907-9.00008-8.
13. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure-activity relationship: an update review. Phytother Res. 2019;33(1):13-40. doi: 10.1002/ptr.6208.
14. Akhtar N, Ayoubi R, Kour V, Gautam U, Mannan AU. Natural products for fungal diseases management and prevention. Nat Prod J. 2022;12(2):60-9. doi: 10.2174/2210315511666210512035847.
15. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients. 2020;12(2):457. doi: 10.3390/nu12020457.
16. Agati G, Brunetti C, Fini A, Gori A, Guidi L, Landi M, et al. Are flavonoids effective antioxidants in plants? Twenty years of our investigation. Antioxidants (Basel). 2020;9(11):1098. doi: 10.3390/antiox9111098.
17. Zahmatkesh K, Akbari Dilmaghani K, Sarveahrabi Y. Synthesis, antimicrobial and molecular docking studies of some new derivatives of 2,3-dihydroquinazolin-4(1H)-one. Acta Chim Slov. 2022;69(3):619-28. doi: 10.17344/acsi.2022.7512.
18. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-91. doi: 10.1002/jcc.21256.
19. Ghameshlouei S, Zarrabi Ahrabi N, Souldozi A, Sarve Ahrabi Y. Evaluation of the antibacterial and investigation of the molecular docking of new derivatives of 1, 3, 4-oxadiazole as inhibitors of quorum sensing system in the human pathogen *Pseudomonas aeruginosa*. Avicenna J Clin Microbiol Infect. 2021;8(1):27-33. doi: 10.34172/ajcmi.2021.06.
20. Omidi B, Sarve Ahrabi Y. In vitro and in silico evaluation of biological properties of some 1, 3, 4-oxadiazole derivatives against *Streptococcus mutans* and their interaction with Gbp-C by molecular docking. Avicenna J Dent Res. 2021;13(4):142-7. doi: 10.34172/ajdr.2021.27.
21. Clemens J, Gold J, Chaffin J. Effect and acceptance of silver diamine fluoride treatment on dental caries in primary teeth. J Public Health Dent. 2018;78(1):63-8. doi: 10.1111/jphd.12241.
22. Peres MA, Macpherson LMD, Weyant RJ, Daly B, Venturelli R, Mathur MR, et al. Oral diseases: a global public health

challenge. Lancet. 2019;394(10194):249-60. doi: 10.1016/s0140-6736(19)31146-8.
23. US Department of Health and Human Services Federal Panel on Community Water Fluoridation. US Public Health Service recommendation for fluoride concentration in drinking water for the prevention of dental caries. Public Health Rep. 2015;130(4):318-31. doi: 10.1177/003335491513000408.
24. Motallaei MN, Yazdanian M, Tebyanian H, Tahmasebi E, Alam M, Abbasi K, et al. The current strategies in controlling oral diseases by herbal and chemical materials. Evid Based Complement Alternat Med. 2021;2021:3423001. doi: 10.1155/2021/3423001.
25. Klein MI, Hwang G, Santos PH, Campanella OH, Koo H. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol. 2015;5:10. doi: 10.3389/fcimb.2015.00010.
26. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The biology of *Streptococcus mutans*. Microbiol Spectr. 2019;7(1):10.1128/microbiolspec.GPP3-0051-2018. doi: 10.1128/microbiolspec.GPP3-0051-2018.
27. Luo H, Liang DF, Bao MY, Sun R, Li YY, Li JZ, et al. In silico identification of potential inhibitors targeting *Streptococcus mutans* sortase A. Int J Oral Sci. 2017;9(1):53-62. doi: 10.1038/ijos.2016.58.
28. Babaeekhou L, Ghane M. Antimicrobial activity of ginger on cariogenic bacteria: molecular networking and molecular docking analyses. J Biomol Struct Dyn. 2021;39(6):2164-75. doi: 10.1080/07391102.2020.1745283.
29. Yao L, Liu W, Bashir M, Nisar MF, Wan CC. Eriocitrin: a review of pharmacological effects. Biomed Pharmacother. 2022;154:113563. doi: 10.1016/j.biopha.2022.113563.
30. Miyake Y, Hiramitsu M. Isolation and extraction of antimicrobial substances against oral bacteria from lemon peel. J Food Sci Technol. 2011;48(5):635-9. doi: 10.1007/s13197-011-0330-3.
31. Zajkani E, Zeighami H, Zaeefjou A. Comparision of the effect of fluoride 0.2% and a combination mouthwash (xylitol and fluoride) on *Streptococcus mutans* and *Lactobacillus acidophilus* growths. J Dent Med. 2017;30(1):57-64. [Persian].
32. Adamczak A, Ożarowski M, Karpiński TM. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J Clin Med. 2019;9(1):109. doi: 10.3390/jcm9010109.