

Preliminary screening of anti-caries active compounds of *Caesalpinia sappan*

Yongliang Jia

Kunming Medical University

Dongdong Zhang

Chinese Academy of Sciences

Hongxing Lu

Kunming Medical University

Yanhong Li

yanhongli_kmu@126.com

Kunming Medical University

Yuehu wang

Chinese Academy of Sciences

Article

Keywords: Dental caries, *Caesalpinia sappan*, *Streptococcus mutans*, Network pharmacology, Molecular docking

Posted Date: October 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7620115/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on April 14th, 2026. See the published version at <https://doi.org/10.1038/s41598-026-47281-4>.

Abstract

Dental caries represents a major global oral health issue, primarily caused by *Streptococcus mutans* (*S. mutans*). While previous studies have indicated that *Caesalpinia sappan* (*CS*) possesses anti-caries activity, its specific anti-caries chemical constituents remain largely unexplored. This study aimed to identify the potential anti-caries constituents of *CS* using an integrated approach combining network pharmacology, ultra-Performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS), bioassay-guided isolation targeting *S. mutans*, and molecular docking. We identified a total of 35 active ingredients in *CS*, sharing 79 potential therapeutic targets associated with dental caries, including AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1. Key pathways implicated included nitrogen metabolism and calcium signaling. Bioassay-guided isolation yielded 33 compounds in the fifth fraction from *CS*. However, only brazilin overlapped with the network pharmacology-predicted key ingredients. Brazilin effectively inhibited biofilm formation and acid production by *S. mutans* and demonstrated strong binding affinity to EGFR, BCL2, and MMP9 in molecular docking analysis, with docking scores all below -7 kcal/mol. Collectively, these findings not only elucidate the anti-caries chemical constituents of *CS* and their potential mechanisms of action but also provide novel insights into the bioactive constituents and mechanisms underlying natural plant-derived medicines for the prevention and treatment of dental caries.

1. Introduction

According to the Global Burden of Disease Study 2021, dental caries ranks as one of the most prevalent oral diseases globally^[1]. As dental caries progresses, it leads to defects in dental hard tissues, impairing masticatory function. Moreover, untreated dental caries can progress to oral diseases such as pulpitis and apical periodontitis, significantly impacting oral health^[2]. Beyond the oral cavity, severe dental caries has been associated with an elevated risk of systemic conditions, including cardiovascular and gastrointestinal diseases, thereby affecting overall systemic health^[3].

Dental caries represents an infectious disease that causes chronic and progressive destruction of the hard tissues of the teeth, mainly by various factors such as bacteria. Currently, *Streptococcus mutans* (*S. mutans*) is still considered the main cariogenic microorganism. *S. mutans* has exceptional acid-producing and acid-resistant properties. It can produce acidic metabolites through glycolysis, resulting in the formation of an acidic environment within the dental plaque^[4]. *S. mutans* can survive in an acidic environment and maintain its glycolysis ability, and ultimately the long-term presence of acidic metabolites causes demineralization of tooth enamel, which ultimately leads to the

development of dental caries^[5]. Furthermore, *S. mutans* can also synthesize large amounts of extracellular polysaccharide (EPS) utilizing sucrose substrate. These EPS assemble into structurally complex biofilms that enhance bacterial adhesion and provide protection for embedded microorganisms^[5, 6].

Currently, two types of anti-caries agents are used clinically to effectively prevent the initiation and progression of dental caries. The first is fluoride, which remains the most effective anti-caries agent in global public health^[7]. Fluoride inhibits *S. mutans* growth and promotes enamel remineralization^[8]. However, excessive fluoride exposure may cause adverse effects of fluorosis, fluoride-resistant microorganisms, and disruption of oral microecological balance^[9-11]. The alternative agent is chlorhexidine (CHX), which is a highly effective cationic surface-active disinfectant with a broad spectrum of antibacterial effects. However, long-term use can cause noticeable staining of the tooth surface and taste alterations, and even cause dysbiosis of the commensal oral flora^[12]. These limitations underscore the need for novel anti-caries therapeutics.

In recent years, with the continuous development of natural plant medicine research, it has garnered significant scientific interest. Natural plants represent promising anti-caries candidates owing to their wide sources, favorable safety profiles, and minimal induction of microbial resistance. Numerous natural plant extracts demonstrate inhibitory activity against common oral pathogens, such as *Galla chinensis*, *Rhodiola*, and *Arnebia* root, which have been used to prevent dental caries^[13-15]. Their primary mechanisms involve: (1) inhibition of the growth, acid production, and biofilm formation of *S. mutans* and (2) preventing demineralization or promoting remineralization of hard tooth tissues. Our group's previous study investigated the inhibitory effect of six common natural plant extracts from Yunnan Province, China, on common dominant bacteria in the oral cavity, and found that the extract of *Caesalpinia sappan* (CSE) had a better inhibitory effect on *S. mutans*^[16]. Nevertheless, the specific bioactive constituents responsible for CS need to be further explored.

Network pharmacology is an emerging interdisciplinary approach integrating pharmacology, bioinformatics, and systems biology. This methodology enables systematic construction of drug-target-disease interaction networks to elucidate bioactive compounds and their mechanisms of action^[17]. Complementary to this, molecular docking serves as a pivotal drug discovery tool that computationally predicts binding affinities and conformational orientations between candidate molecules and target proteins^[18, 19].

This study aims to elucidate the anti-caries compounds and molecular mechanisms using integrated network pharmacology and molecular docking, and further screen the possible anti-caries active components in CS through bioassay-guided isolation based on the

inhibitory activity of *S. mutans*, in order to provide a foundation for dental caries-preventive studies of *CS*, as well as contribute to the public health endeavor of caries prevention and treatment.

2. Materials and methods

2.1 Network pharmacology analysis

2.1.1 Data acquisition

We obtained the compounds for *CS* from TCMSP databases (<https://www.tcmsp-e.com/tcmsp.php>) and HERB databases (<http://herb.ac.cn/>), obtained the compounds's Simplified Molecular Input Line Entry Specification (SMILES) from Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>), and obtained the related targets from Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>)^[20, 21]. We limited our search to the "Homo sapiens" species and probability "0" to find the targets of compounds. We obtained the target genes for dental caries from the GeneCards (<https://www.genecards.org/>), TTD (<https://db.idrblab.net/ttd/>), and OMIM (<https://omim.org/>) database using "dental caries" and "caries" as search keywords. We used the online Venn platform (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to visualize the results.

2.1.2 PPI network construction

In order to explore the core targets from coincident targets of *CS* and dental caries, the coincident targets were input into the STRING 12.0 database (<https://cn.string-db.org/>). The biological was set to "Homo sapience", the meaning of network edges was set to "confidence", the minimum required interaction score was set to "medium confidence (0.400)". Then, the Cytoscape version 3.10.0 was used to construct the Protein-Protein Interaction (PPI) network among targets. In this network analysis, the degree value is used to reflect the interaction intensity between targets.

2.1.3 GO and pathway enrichment analyses

The targets were input into Metascape database (<https://metascape.org/>) to identify the term enrichment and analyze the share genes. The species was set as "Homo sapience" and the statistical significance of enrichment was set as "P value cut off = 0.01". We limited the minimum number of pathways of 3, showing only the top 20. Then, the online Weishengxin platform (<http://www.bioinformatics.com.cn/>) was used to visualize the analytical results. This analysis included Gene Ontology (GO) enrichment for Biological Process (BP), Molecular Function (MF), Cellular Component (CC), and KEGG metabolic pathways.

2.1.4 Construction of the target-drug-pathways network

The Cytoscape version 3.10.0 was used to construct the interaction network of target-drug-pathways.

2.2 Bioassay-guided isolation of compounds from *CS* against *S. mutans*

2.2.1 Chemicals and reagents

The heartwood of *CS* was purchased from Luosiwan market (Kunming, China). Ethyl alcohol (EtOH), petroleum ether (PE), ethyl acetate (EtOAc), *n*-butanol was purchased from Chuandong Chemical Co., LTD (Chongqing, China). *S. mutans* UA159 was provided by the

Yunnan Key Laboratory of Stomatology (China). Crystal violet (CV) and sucrose were purchased from Yuanye (Shanghai, China) and Solarbio (Beijing, China). Brain heart infusion (BHI) broth and agar were from Bacton, Dickinson and Company.

2.2.2 Plant materials and extracts preparation

The *CS* powders were ultrasonically extracted for 30 min with different concentration EtOH (0–90%) at 60°C. Gradient EtOH crude extract were obtained by concentrated the solvent. The 70% EtOH extract were suspended in water and then successively extracted with petroleum ether, ethyl acetate, and *n*-butanol to yield the petroleum ether-soluble, ethyl acetate-soluble, and *n*-butanol-soluble extracts, and the remaining water phase part. Then, the EtOAc extract were subjected to silica gel column chromatography (PE-EtOAc, 1:0→0:1) to yield fractions 1–10 (Fr. 1–Fr. 10).

2.2.3 Bacterial strains culture conditions

S. mutans UA 159 was recovered and inoculated on BHI agar plates, and single bacterial colony was added into BHI broth grown in anaerobic chamber (10% H₂, 5% CO₂, and 85% N₂) at 37°C. BHI broth was replaced with BHI supplemented with 1% (w/v) sucrose (BHIS) in biofilm assays and glycolytic pH drop assay.

2.2.4 Determination of the minimum inhibitory concentration (MIC)

The antimicrobial activity of different extracts was evaluated by MIC^[22]. Different extracts mother solutions were diluted with BHI medium to different concentrations by the doubled dilution method. *S. mutans* was cultured to mid-log phase and diluted with BHI medium

according to the volume ratio of 1:100, and then anaerobically cocultured with different extracts at 37°C for 24 h, the lowest concentration with no bacterial growth was defined as the MIC.

2.2.5 Biofilm formation assays

The effect of different extracts on *S. mutans* was analyzed using crystal violet (CV) staining^[23]. Briefly, *S. mutans* was diluted using BHIS medium and co-cultured anaerobically with different extracts at 37°C for 24 h. The biofilm was rinsed three times with sterile water. The bacterial cells were fixed with 4% (w/v) paraformaldehyde for 10 min and then stained with 0.1% (w/v) CV solution for 10 min in each well. The solution was removed and rinsed repeatedly and gently with sterile water. The dye was solubilized by adding 33% (v/v) acetic acid solvent to the well plates for 10 min. Finally, the 100 µL of solution per well was pipetted into a new 96-well plate and the optical density (OD) was read at 575 nm using a Microplate reader.

2.2.6 Glycolytic pH drop assay

The effect of different extracts on acid production for *S. mutans* biofilm was assessed by determining the pH of the medium^[24]. *S. mutans* was co-cultured with different extracts in 24-well plates, the initial pH was adjusted to 7.0 and anaerobically incubated at 37°C for 24 h. The decrease in pH of each well was measured using a pH meter (Mettler Toledo, Switzerland).

2.2.7 UPLC-MS/MS analysis of the extract

In order to further explore the composition of active components, Fr. 5 was analyzed by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS)^[25]. The Fr. 5 powder was redissolved in methanol with a concentration of 4 mg/mL by ultrasonication, centrifuge at 13,000 rpm/min for 10 min and take the supernatant for analysis, storage time should not exceed 24 h. UltiMate 3000 UHPLC and Thermo Hypersil gold C18 (2.1 mm×100 mm, 1.9 µm) were used in liquid phase separation, the liquid phase was 0.1% formic acid/methanol (B)-0.1% formic acid/water (A), the gradient elution procedure of the solution, as detailed in Table 1. Chromatographic condition: 0 – 20 min, flow rate was 0.3 mL/min.

Table 1
Mobile phase gradient
elution procedure

Time (min)	A	B
0	90	10
1	90	10
13	0	100
17	0	100
17.1	90	10
20	90	10

The mass spectrometer was a Q-Exactive (Thermo Fisher Scientific, CA, USA) HESI source with an ion source temperature of 310°C, a capillary temperature of 320°C, a sheath gas flow rate of 30 units, an auxiliary gas flow rate of 10 units, and a spray voltage of 2.8 kV for the negative ion mode.

The analysis was performed using data-dependent scanning analysis (DDA) with the loop count set to 10, and the HCD energies were step-wise normalized collision energies set to 10, 28, and 35 eV. The primary mass spectrometry was scanned over a range of 100–1500 *m/z*, with the resolution set to 70000, the AGC target set to 3E6, and the injection time set to 200 ms. The resolution of the secondary mass spectrum was set to 17500, the AGC target was set to 1E5, and the injection time was set to 50 ms.

2.3 Molecular Docking

We used molecular docking to test the reliability of potential targets for dental caries treatment. The PDB files of the targets were obtained from PDB database (<https://www.rcsb.org/>) and the SDF files of the compound 2D structure were obtained from Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>). We used the AutoDock Vina software to perform the molecular docking, and the PyMol software to visualize the results.

2.4 Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.5.1 (California, USA). T-test was used for comparisons between two groups. One-way ANOVA was performed to assess variance among multiple groups, and Tukey's multiple comparison test was used for multiple comparisons. All significant differences were identified at 0.05.

3. Results

3.1 Network pharmacology analysis

3.1.1 Active ingredients of *CS*

After excluding active ingredients without predicted targets, a total of 35 active ingredients were obtained (Table 2)

Table 2
Information on the main active ingredients of the *CS*

Code number	Ingredient ID	Ingredient name	Ingredient formula
C1	HBIN001020	1,3,4,5-tetracaffeoylquinic acid	C ₄₃ H ₃₆ O ₁₈
C2	HBIN002875	1-octacosanol	C ₂₈ H ₅₈ O
C3	HBIN007845	3,9-dihydroxy-8-methoxydibenzo [b, d] pyran-6-one	C ₁₄ H ₁₀ O ₅
C4	HBIN008435	3-deoxysappanchalcone	C ₁₆ H ₁₄ O ₄
C5	HBIN008436	3'-deoxysappanol	C ₁₆ H ₁₆ O ₅
C6	HBIN008437	3-deoxysappanone b	C ₁₆ H ₁₄ O ₅
C7	HBIN008792	3'-methoxy-4',5,7-trihydroxyflavone	C ₁₆ H ₁₂ O ₆
C8	HBIN012903	7,3',4'-trihydroxy-3-benzyl-2 <i>H</i> -chromene	C ₁₆ H ₁₄ O ₄
C9	HBIN013224	7-hydroxy-3-(4'-hydroxybenzylidene)-chroman-4-one	C ₁₆ H ₁₂ O ₄
C10	HBIN018719	bonducellin	C ₁₇ H ₁₄ O ₄
C11	HBIN018818	brazilein	C ₁₆ H ₁₂ O ₅
C12	HBIN018819	brazilin	C ₁₆ H ₁₄ O ₅
C13	HBIN024649	(e)-2-nonenal	C ₉ H ₁₆ O
C14	HBIN025418	episappanol	C ₁₆ H ₁₆ O ₆
C15	HBIN027030	gallic acid	C ₇ H ₆ O ₅
C16	HBIN028989	hematein	C ₁₆ H ₁₂ O ₆
C17	HBIN034745	menthol	C ₁₀ H ₂₀ O
C18	HBIN035418	(-)-methyl selina-3,11-dien-14-oate	C ₁₆ H ₂₄ O ₂
C19	HBIN036718	neoprotosappanin	C ₃₂ H ₂₆ O ₁₀
C20	HBIN038026	oleic acid	C ₁₈ H ₃₄ O ₂
C21	HBIN038089	ombuin	C ₁₇ H ₁₄ O ₇
C22	HBIN039794	phytol	C ₂₀ H ₄₀ O

Code number	Ingredient ID	Ingredient name	Ingredient formula
C23	HBIN040939	protosappanin A	C ₁₅ H ₁₂ O ₅
C24	HBIN040941	protosappanin A dimethyl acetal	C ₁₇ H ₁₈ O ₆
C25	HBIN040942	protosappanin B	C ₁₆ H ₁₆ O ₆
C26	HBIN040948	protostemonine	C ₂₃ H ₃₁ NO ₆
C27	HBIN041495	quercetin	C ₁₅ H ₁₀ O ₇
C28	HBIN042158	rhamnetin	C ₁₆ H ₁₂ O ₇
C29	HBIN043129	sappanchalcone	C ₁₆ H ₁₄ O ₅
C30	HBIN043131	sappanol	C ₁₆ H ₁₆ O ₆
C31	HBIN043133	sappanone b	C ₁₆ H ₁₄ O ₆
C32	HBIN043148	sarcosine	C ₃ H ₇ NO ₂
C33	HBIN044730	stearic acid	C ₁₈ H ₃₆ O ₂
C34	HBIN045541	Taraxerol	C ₃₀ H ₅₀ O
C35	HBIN046012	tetraacetylbrazilin	C ₂₄ H ₂₂ O ₉

3.1.2 Potential targets prediction

Using a probability threshold > 0 as the selection criterion, 348 putative targets were obtained for the 35 active compounds, and 2568 dental caries targets were retrieved. Through Venn mapping, 79 common targets of *CS* and dental caries were obtained (Fig. 1).

3.1.3 PPI network construction and analysis

The interaction among 79 overlapping targets were analyzed by Protein-protein interaction (PPI) network. The number of edges in PPI network is 444, and the degree value of average nodes is 11.53. In addition, the degree values of 34 targets were exceeded the average value (Fig. 2), identifying AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1 as topologically significant hub nodes. These high-degree targets represent potential key mediators of *CS*'s anti-caries mechanisms.

3.1.4 GO function and KEGG pathway enrichment

Functional enrichment analysis identified 881 significant GO terms and 113 KEGG pathways. The results show that the most significant biological processes were associated with cellular response to nitrogen compound and cellular response to hormone stimulus, the most significant cellular component was associated with extracellular matrix, and the most significant molecular function was related to protein kinase activity (Fig. 3A). Moreover, KEGG pathway enrichment analysis demonstrated that the overlapped targets were mainly enriched in the nitrogen metabolism, pathways in cancer, prostate cancer, microRNAs in cancer, calcium signaling pathway (Fig. 3B).

3.1.5 Target-drug-pathways (T-D-P) network in dental caries construction and analysis

The final interaction network was constructed using the previously obtained information of 79 overlapping targets, 17 KEGG pathways involved in the anti-caries mechanism of *CS*. The results of the T-D-P network revealed five hub pathways with degree centrality exceeding the network average (12.53): prostate cancer, pathways in cancer, calcium signaling pathway, microRNAs in cancer, and nitrogen metabolism. Concurrently, the 11 active compounds were prioritized based on the degree value of average nodes (7.86), including C7, C4, C3, C1, C6, C15, C8, C27, C35, C19, and C12 (Fig. 4).

3.2 Bioassay-guided isolation of active ingredients from *CS* against *S. mutans*

3.2.1 Effect of ethanol extracts on biofilm formation and acid production in *S. mutans*

According to the results, the MIC of the 0% ethanol extract was 1.25 mg/mL, while extracts prepared with 30–90% ethanol exhibited a lower MIC of 0.625 mg/mL. The effect of ethanol extracts on *S. mutans* biofilm formation is shown in Fig. 5A. The crystal violet staining experiment revealed that compared to the control group, the biomass of *S. mutans* biofilms could be effectively decreased by 70–90% ethanol extracts at 1/2 MIC concentration (89–93% reduction). Furthermore, as shown in Fig. 5B, all ethanol extracts at 1/2 MIC concentration could inhibit acid production by *S. mutans* compared to the control.

3.2.2 Effect of EtOAc, *n*-Butanol, and water extracts on biofilm formation and acid production in *S. mutans*

EtOAc, *n*-butanol, and water extracts were obtained by further fractionating the 70% ethanol extract with various solvents. A PE fraction was not obtained in significant yield. The MIC value of the EtOAc extract was 0.625 mg/mL, the MIC value of the *n*-butanol extract was 0.3125 mg/mL, and the MIC value of the water extract was more than 5 mg/mL. CV staining revealed that only the EtOAc extract significantly inhibited *S. mutans* biofilm formation (Fig. 6A, B). Furthermore, assessment of acid production inhibition

showed that only the EtOAc extract significantly inhibited *S. mutans* acid production (Fig. 6C, D), consistent with the biofilm formation results.

3.2.3 Effect of different fractions on biofilm formation and acid production in *S. mutans*

The EtOAc extract was further fractionated to yield 10 fractions (Fr. 1-Fr. 10), of which the MIC value of Fr. 5 was 0.625 mg/mL, Fr. 8, Fr. 9 and Fr. 10 were 2.5 mg/mL, and the other fractions were more than 5 mg/mL. The inhibition of biofilm formation of *S. mutans* by different fractions was investigated by CV staining and it was found that Fr. 5, Fr. 8, and Fr. 10 were effective in inhibiting the biofilm formation at the 1/2 MIC and 1/4 MIC, and Fr. 9 could significantly inhibit the biofilm formation at only 1/2 MIC (Fig. 7A). However, in acid production, Fr. 5 and Fr. 10 could effectively inhibit the acid production by *S. mutans*, Fr. 8 and Fr. 9 could significantly inhibit the acid production at only 1/2 MIC (Fig. 7C). Interestingly, Fr. 2 and Fr. 6 inhibit the acid production at 2.5 mg/mL (Fig. 7D). These results suggest that different components may influence the cariogenicity of *S. mutans* through distinct mechanisms.

3.2.4 The ingredients analysis of F5 by UPLC-MS/MS

Due to its superior inhibitory activity against *S. mutans* biofilm formation and acid production at lower concentrations compared to other fractions, F5 was selected for compositional analysis. According to the retention time, the high-resolution accurate molecular weight and MS/MS fragment information of each chemical component were obtained by UPLC-MS detection. A total of 33 constituents were tentatively identified in F5. The total ion chromatogram (TIC) in negative ion mode is presented in Fig. 8. Based on relative peak abundance, the major compounds were tentatively identified as brazilin, sappanol, and 3-deoxysappanone B (Table 3). Comparison with the network pharmacology-predicted anti-caries components in CS revealed brazilin as the sole overlapping constituent. This suggests brazilin is a key anti-caries component in CS (Fig. 9).

Table 3
List of ingredients detected from Fr. 5.

No	RT (min)	Molecular formula	Measured mass [M – H] ⁺ (m/z)	Putative Identification	Peak area (%)
1	6.594	C ₁₆ H ₁₄ O ₅	285.07718	brazilin	23.31
2	3.361	C ₁₆ H ₁₆ O ₆	303.0878	sappanol	20.73
3	4.02	C ₁₆ H ₁₄ O ₅	285.07712	3-deoxysappanone B	18.26
4	5.604	C ₁₆ H ₁₂ O ₅	283.06155	brazilein	9.03
5	5.805	C ₁₆ H ₁₄ O ₆	301.07199	hematoxylin	7.80
6	5.709	C ₁₅ H ₁₂ O ₅	271.06149	protosappanin A	1.90
7	7.47	C ₁₅ H ₁₂ O ₅	271.06149	3',4',7-trihydroxyflavanone	1.38
8	7.006	C ₁₅ H ₁₀ O ₇	301.03577	quercetin	0.77
9	3.388	C ₇ H ₆ O ₃	137.02348	2,5-dihydroxybenzaldehyde	0.74
10	7.256	C ₁₆ H ₁₄ O ₄	269.08221	10,11-dihydroxydracaenone C	0.73
11	1.929	C ₈ H ₈ O ₄	167.03436	6-methoxysalicylic acid	0.49
12	8.798	C ₁₇ H ₁₄ O ₇	329.06702	ombuin	0.32
13	12.04	C ₁₈ H ₃₂ O ₄	293.21265	9-HpODE	0.30
14	7.242	C ₁₆ H ₁₂ O ₇	315.05148	isorhamnetin	0.29
15	5.682	C ₁₅ H ₁₂ O ₇	303.05133	taxifolin	0.28
16	4.703	C ₈ H ₈ O ₂	135.04424	2-methylbenzoic acid	0.14
17	8.513	C ₁₆ H ₁₂ O ₆	299.05634	tectorigenin	0.10
18	5.187	C ₉ H ₈ O ₃	163.03932	2-hydroxycinnamic acid	0.07
19	6.903	C ₇ H ₆ O ₃	137.02338	3-hydroxybenzoic acid	0.06
20	3.837	C ₈ H ₈ O ₄	167.03421	isovanillic acid	0.06
21	2.24	C ₈ H ₁₀ O ₃	153.05489	3,4-dihydroxyphenylethanol	0.06
22	15.953	C ₁₄ H ₃₀ O ₄	293.17972	myristyl sulfate	0.05

No	RT (min)	Molecular formula	Measured mass [M – H] ⁺ (m/z)	Putative Identification	Peak area (%)
23	18.608	C ₃₀ H ₄₈ O ₃	455.35391	ursolic acid	0.05
24	3.877	C ₁₀ H ₈ O ₄	191.03423	7,8-dihydroxy-4-methylcoumarin	0.05
25	5.807	C ₈ H ₈ O ₃	151.03908	2-hydroxyphenylacetic acid	0.04
26	11.255	C ₁₆ H ₂₂ O ₄	277.14481	dibutyl phthalate	0.02
27	5.307	C ₉ H ₁₀ O ₅	197.04507	ethyl gallate	0.02
28	6.145	C ₁₁ H ₁₂ O ₄	207.0659	sinapoyl aldehyde	0.02
29	3.728	C ₉ H ₆ O ₄	177.01862	7,8-dihydroxycoumarin	0.02
30	1.103	C ₁₆ H ₁₂ O ₆	299.05649	hematein	0.02
31	3.567	C ₉ H ₁₀ O ₃	165.05493	3-hydroxy-1-(4-hydroxyphenyl) propan-1-one	0.01
32	9.279	C ₁₅ H ₂₀ O ₃	247.13405	atractylenolide III	0.01
33	9.477	C ₁₄ H ₂₂ O ₂	221.15451	2,5-di-tert-butylhydroquinone	0.01

3.2.5 Effect of brazilin on biofilm formation and acid production in *S. mutans*

The aim of this study was to assess the effect of brazilin on *S. mutans* biofilm formation and acid production. The results demonstrated that brazilin at 1/2 MIC significantly inhibited biofilm formation, reducing total biomass by approximately 36% compared to the control (Fig. 10A, B). Furthermore, brazilin effectively reduced acid production, as evidenced by a higher final pH compared to the control (Fig. 10C).

3.3 Validation of key targets using molecular docking

Molecular docking analysis was performed between brazilin and seven key targets (AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1). Generally, lower docking scores indicate stronger predicted binding affinity, with docking scores < -7 kcal/mol typically considered indicative of strong binding. The interaction of brazilin and keys targets was shown in Fig. 11, the results showed docking scores were – 6.8, -8.3, -7.8, -9.3, -8.5, -6.3,

-7.9 kcal/mol respectively, indicating that brazilin demonstrated stronger predicted binding affinity towards EGFR, BCL2, PTGS2, MMP9, and HSP90AA1.

4. Discussion

Dental caries remains a global public health concern, adversely impacting both oral and systemic health while imposing significant economic burdens. Natural plant medicine shows considerable promise for dental caries management. In this study, we investigate the underlying targets and molecular mechanisms of *CS* in dental caries through a network pharmacology approach. Our analysis identified key targets (AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1) and pathways (nitrogen compound metabolic process, calcium signaling pathway) potentially modulated by *CS*. Given that *S. mutans* is the primary microorganism responsible for the occurrence of dental caries, we subsequently isolated the active component(s) of *CS* through bioassay-guided fractionation targeting *S. mutans*. This approach coupled with network pharmacology led to the identification of brazilin, which effectively inhibited *S. mutans* acid production and biofilm formation. Consequently, brazilin was identified as an active compound within *CS* contributing to dental caries prevention. Furthermore, molecular docking analysis suggests that its anti-caries effects may be mediated through potential interactions with EGFR, BCL2, PTGS2, MMP9, HSP90AA1.

Gene ontology enrichment analysis indicated that the molecular function of protein kinase activity is related to *CS* against dental caries. The activities of numerous protein kinases are critically involved in caries pathogenesis, primarily by modulating *S. mutans* virulence factors. Specifically, serine/threonine protein kinases (STPKs) and histidine kinase (HK) are involved in two-component system (TCS) signaling of *S. mutans* and can regulate the expression of metabolism-related genes in the cell wall by sensing environmental signals and activating response regulators^[26, 27]. KEGG has been used in biological big data analysis, for example, for uncovering pathway through the KEGG mapping procedure^[28]. Concurrently, nitrogen metabolism and calcium signaling are two pathways enriched related to dental caries in KEGG analysis. Nitrogen metabolism is essential for the growth and survival of bacteria, and the bacteria can provide the essential materials by metabolizing nitrogen-containing compounds^[29, 30]. Furthermore, the first gene of the *ciaRH* operon in *S. mutans* is a calcium sensing signal peptide that allows the CiaRH system to regulate the cariogenicity of *S. mutans* by modulating the expression of its own operon in response to calcium ions in the oral environment^[31]. *CS* contains a variety of active compounds with obvious antibacterial effects^[32]. Our integrated analyses suggest that *CS* may interfere with nitrogen metabolism and the calcium signaling pathway to prevent dental caries.

Traditional separation methods often face challenges in efficiently isolating compounds with specific biological activities. Bioassay-guided isolation addresses this limitation by integrating activity assessment throughout the separation process, thereby increasing the probability of obtaining bioactive compounds and enhancing their practical utility^[33]. The cariogenicity of *S. mutans* is primarily related to the formation of dental plaque biofilm and the production of acidic metabolites. Therefore, the inhibitory effects of different extracts on *S. mutans* biofilm formation and acid production were determined during the isolation process. Initial extraction employed ethanol as the solvent, and the results indicated that 70–90% ethanol extracts exhibited significant inhibitory effect on *S. mutans*. Given that lower solvent concentrations can better preserve compound activity^[34]. Therefore, the 70% ethanol extract was selected for further fractionation. Further, UPLC-MS/MS analysis of the Fr. 5 identified brazilin as the predominant compound. Combined with the potential active compounds screened in the network pharmacology analysis, only brazilin overlapped with the Fr. 5 of UPLC-MS/MS analysis, and brazilin was the main compound obtained the heartwood of *CS*^[35]. Brazilin was identified as the key compound within *CS* responsible for dental caries prevention. Furthermore, its demonstrated efficacy in inhibiting *S. mutans* biofilm formation and acid production substantiates its anti-caries potential.

Analysis of potential targets identified AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1 as potential key targets. Molecular docking analysis further assessed the interactions between brazilin and these proteins. Lower docking scores indicate stronger predicted binding affinity. Notably, the docking scores for EGFR, BCL2, PTGS2, MMP9, and HSP90AA1 were all below – 7 kcal/mol, suggesting strong binding potential. EGFR plays a critical role in proliferation, differentiation, development, and promoting downstream signaling, the expression of EGFR is up-regulated in dental caries, which may be involved in the extracellular matrix (ECM) degradation process^[36, 37]. Interestingly, brazilin has been shown to reduce ECM synthesis, suggesting a potential counteracting mechanism^[38]. BCL2 is involved in apoptosis through multiple pathways, and has been reported to be overexpressed in teeth with advanced dental caries^[39, 40]. A study showed that brazilin can suppress the expression of BCL2^[41]. PTGS2, also known as cyclooxygenase-2 (COX-2), is an inducible enzyme associated with prostaglandin synthesis and also plays an important role in inflammatory responses, cell proliferation, and apoptosis^[42, 43]. However, PTGS2 is mainly related to dental pulp inflammation in dental caries research, the AgI/II protein produced by *S. mutans* is capable of binding to receptors on the host cell surface, which is responsible for producing a variety of inflammatory mediators and causing the occurrence of dental pulp inflammation^[44, 45]. MMP9 is a protein of the matrix metalloproteinase family that plays an essential role in the proteolysis of the ECM, and may be involved in the destruction of the dentin organic

matrix during caries^[46]. A study showed that brazilin can suppress MMP9^[41]. HSP90AA1 encodes heat shock protein 90 α (Hsp90 α), involved in cell cycle control, signal transduction, and other physiological processes^[47]. However, it also promotes inflammation induced by *Porphyromonas gingivalis* lipopolysaccharide via autophagy regulation^[48]. It is hypothesized that HSP90AA1 might similarly influence caries-associated bacteria through autophagy. Based on the stronger evidence linking their functions to caries pathogenesis and the direct experimental evidence of brazilin's modulation, EGFR, BCL2, and MMP9 emerge as the most plausible primary targets for brazilin's anti-caries effects.

Finally, this study provides insights into the potential of *CS* for dental caries prevention and treatment and proposes potential mechanism. However, our findings require further experimental validation. An additional limitation of this study is that although only *S. mutans* was selected for the activity screening, considering its important role in the occurrence and development of dental caries. Nevertheless, brazilin, identified as a key active component of *CS*, demonstrated significant potential anti-caries effects against this key cariogenic bacterium.

5. Conclusion

In conclusion, we propose that *CS* exerts its anti-caries effects potentially through modulation of seven key targets (AKT1, EGFR, BCL2, PTGS2, MMP9, ERBB2, and HSP90AA1) and two pathways (nitrogen metabolism and calcium signaling pathway). Furthermore, brazilin was identified as the active compound within *CS* responsible for caries prevention and treatment. Molecular docking analysis revealed EGFR, BCL2, and MMP9 as its core interacting targets, with spontaneous binding predicted for each. Therefore, brazilin represents a promising candidate compound for dental caries prevention, likely acting through multi-target and multi-pathway mechanisms.

Declarations

Author contributions

Yongliang Jia: conceptualization, performed the experiments, data curation, formal analysis, visualization, validation, writing- original draft.

Yongliang Jia, Hongxing Lu: performed the experiments, data curation, formal analysis, visualization.

Dongdong Zhang: conceptualization, supervision, writing- review & editing.

Yanhong Li, Yuehu Wang: conceptualization, funding acquisition, project administration, supervision, validation, writing- review & editing.

Conflicts of interest

The author(s) declare no competing interests.

Data availability statement

Data available on request due to privacy or ethical restrictions. If someone wants to request the data from this study, please contact the Yanhong Li.

Funding

This work was supported by the National Natural Science Foundation of China (grant number 82160179), the "Xingdian Talent Support Plan" of Yunnan Province-Medical and Health Talents Special Project (grant number XDYC-YLWS-2023-0047), the Major Science and Technology Projects in Yunnan Province (grant number 202302AA310038), the Degree and Graduate Education Innovation Fund Project of Kunming Medical University (grant number 2025B007), and the National Key Clinical Specialty Development Project of Pediatric Dentistry Division (grant number 20230610).

References

1. Bernabe E. et al. Trends in the global, regional, and national burden of oral conditions from 1990 to 2021: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet*. 405(10482), 897-910 (2025).
2. Pitts N B. et al. Dental caries. *Nature Reviews Disease Primers*. 25, 3, 17030; 10.1038/nrdp.2017.30 IF: 60.6 Q1 (2017).
3. Wang X H, Xu Z Q, Bian Z, Meng L Y. Research progress on the correlation between dental caries and systemic diseases. *Zhong Hua Kou Qiang Yi Xue Za Zhi*. 59(1), 99-104 (2024).
4. Soeta B de J J, Kilian Nyvad M, Strain-related acid production by oral streptococci. *Caries Res*. 34, 486-490 (2000).
5. Thayumanavan T, Harish B S, Subashkumar R, Shanmugapriya K, Karthik V. Streptococcus mutans biofilms in the establishment of dental caries: a review. *3 Biotech*. 15(3), 62 (2025).
6. Koo H, Allan R N, Howlin R P, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. *Nature Reviews Microbiology*. 15(12), 740-755 (2017).

7. Whelton H P, Spencer A J, Do L G, Rugg-Gunn A J. Fluoride Revolution and Dental Caries: Evolution of Policies for Global Use. *Journal of Dental Research*. 2019, 98(8), 837-846 (2019).
8. Schiffner U. Verwendung von Fluoriden zur Kariesprävention. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 64(7): 830-837 (2021).
9. Martinez-Mier E A. et al. Relationship between enamel fluorosis severity and fluoride content. *Journal of Dentistry*. 46, 42-46 (2016).
10. Liao Y et al. Fluoride resistance in *Streptococcus mutans*: a mini review. *Journal of Oral Microbiology*. 9(1), 1344509 (2017).
11. Zhang Q et al. Application of fluoride disturbs plaque microecology and promotes remineralization of enamel initial caries. *Journal of Oral Microbiology*. 14(1): 2105022; 10.1080/20002297.2022.2105022 (2022).
12. Brookes Z L S, Bescos R, Belfield L A, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. *Journal of Dentistry*. 103, 103497; 10.1016/j.jdent.2020.103497(2020).
13. Zhang T, Chu J, Zhou X. Anti-cariogenic Effects of *Galla chinensis*: A Systematic Review. *Phytother Res*. 29(12): 1837-1842 (2015).
14. Zhang Z et al. *Rhodiola rosea* extract inhibits the biofilm formation and the expression of virulence genes of cariogenic oral pathogen *Streptococcus mutans*. *Archives of Oral Biology*. 116, 104762; 10.1016/j.archoralbio.2020.104762 (2020).
15. Wu Z, Song J, Zhang Y, Yuan X, Zhao J. Inhibitory and preventive effects of *Arnebia euchroma* (Royle) Johnston. root extract on *Streptococcus mutans* and dental caries in rats. *BDJ Open*. 10(1),15 (2024).
16. Li YH, Liu J, Yang LC, Zhang CH, Li G. Antibacterial activity determination of six kinds of natural herbs in yunnan on normal oral predominant. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 28(2),199-207 (2010).
17. Nogales C et al. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*. 43(2), 136-150 (2022).
18. Duan X, Wang N, Peng D. Application of network pharmacology in synergistic action of Chinese herbal compounds. *Theory in Biosciences*. 143(3), 195-203 (2024).
19. Paggi J M, Pandit A, Dror R O. The Art and Science of Molecular Docking. *Annual Review of Biochemistry*. 93(1), 389-410 (2024).
20. Kim S et al. PubChem 2023 update. *Nucleic Acids Research*. 51(D1), D1373-D1380 (2023).
21. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*. 47(W1), W357-W364 (2019).

22. Chen H et al. Effects of *S. mutans* gene-modification and antibacterial monomer dimethylaminohexadecyl methacrylate on biofilm growth and acid production. *Dental Materials*. 36(2), 296-309 (2020).
23. Aqawi M, Sionov R V, Gallily R, Friedman M, Steinberg D. Anti-Biofilm Activity of Cannabigerol against *Streptococcus mutans*. *Microorganisms*. 9(10), 2031 (2021).
24. Wang Y et al. Antimicrobial peptide GH12 suppresses cariogenic virulence factors of *Streptococcus mutans*. *Journal of Oral Microbiology*. 10(1), 1442089; 10.1080/20002297.2018.1442089 (2018).
25. Li P et al. Therapeutic effects and mechanisms study of Hanchuan Zupa Granule in a Guinea pig model of cough variant asthma. *Journal of Ethnopharmacology*. 269, 113719; 10.1016/j.jep.2020.113719 (2021).
26. Wang L, Liu P, Wu Y, Pei H, Cao X. Inhibitory effect of *Lonicera japonica* flos on *Streptococcus mutans* biofilm and mechanism exploration through metabolomic and transcriptomic analyses. *Frontiers in Microbiology*. 15, 1435503; 10.3389/fmicb.2024.1435503 (2024).
27. Reck M et al. The Biofilm Inhibitor Carolacton Disturbs Membrane Integrity and Cell Division of *Streptococcus mutans* through the Serine/Threonine Protein Kinase PknB. *Journal of Bacteriology*. 193(20), 5692-5706 (2011).
28. Kanehisa M, Furumichi M, Sato Y, Matsuura Y, Ishiguro-Watanabe M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res*. 53(D1), D672-D677 (2025).
29. Chen P-M et al. Role of GlnR in Acid-Mediated Repression of Genes Encoding Proteins Involved in Glutamine and Glutamate Metabolism in *Streptococcus mutans*. *Applied and Environmental Microbiology*. 76(8), 2478-2486 (2010).
30. Liao Y, Zhang M, Lin X, Yan F. Diaryl Urea Derivative Molecule Inhibits Cariogenic *Streptococcus mutans* by Affecting Exopolysaccharide Synthesis, Stress Response, and Nitrogen Metabolism. *Frontiers in Cellular and Infection Microbiology*. 12, 904488; 10.3389/fcimb.2022.904488 (2022).
31. He X et al. The *cia* operon of *Streptococcus mutans* encodes a unique component required for calcium-mediated autoregulation. *Molecular Microbiology*. 70(1), 112-126 (2008).
32. Vij T et al. A Comprehensive Review on Bioactive Compounds Found in *Caesalpinia sappan*. *Molecules*. 28(17), 6247; 10.3390/molecules28176247 (2023).
33. Lu X, Xu H, Cheng Y, Liu W, Wang Y. Switchable Separation Strategy via Host-Guest Locks. *Langmuir*. 39(7), 2779-2786 (2023).
34. Ghendov-Mosan A et al. Chemometric Optimization of Biologically Active Compounds Extraction from Grape Marc: Composition and Antimicrobial Activity. *Molecules*. 27(5), 1610; 10.3390/molecules27051610 (2022).

35. Nava-Tapia D A et al. Brazilin: Biological activities and therapeutic potential in chronic degenerative diseases and cancer. *Pharmacological Research*. 175, 106023; 10.1016/j.phrs.2021.106023 (2022).
36. Sabbah D A, Hajjo R, Sweidan K. Review on Epidermal Growth Factor Receptor (EGFR) Structure, Signaling Pathways, Interactions, and Recent Updates of EGFR Inhibitors. *Current Topics in Medicinal Chemistry*. 20(10), 815-834 (2020).
37. Oliveira S G et al. Differential expression reveals inflammatory response and oxidative stress genes in dentin caries. *Archives of Oral Biology*. 175, 106274; 10.1016/j.archoralbio.2025.106274 (2025) .
38. Li Z-Y et al. Brazilin Ameliorates Diabetic Nephropathy and Inflammation in db/db Mice. *Inflammation*. 40(4), 1365-1374 (2017).
39. Vogler M et al. The BCL2 family: from apoptosis mechanisms to new advances in targeted therapy. *Signal Transduction and Targeted Therapy*. 10(1), 91; 10.1038/s41392-025-02176-0 (2025).
40. Loreto C, Psaila A, Musumeci G, Castorina S, Leonardi R. Apoptosis activation in human carious dentin. An immunohistochemical study. *European Journal of Histochemistry*. 59(3), 2513; 10.4081/ejh.2015.2513 (2015).
41. Jenie R I, Handayani S, Susidarti R A, Udin L Z, Meiyanto E. The Cytotoxic and Antimigratory Activity of Brazilin-Doxorubicin on MCF-7/HER2 Cells. *Advanced Pharmaceutical Bulletin*. 8(3), 507-516 (2018).
42. Tanaka M et al. Endothelial ROBO4 suppresses PTGS2/COX-2 expression and inflammatory diseases. *Communications Biology*. 7(1), 599; 10.1038/s42003-024-06317-z (2024).
43. Lin X-M et al. The Role of Prostaglandin-Endoperoxide Synthase-2 in Chemoresistance of Non-Small Cell Lung Cancer. *Frontiers in Pharmacology*. 10, 836; 10.3389/fphar.2019.00836 (2019).
44. Cha S-M, Cha J-D, Jang E-J, Kim G-U, Lee K-Y. Sophoraflavanone G prevents Streptococcus mutans surface antigen I/II-induced production of NO and PGE2 by inhibiting MAPK-mediated pathways in RAW 264.7 macrophages. *Archives of Oral Biology*. 68, 97-104 (2016).
45. Chang M-C et al. Inducing phospholipase A2 and cyclooxygenase-2 expression and prostaglandins' production of human dental pulp cells by activation of NOD receptor and its downstream signaling. *International Journal of Biological Macromolecules*. 292, 139193; 10.1016/j.ijbiomac.2024.139193 (2025).
46. Antunes L A et al. Analysis of the association between polymorphisms in MMP2, MMP3, MMP9, MMP20, TIMP1, and TIMP2 genes with white spot lesions and early childhood caries. *International Journal of Paediatric Dentistry*. 26(4), 310-319 (2015).

47. Zuehlke A D, Beebe K, Neckers L, Prince T. Regulation and function of the human HSP90AA1 gene. *Gene*. 570(1), 8-16 (2015).
48. Zhang H, Huang J, Fan X, Miao R, Wang Y. HSP90AA1 promotes the inflammation in human gingival fibroblasts induced by Porphyromonas gingivalis lipopolysaccharide via regulating of autophagy. *BMC Oral Health*. 22(1), 366; 10.1186/s12903-022-02304-0 (2022).

Figures

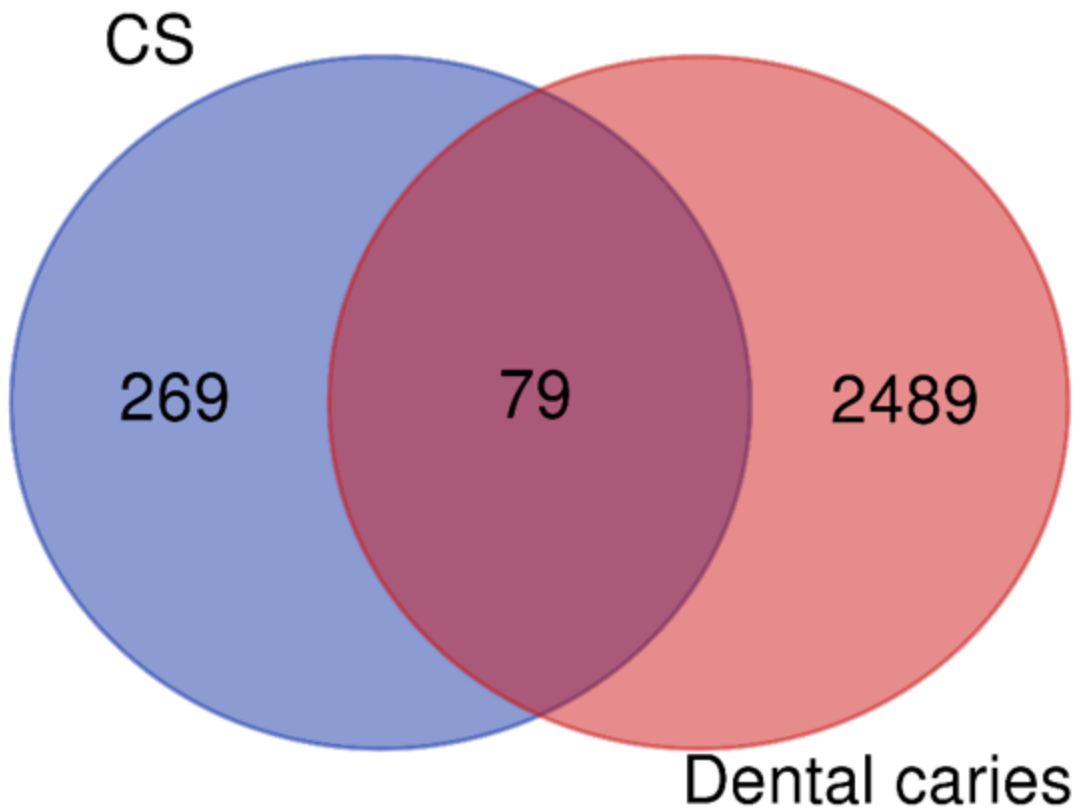


Figure 1

Venn diagram of the intersection of the CStargets and dental caries targets.

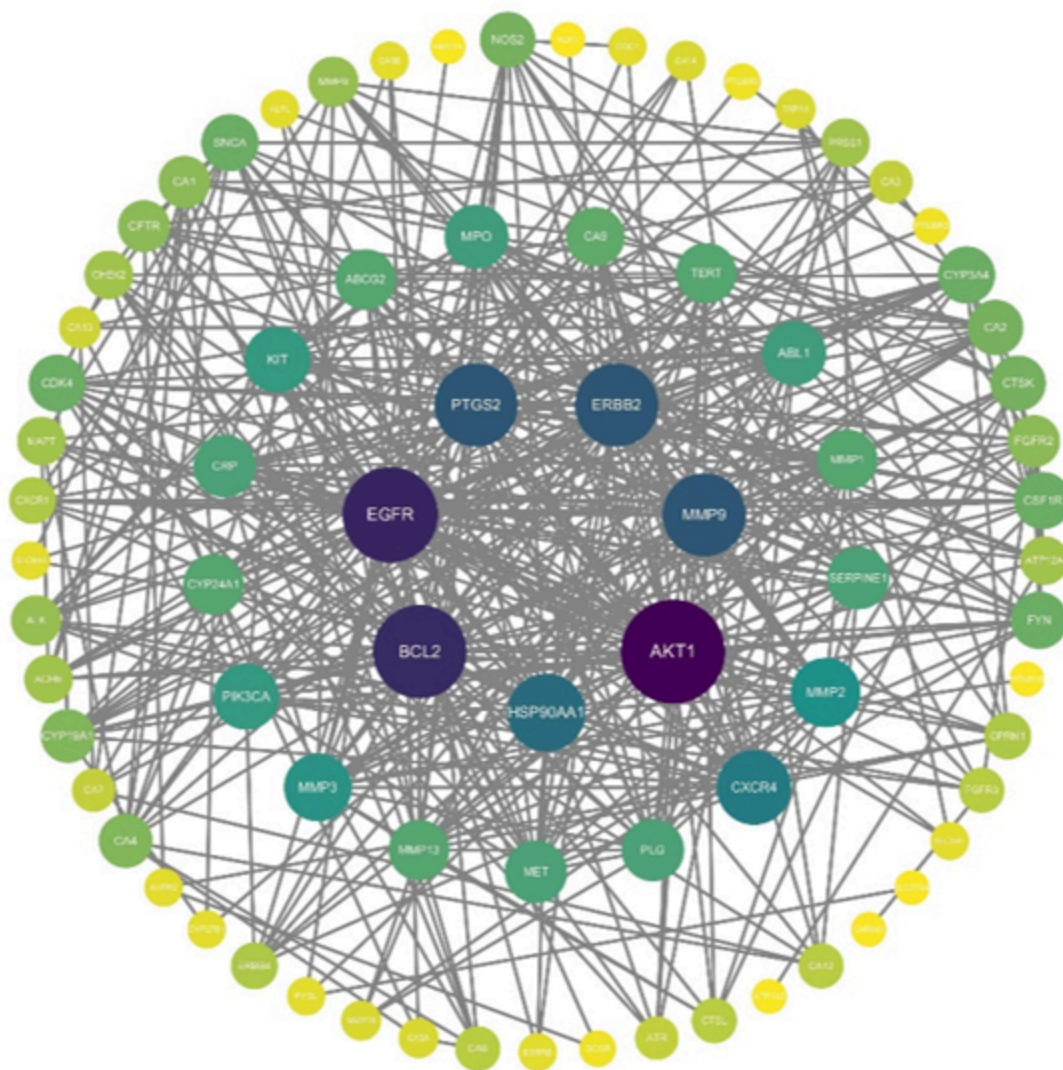


Figure 2

PPI network of the 79 potential therapeutic targets. Each node represents a relevant gene, and the edge thickness indicates the strength of the data support.

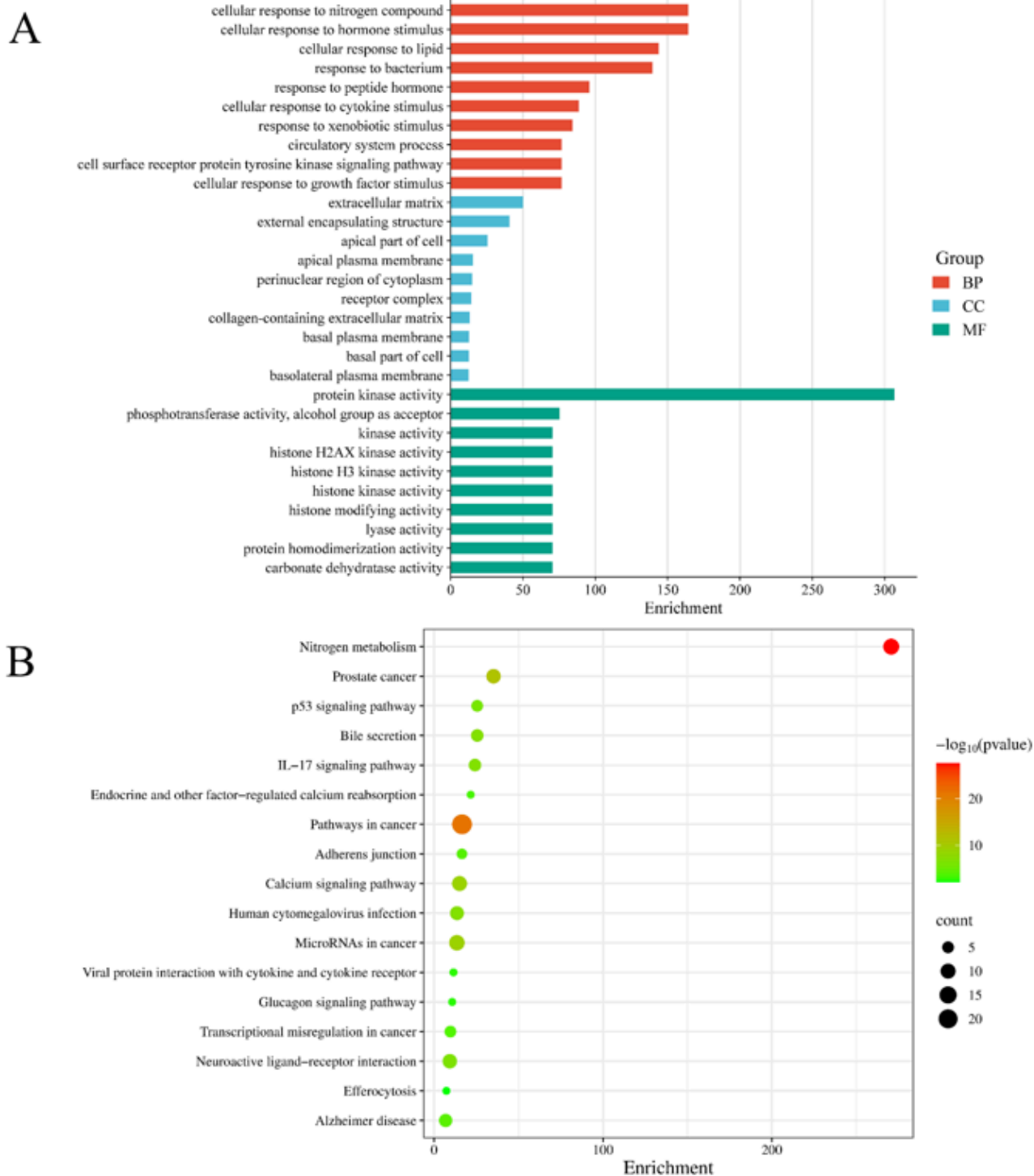


Figure 3

GO enrichment and KEGG pathway enrichment analysis. (A) GO enrichment analysis: the top 10 Biological Process (BP), Cellular Component (CC) and Molecular Function (MF); (B) Bubble chart of the KEGG pathways.

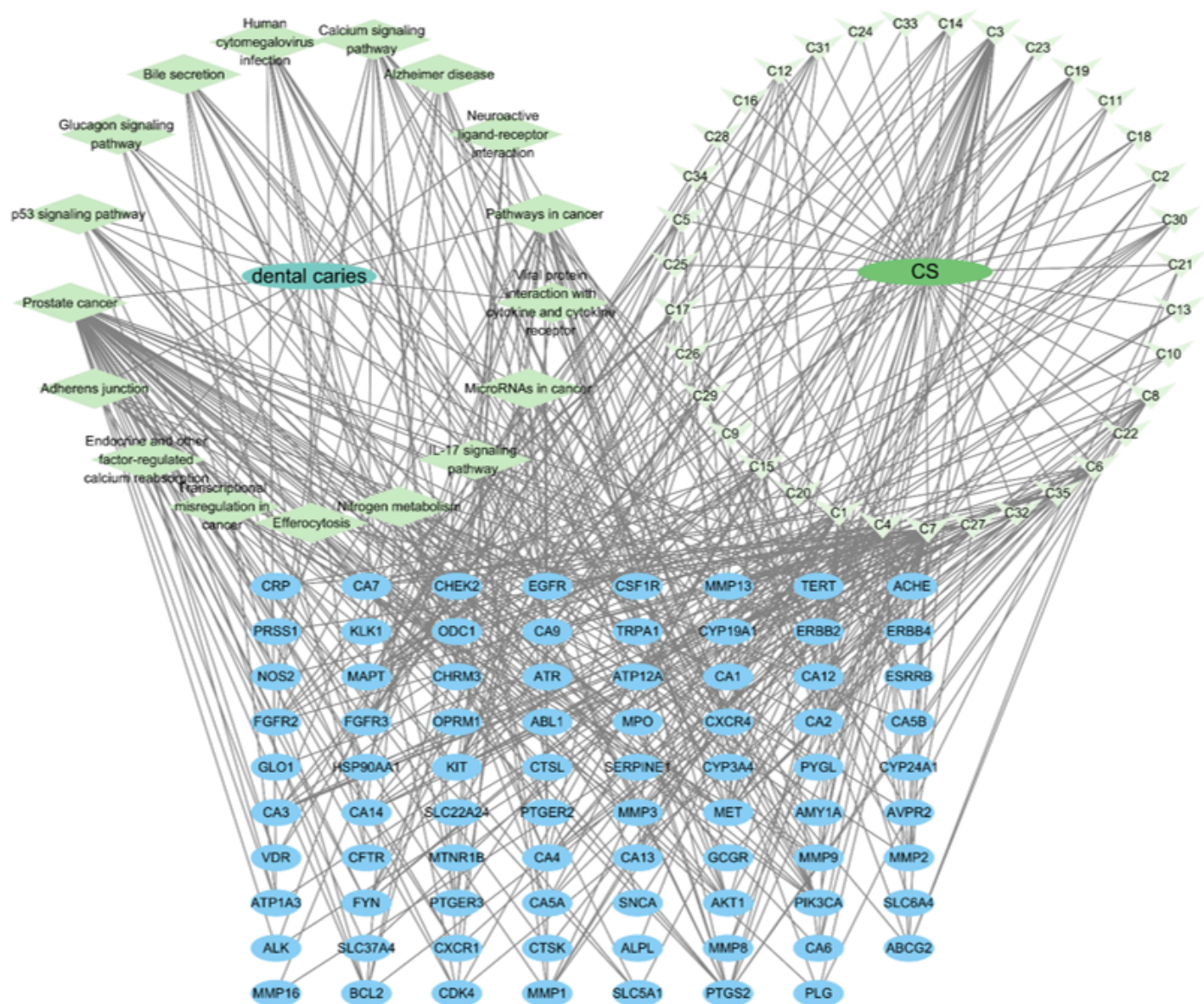


Figure 4

T-D-P network. The diamonds nodes indicate metabolic pathways, the triangle nodes indicate active ingredients of *CS*, the blue oval nodes indicate targets. The lines indicate interactions.

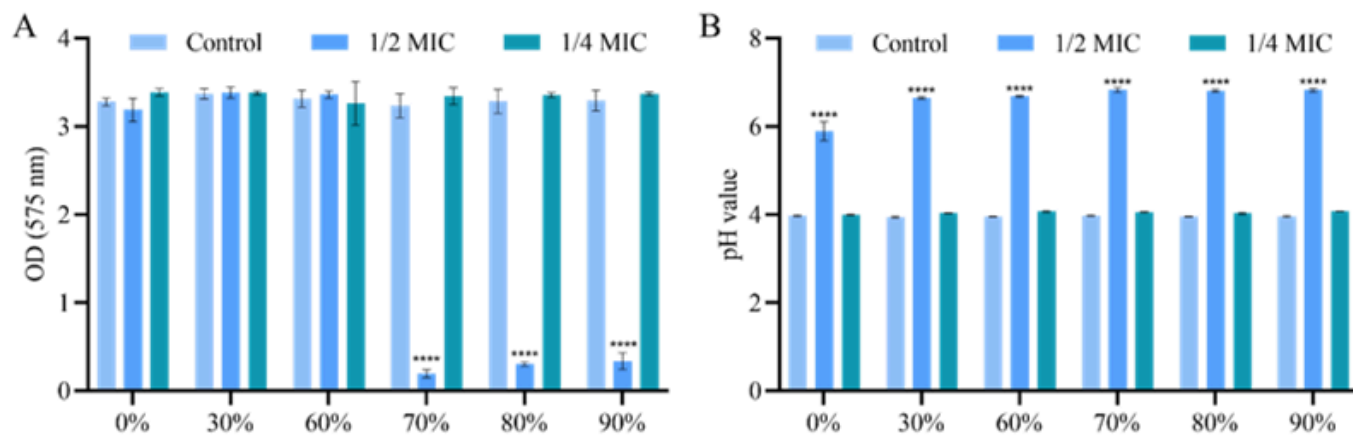


Figure 5

Different ethanol extracts on biofilm formation and acid production in *S. mutans*. (A) Quantitative analysis by crystal violet staining. (B) *S. mutans* medium pH measurement.

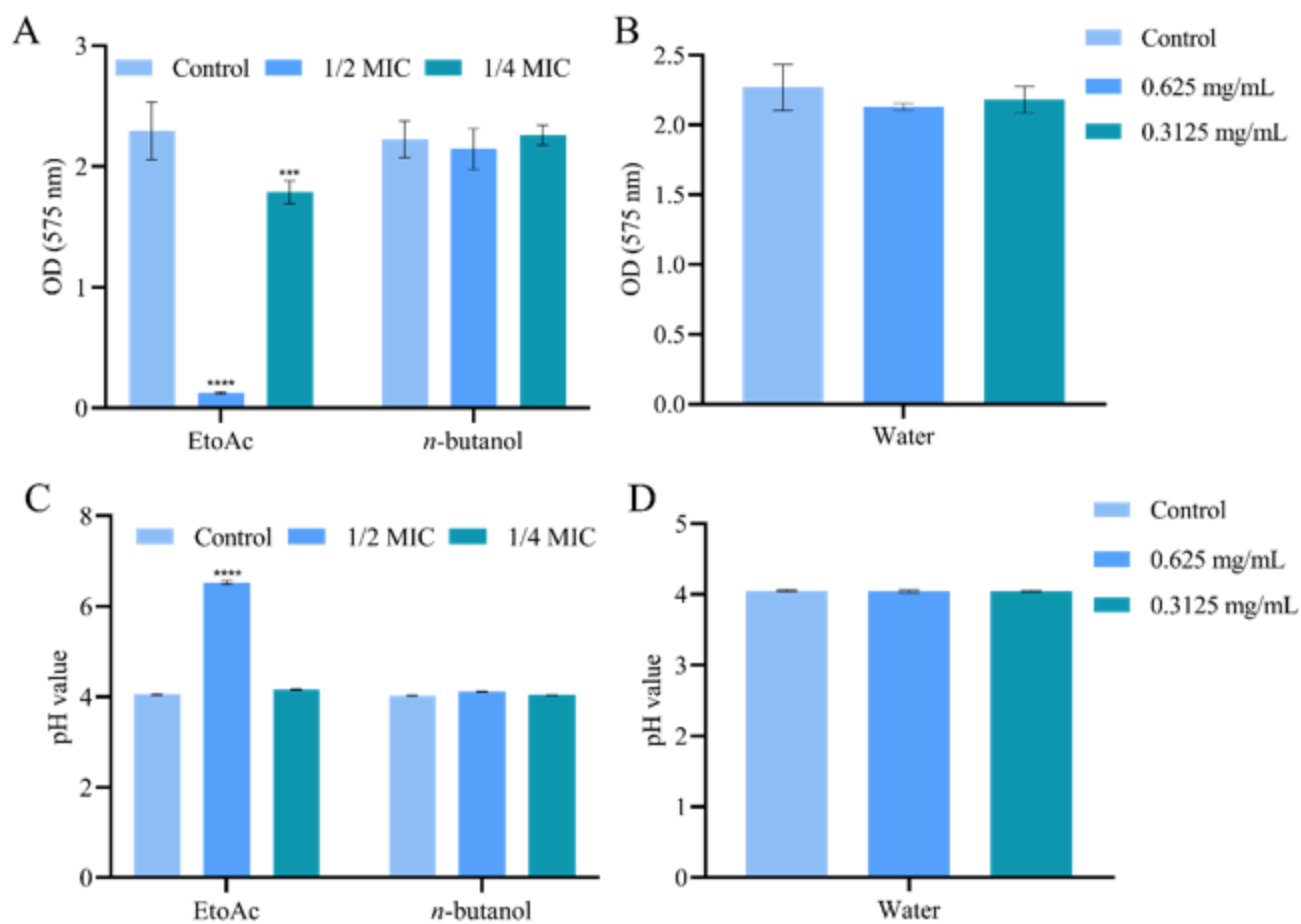


Figure 6

EtoAc, *n*-butanol, and water extracts on biofilm formation and acid production in *S. mutans*. (A, B) Quantitative analysis by crystal violet staining. (C, D) *S. mutans* medium pH measurement.

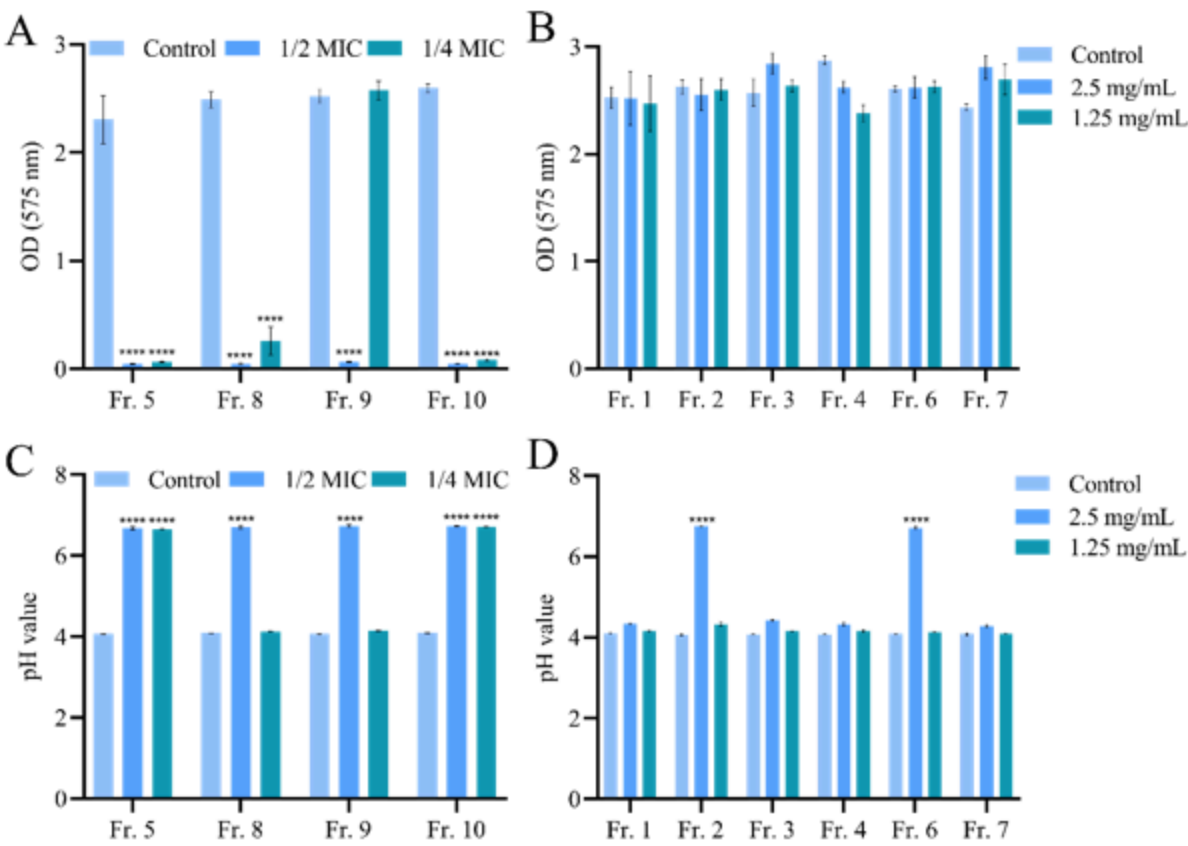


Figure 7

Different fractions on biofilm formation and acid production in *S. mutans*. (A) Quantitative analysis by crystal violet staining. (B) *S. mutans* medium pH measurement.

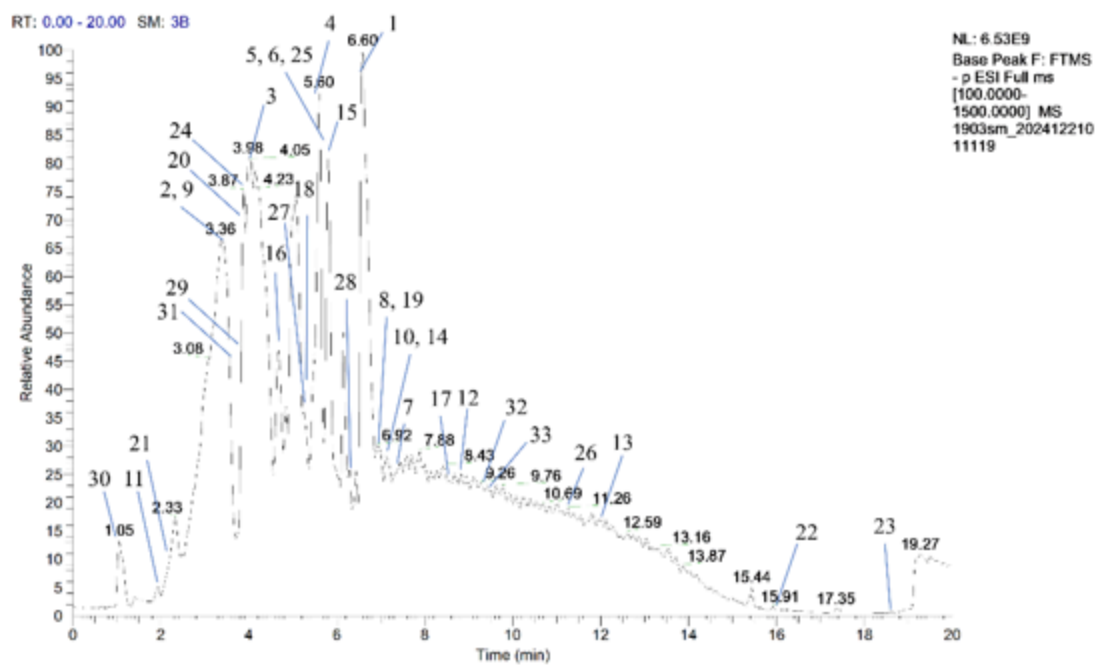


Figure 8

The total ion current map of Fr. 5 in the negative ion mode.

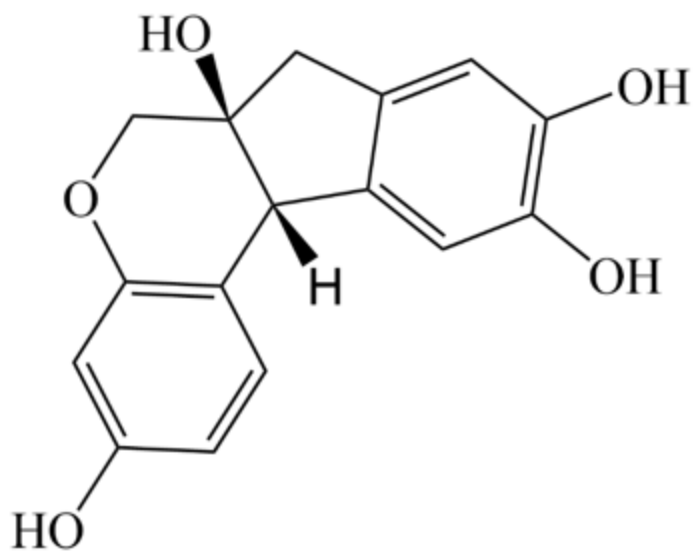


Figure 9

The chemical structural formula of brazilin.

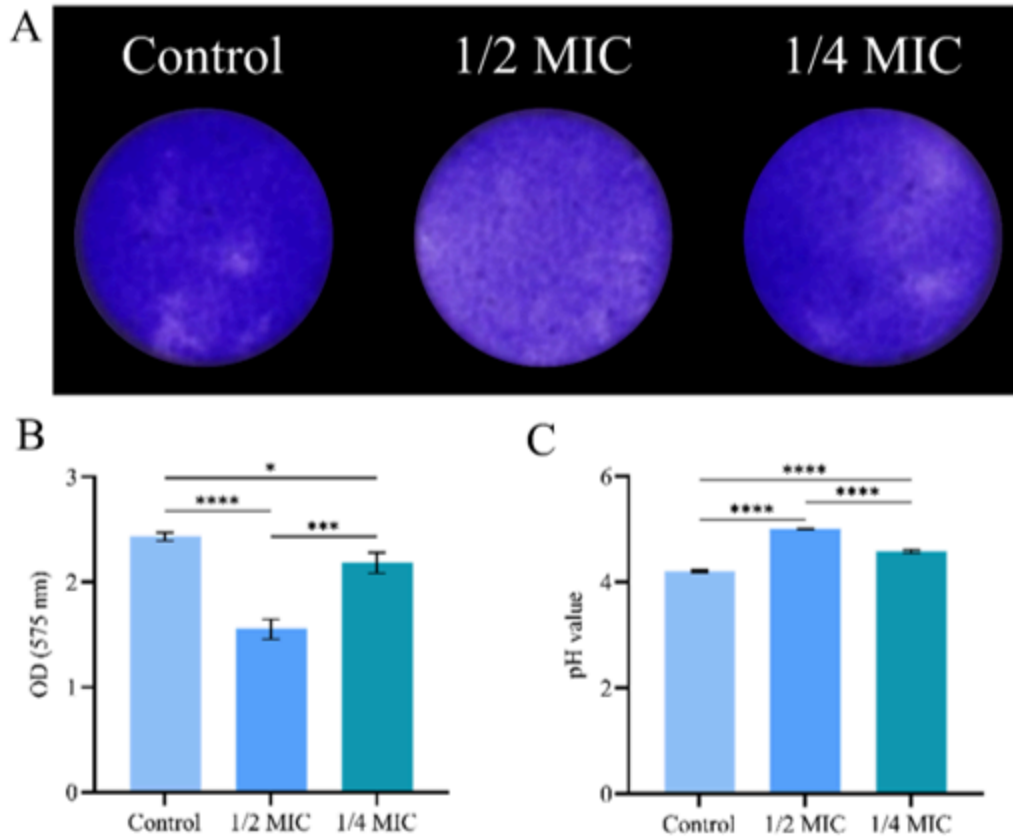


Figure 10

Effect of brazilin on biofilm formation and acid production in *S. mutans*. (A) Crystal violet staining. (B) Quantitative analysis by crystal violet staining. (C) *S. mutans* medium pH measurement.

