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Original Article

Antibacterial activity of *Stachytarpheta jamaicensis* (L.) Vahl roots extract on some bacteria proteins: An *in silico* and *in vitro* study

[Actividad antibacteriana del extracto de raíces de *Stachytarpheta jamaicensis* (L.) Vahl sobre algunas proteínas bacterianas: un estudio *in silico* e *in vitro*]

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Abstract

Context: *Stachytarpheta jamaicensis* (L.) Vahl plant is used for traditional therapy because of its content, including flavonoids, alkaloids, tannins, saponins, terpenoids, and coumarins.

Aims: To determine the antibacterial ability of *S. jamaicensis* roots extract (SJRE) on some selected mouth bacteria through *in vitro* and *in silico* studies.

Methods: Phytochemical analysis and liquid chromatography-high resolution mass spectrometry (LC-HRMS) were done to explore the active compounds on SJRE. Absorption, distribution, metabolism, excretion and toxicity prediction, molecular docking simulation and visualization of luvangetin, and xanthyletin as anti-inflammatory and antibacterial were investigated *in silico*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SJRE against *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, and *Actinomyces* spp. were calculated.

Results: Luvangetin and xanthyletin are good candidate drug molecules with low toxicity. Xanthyletin has higher binding activity than luvangetin to TNF-α, IL-6, IL-10, peptidoglycan, flagellin, and dectin protein. SJRE exhibited a high antibacterial ability, and MIC. This extract inhibits the growth of *A. actinomycetemcomitans, E. faecalis and Actinomyces* spp. at various concentrations 2000, 8000, and 8000 µg/mL, respectively, with statistically significant differences ($p = 0.0001$; $p<0.05$).

Conclusions: SJRE has an antibacterial ability, and 2000 µg/mL SJRE may act as an antibacterial agent *in vitro.* In addition, xanthyletin in SJRE has a potential role as an antibacterial and anti-inflammatory *in silico*.

Keywords: communicable disease; dentistry; infectious disease; medicine; periodontal disease.

Resumen

Contexto: La planta de *Stachytarpheta jamaicensis* (L.) Vahl se utiliza para la terapia tradicional por su contenido, que incluye flavonoides, alcaloides, taninos, saponinas, terpenoides y cumarinas.

Objetivos: Determinar la capacidad antibacteriana del extracto de raíces de *S. jamaicensis* (SJRE) sobre algunas bacterias bucales seleccionadas mediante estudios *in vitro* e *in silico*.

Métodos: Se realizaron análisis fitoquímico y cromatografía líquida-espectrometría de masas de alta resolución (LC-HRMS) para explorar los compuestos activos en SJRE. Se investigaron *in silico* la absorción, la distribución, el metabolismo, la excreción y la predicción de la toxicidad, la simulación de acoplamiento molecular y la visualización de la luvangetina y la xantiletina como antiinflamatorios y antibacterianos. Se calcularon la concentración inhibitoria mínima (MIC) y la concentración bactericida mínima (MBC) de SJRE contra *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis* y *Actinomyces* spp.

Resultados: Luvangetin y xanthyletin son buenas moléculas candidatos a fármacos y tienen baja toxicidad. La xantiletina tiene una mayor actividad de unión que la luvangetina a TNF-α, IL-6, IL-10, peptidoglicano, flagelina y proteína dectina. SJRE exhibió una alta capacidad antibacteriana y MIC. Este extracto inhibe el crecimiento de *A. actinomycetemcomitans*, *E. faecalis* y *Actinomyces* spp. a varias concentraciones 2000, 8000 y 8000 µg/mL, respectivamente, con diferencias estadísticamente significativas ($p = 0,0001$; $p<0,05$).

Conclusiones: SJRE tiene una capacidad antibacteriana y a 2000 µg/mL SJRE puede actuar como un agente antibacteriano *in vitro*. Además, la xantiletina en SJRE tiene un papel potencial como antibacteriano y antiinflamatorio *in silico*.

Palabras Clave: enfermedad infecciosa; enfermedad periodontal; enfermedad transmisible; odontología; medicamento.

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INTRODUCTION

A wide range of oral bacteria species significantly affects oral health. Sometimes they will bring many benefits for balancing oral conditions such as normal oral flora. Another side, there are some bacteria with pathogenic characteristics that bring detrimental effects that lead to systemic disease. Pathogenic microbes can infect host cells and lead to an inflammation process in the tissue. For example, periodontal disease as an inflammatory response to tissue infection caused by pathogenic mouth bacteria (Nugraha et al., 2022a; Ramadhani et al., 2020a). Based on Basic Health Research by the Indonesian Ministry of Health in 2018, the prevalence of periodontitis in Indonesia is 74.1%. Periodontal disease can affect the teeth' supporting tissues, which can cause loose teeth and even fall out (Ticoalu et al., 2016). While the prevalence of apical periodontitis varied between 7 and 86%, while that of posttreatment apical periodontitis varied between 10 and 62% (Ozok et al., 2012).

Classification of periodontal disease is based on disease progression, namely chronic periodontitis, and aggressive periodontitis. Chronic periodontitis (CP) cases are associated with a plethora of plaque, calculus, and endodontic infection (Mehrotra and Singh, 2022). Periodontal disease is a common oral infectious disease that is related to some bacteria such as *A. actinomycetemcomitans*, *Enterococcus faecalis* and *Actinomyces* spp. A previous study presented *E. faecalis* as the main microorganism associated with endodontic failure (Prada et al., 2019). Actinomyces are detected in root canals associated with apical periodontitis (Ozok et al., 2012).

Regarding the aseptic nature of the root canal system in its healthy and intact state, any bacteria present can be regarded as an endodontic pathogen. Aggressive periodontitis is caused by an inadequate immune system against pathogenic organisms. The dominant bacterium that plays a role in aggressive periodontitis is *Aggregatibacter actinomycetemcomitans* (Aberg et al., 2015; Ramadhani et al., 2020b).

The bacterium has the ability to express some proteins that act as important virulence properties. The toxin kills white blood cells in a variety of ways, and leukocyte destruction is essential for subsequent bacterial growth and stimulation of the host inflammatory response. There is high expression of proinflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6, IL-12, tumor necrosis factor-alpha (TNF-α), and regulatory cytokines such as IL-4, IL-1(RA) receptor antagonist, IL-10, and induced protein (IP)-10 in the case of periodontitis (Nugraha et al., 2022b; 2022c; Ramadan et al., 2020).

Flagellin is a major structural flagellar protein commonly expressed by commensal and pathogenic bacteria. Flagellin interacts with the pattern recognition receptors toll-like receptor 5 (TLR5) and nucleotide-binding oligomerization domain-like receptor (NLR) family caspase activation and recruitment domain (CARD) domain containing 4, leading to the production of proinflammatory cytokines and chemokines (Ridwan, 2012; Cook et al., 2020). Peptidoglycan (PG) composes the bacteria cell wall and is a vital molecule providing a protective function in bacteria (Irazoki et al., 2019). The other protein that can be expressed as a response to microorganisms is dectin-1. It was originally described as the β-glucan receptor expressed in myeloid cells (Mata-Martinez et al., 2021). These biomarkers have an important role as drug target therapy of antibacterial and anti-inflammatory activity.

Treatment with antibiotics is also often chosen to eliminate pathogenic bacteria and supportive therapy for immunomodulators (Ridwan et al., 2017). The organism cannot rule out the side effects of antibiotics, so many people switch chemical drugs to herbal medicines. One of the wild plants easily found in the tropics, including South Kalimantan that can be used as a medicinal plant, is *Stachytarpheta jamaicensis* (L.) Vahl, *Verbenaceae* family (Ticoalu et al., 2016). This is a wild plant and a weed on agricultural land that grows in tropical areas such as Indonesia. Ethnomedically *S. jamaicensis* is used for allergies, respiratory disorders, fever, constipation, and digestive complications (Liew and Yong, 2016; Suhirman et al., 2015). People in Kalimantan generally use the flowers and roots of *S. jamaicensis* as a traditional medicine to relieve sore throat and cough by boiling and drinking it, while the leaves are used as toothache medicine (Liew and Yong, 2016; Mingga et al., 2019). On the other hand, *Stachytarpheta cayenensis*, a member of the same genus and family, can act as an antioxidant by scavenger of mitochondrial reactive oxygen species (de Souza et al., 2011).

Also, it has been studied that *S. jamaicensis* contains secondary metabolites such as flavonoids, phenols, saponins, tannins, terpenoids and coumarins (Liew and Yong, 2016; Yuliana et al., 2019). *S. jamaicensis* leaves also showed secondary metabolites such as alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and quinones (Wahyudi et al., 2019). In another study was found that the leaf extract of *S. jamaicensis* could inhibit the growth of several bacteria *Eschericia coli, Enterococcus faecalis, Mycobacterium variants, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus agalactiae, Serratia marcescens* and *Salmonella typhi-*

murium at concentrations of 250, 500, and 1000 µg/mL with different zones of inhibition (Ololade et al., 2017).

SJRE may be promising phytotherapy that acts as an antioxidant, anti-inflammatory, and antibacterial that can treat oral infectious diseases. However, the study about ethanol extract of SJRE as an antibacterial in mouth bacteria is still limited. Furthermore, this study investigated SJRE as an antibacterial on some selected mouth bacteria through *in vitro* study and its potential as antibacterial and anti-inflammatory through *in silico* study.

MATERIAL AND METHODS

Ethical clearance statement

All methods in this research were performed following the relevant guidelines and regulations by the Ethics Committee, Faculty of Dentistry, University of Lambung Mangkurat, Surabaya, Indonesia, with number No. 079/KEPKG-FKGULM/EC/IX/2021 and No. 080/KEPKG-FKGULM/EC/IX/2021.

Stachytarpheta jamaecensis roots extract (SJRE) sample preparation

S. jamaicensis was collected from Kiram Park, Sungai Andai village, Cempaka District, Banjarbaru City (3.4572° S, 114.8103° E). Fresh *S. jamaicensis* without any indication of damage (physical or disease) were collected by picking and then stored in dark plastic samples and stored in a clean box. The samples were prepared at the Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University. The roots were cleaned by washing using running water to remove the attached impurities, and then the water bundle was dried and cut into small pieces.

S. jamaicensis roots extraction process

Five hundred and fifty grams of *S. jamaecensis* roots were carried out by a single solvent maceration method using ethanol in a ratio of 1:2 (w/v) with several solvent changes due to solvent saturation. The filtrate and residue were prepared by filtering the maceration products with filter paper. A vacuum rotary evaporator was used to evaporate the filtrate at 4°C. The thick extract obtained from the evaporation results was desalted using the decantation technique, mixing the thick extract with ethanol and allowing the salt to settle. Finally, the procedure was repeated until the white tint indicated salt in the solvent was no longer visible. The solvent was then evaporated in a vacuum rotary evaporator. Then, the extract of *S. jamaicensis* roots was concentrated and stored at 4°C for further examination.

Metabolite profiling by LC-HRMS

The metabolites in the ethanol extract were characterized using phytochemical tests and liquid chromatography-high resolution mass spectrometry (LC-HRMS). Phytochemical tests were performed to detect compounds from flavonoids, phenols, saponins, tannins, and terpenoids (Liew and Yong, 2016; Utami et al., 2019).

Metabolite profiling was performed using two tools**:** liquid chromatography (LC) and mass spectrometry (MS). LC analysis was performed using the UltiMate™ 3000 RSLCnano System with a microflow meter (Thermo Scientific, USA). The column used was Hypersil GOLD aQ 50 × 1 mm × 1.9 µm particle size with a flow rate of 40 µL/min for 30 min. The mobile phase used was 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Metabolite profiling of *S. jamaecensis* roots' extract analysis was performed using Q Exactive Mass Spectrometers (Thermo Scientific Q Exactive, US). Compounds were screened with a resolution of 70.000 dab 17.500 for 30 min. The compound predictive analysis was performed using the mzCloud MS/MS Library.

An *in vitro* study

Bacteria culture

The antibacterial activity of SJR crude extract was investigated by employing a dilution method. The method was carried out with three bacteria species, including the Gram-negative bacteria *Agreggagibacter actinomycetemcommitans* ATCC 43718 (Biomedicine Laboratory, Faculty of Dentistry University of Lambung Mangkurat) and the Gram-positive bacteria *Enterococcus faecalis* ATCC 19433 (Embrio Biotekindo Collection) and *Actinomyces* spp. ATCC 49338 (Biomedicine Laboratory, Faculty of Dentistry University of Lambung Mangkurat).

Several colonies of mouth pathogenic bacteria from pure isolates were put into the BHIB media using a sterile loop. The BHIB media were put into an anaerobic incubator and incubated for 1 × 24 hours at 37°C. After the bacteria sample was cultured on BHIB media, then a suspension was made by taking bacteria from the media using a sterile tube and then putting it into a test tube containing 1 mL of sterile BHIB, then incubated under anaerobic conditions in 5% CO_2 at 37°C for 1 × 24 h. After that, dilute sterile distilled water and homogenize until the turbidity is comparable to the standard Mc Farland 0.5 (1.5×10^8) (Nugraha et al., 2022a). Metronidazole gel (25%) and sodium hypochlorite (2.5%) were used to assess the MIC values of the reference strains. MIC and MBC values were defined as the lowest concentration of each

group, which completely inhibited growth or yielded no viable microorganisms, respectively. The results were expressed in micrograms per milliliter.

Dilution method

The crude SJRE was diluted with distilled water to obtain concentrations of 125, 250, 500, 1000, 2000, 4000, and 8000 µg/mL to test the antibacterial ability of SJRE using solid and liquid dilution methods. Then, the sterile test tubes were covered with sterile cotton and homogenized with a vortex mixer. After that, 1 mL of standardized bacterial suspension with McFarland turbidity 0.5 (1.5×10^8) CFU/mL was put into each test tube containing 1 mL of the extract with eight different concentrations: 125, 250, 500, 1000, 2000, 4000, 8000 µg/mL and each positive control. The test tube was then cultured to determine the effect of SJRE on the growth of bacteria, after which the absorbance of the tube was measured using a 722AP UV-Vis Spectrophotometer (λ = 460 nm) (Nugraha et al., 2022a).

Determination of MIC and MBC

The MIC measurement was determined by comparing the absorbance after incubation minus before incubation. If there was a negative value, we could say that bacterial growth was inhibited (MIC). If the value of delta Optical Density (OD) was positive, so bacterial growth in the media was still present. After obtaining the MIC value, a further test was carried out to determine the MBC by taking 100 µL of the concentration showing the MIC added to a Petri dish containing sterile NA media, and then was incubated for 24 hours at 37°C. After that, the number of bacteria was counted by a colony counter. If the result of relying on the number of bacterial colonies was zero (no bacteria), then the MBC was obtained (Nugraha et al., 2022a).

Statistical analysis

Statistical analysis was performed with an SPSS version 17.0 software program (SPSS, Inc., released 2008; SPSS Statistics for Windows, version 17.0; SPSS, Inc., Chicago, IL, USA). Data were analyzed using Kruskal-Wallis followed by the Mann-Whitney post hoc test ($p<0.05$). The results were expressed as mean ± SD.

Bioinformatic approach, an *in silico* study

Sample preparation

This study used chemical compounds containing *S. jamaicensis,* which consisted of luvangetin and xanthyletin. The Canonical 3D and SMILE structures of the two compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The targets used in this study were TNF-α, NF-κB, RANKL-RANK, IL-6, IL-10, peptidoglycan, flagellin, and dectin. 3D structure information, visualization method, Uniprot ID, PDB ID, resolution (Å), weight (kDa), sequence length (mer), and chain were obtained from the RCSB PDB database (https://www.rcsb.org/) (Nugraha et al., 2022a).

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction

Predictions of absorption, distribution, metabolism, excretion, and toxicity of *S. jamaicensis* chemical compounds were carried out on Swiss ADME (http://www.swissadme.ch/) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction). Physicochemical properties, water solubility, drug-likeness and toxicity are used to predict the ability of query compounds as good candidate drug molecules in general (Berniyanti et al., 2022; Utami et al., 2022).

Virtual screening

The ability of the query compound activity to bind to the target protein in this study was predicted through molecular docking simulations. Molecular docking can be used to determine the type of activity of a ligand and the pattern of molecular interactions when it binds to the target protein based on the value of binding affinity, the type of binding activity is inhibition or increase according to the research objectives (Fahmi et al., 2021; Kharisma et al., 2021; Wijaya et al., 2021). This study used PyRx 0.9.9 version software to identify the binding ability of *S. jamaicensis* compounds to eight target proteins.

Ligand-protein interaction

Identification of positions and types of molecular interactions in this study were identified through the Discovery Studio 2016 version of the software. Types of chemical bond interactions such as Van der Waals, hydrogen, hydrophobic, electrostatic, and pi are found in the docked molecular complexes. The interactions formed are weak bonds that play a role in triggering the activity of the target protein (Fahmi et al., 2021; Ramadhani et al., 2022).

Molecular visualization

Virtual prediction analysis and visualization of protein-ligand complexes from the docking step were analyzed and visualized using Discovery Studio. The interaction site was analyzed based on the ligand–residue interaction and structural conformation. The software works with python programming and is used for the structural selection or coloring of docked

molecular complexes (Ardani et al., 2022; Luqman et al., 2020).

RESULTS

The metabolite profiling analysis of SJRE presented in chromatogram data depicted seven major compound peaks with two compounds that have the best peak formation. They were luvangetin and xanthyletin (Fig. 1). In this study, the SJRE ethanol extract exhibited MIC, MBC and can inhibit the growth of *A. actimomycetemcomitans*, *Enterococcus faealis,* and *Actinomyces* spp. (Tables 1 and 2). The most MIC of *A. actimomycetemcomitans* was found in metronidazole treatment followed by SJRE of 8000, 4000, and 2000 µg/mL with significant differences ($p= 0.0001$; $p<0.05$) (Fig. 2A-B). Meanwhile, the most extensive MBC of *A. actimomycetemcomitans* was found in metronidazole treatment but did not follow by SJRE concentration. The MIC of SJRE on *Actinomyces* spp. and *E. faecalis* bacteria growth was 8000 µg/mL (Figs. 2C and E). However, the MBC on the growth of *Actinomyces* spp. and *E. faecalis* bacteria was not found at that concentration (Figs. 2D and F).

Eight target proteins have been obtained from the database (Table 3), based on ADMET predictions that luvangetin and xanthyletin compounds meet several drug-likeness parameters, then both compounds can be soluble and allow them to pass through the selectively permeable cell layer. In pharmacokinetics analysis, LD_{50} of luvangetin and xanthyletin have a value near under 2500 mol/kg, while hepatotoxicity and AMES toxicity showed no toxicity effects (Table 4).

The results of molecular docking simulations show that xanthyletin has higher activity than luvangetin. This is based on the binding affinity value formed when it binds to all target proteins (Table 5). The docked molecular complexes in this study were displayed using Discovery Studio version software with structural selection and staining (Figs. 3 and 4). The binding location of the docked protein-ligand complex (Figs. 5 and 6) revealed that binding xanthyletin compounds to all target proteins resulted in non-covalent bond interactions consisting of Van der Waals, pi, and hydrogen bonds (Table 6).

DISCUSSION

Microorganisms are important agents that play a role in the incidence of oral diseases. Pathogenic bacteria will be aggressive and cause inflammation in the tissues of the mouth. *A. actinomycetemcomitans* produces large amounts of exotoxin proteins and leukotoxins that play an important role in the pathogenicity of these bacteria (Krueger and Brown, 2020). One of the *A. actinomycetemcomitans* strains was also reported to produce a cytolethal distending toxin (Cdt) to deliver peptidoglycan to the cytosol and initiate NOD1-dependent NF-κB activation (Nice et al., 2018). *E. faecalis* induced an NF-κB inflammatory response and impaired DNA damage response and cell cycle control gene expression (Strickertsson et al., 2013). This bacterium has cytolysin and hyaluronidase, which could facilitate migration and collagen fragmentation related to inflammation in host cells (Asmah, 2020). The previous study revealed that the infection of the macrophage precursors by *E. faecalis* could restrict the plasticity of macrophage change into M2 macrophages (Elashiry et al., 2021). *Enterococcus faecalis* is considered the main cause of intraradicular apical persistence infections and endodontic failures. *Actinomyces* spp. is associated with persistent extraradicular endodontic infections, with possible involvement of the soft tissues of the maxillofacial district (Dioguardi et al., 2020).

The use of *S. jamaicensis* has been appreciated since remote times and has been widely used by society. It was verified that this extract was effective in eliminating microorganisms. The antimicrobial activity of SJRE on three bacteria colonies, *A. actinomycetemcomitans*, *E. faecalis,* and *Actinomyces* spp. was observed. The effective action of *S. jamaicensis* leaves extract on some bacteria such as *E. coli, E. faecalis, M. varians, S. aureus, K. pneumonia, P. aeruginosa, P. mirabilis, S. agalactiae, S. marcescens,* and *S. typhimurium* were also reported (Ololade et al., 2017). This fact might contribute to treating some diseases caused by these types of microorganisms present in the mouth. The real mechanism of action of SJRE has not yet been elucidated in the literature.

The decrease in absorbance value indicates the antibacterial activity of SJRE secondary metabolites, including flavonoid compounds, tannins, terpenoids, saponins, phenols, and coumarins. The inhibitory activity of SJRE on mouth pathogenic bacteria was attributed to these compounds, particularly saponins. Previous studies showed that the most dominant content of SJRE is saponin compounds (31.602%) (Liew and Yong, 2016, Utami et al., 2021). The mechanism of action of saponins as an antibacterial is by lowering the surface tension, resulting in increased permeability of cell leakage and the release of intracellular compounds. These compounds diffuse through the outer membrane and cell wall, then bind to the cytoplasmic membrane and cause cytoplasmic leakage, which results in cell death (Chinonye et al., 2019). Flavonoids, as antibacterial, have a mechanism by inhibiting nucleic acid synthesis, inhibiting bacterial motility, inhibiting the function of bacterial membranes, resulting in impaired bacterial cell permeability function and causing bacterial cell death. The nu-

cleic acid in the hydrogen bonding process causes a reduction in DNA and RNA synthesis, which causes bacteria to lyse and die (Babii et al., 2018; Tagousop et al., 2018; Zhang et al., 2020).

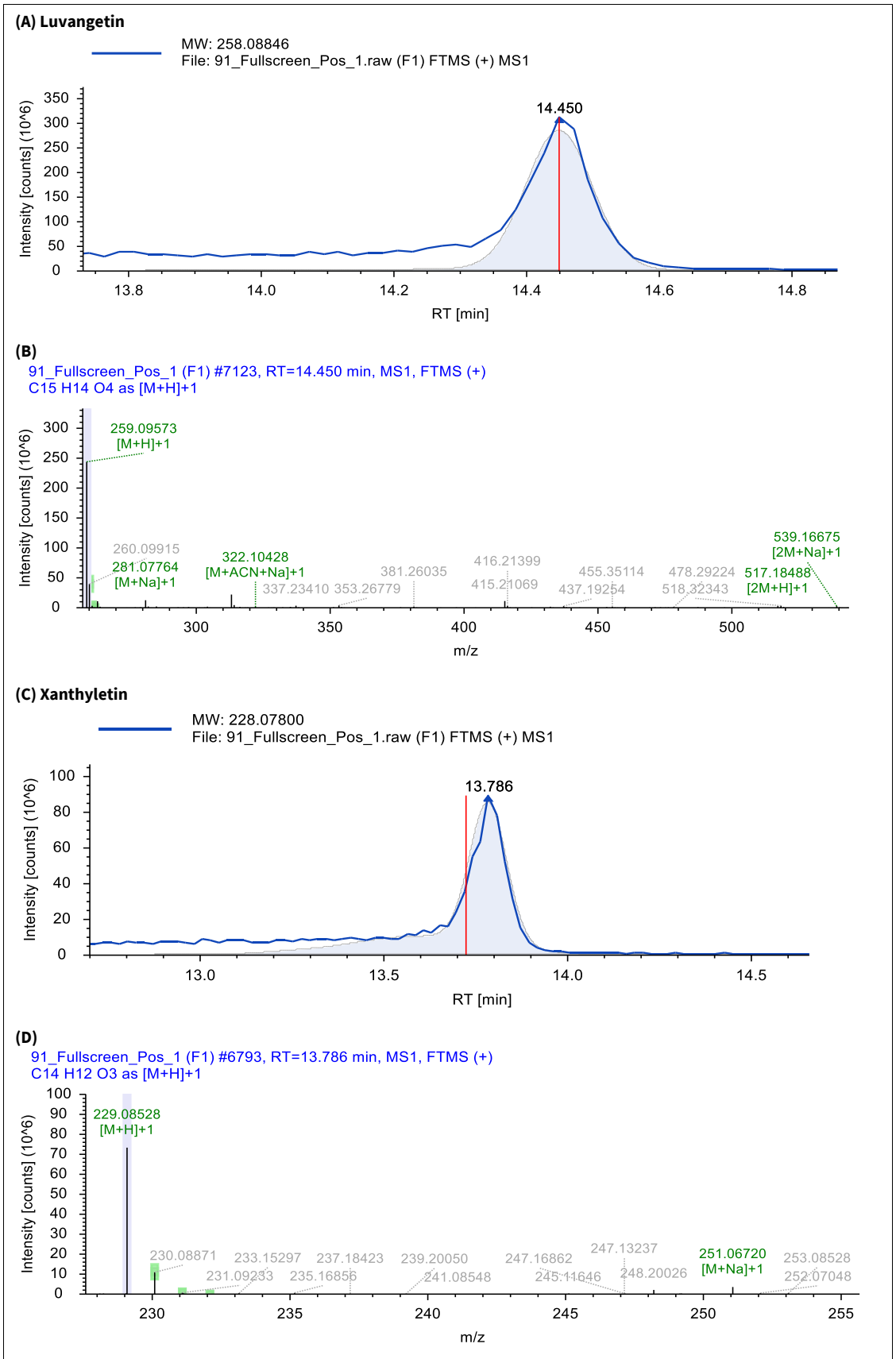


Figure 1. Luvangetin chromatogram **(A)** and product ion mass spectra **(B)**; Xanthyletin chromatogram **(C)** and product ion mass spectra **(D)**.

Table 1. Inhibitory activity of various concentrations of *S. jamaicensis* roots extract (SJRE) on some selected mouth pathogenic bacteria.

Bacteria	Absorbance value							Positive controls	
	125 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)	2000 (µg/mL)	4000 (µg/mL)	8000 (µg/mL)	MTZ	NaClO
A. actinomycetemcomitans	0.626	0.570	0.331	0.247	-0.091	-0.454	-1.158	-1.158	-
E. faecalis	0.391	0.356	0.191	0.186	-0.162	-0.551	-0.580	-	-0.661
Actinomyces spp.	0.467	0.451	0.361	0.270	0.186	0.165	-0.133		-0.067

MTZ: Metronidazole 25%; NaClO 2.5%.

Table 2. Total colony of various concentrations of *S. jamaicensis* roots extract (SJRE) on some selected mouth pathogenic bacteria.

Bacteria	Bacteria colony							Positive controls	
	125 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)	2000 (µg/mL)	4000 (µg/mL)	8000 (µg/mL)	MTZ	NaClO
A. actinomycetemcomitans	2071.86	1688.43	1377.57	1269.29	1069.71	962.29	754.00	0	-
E. faecalis	944.00	724.50	594.43	431.71	372.36	257.36	151.21	-	0
Actinomyces spp.	5280.71	4815.00	3734.29	277.29	1577.50	843.93	569.43	-	0

MTZ: Metronidazole 25%; NaClO 2.5%.

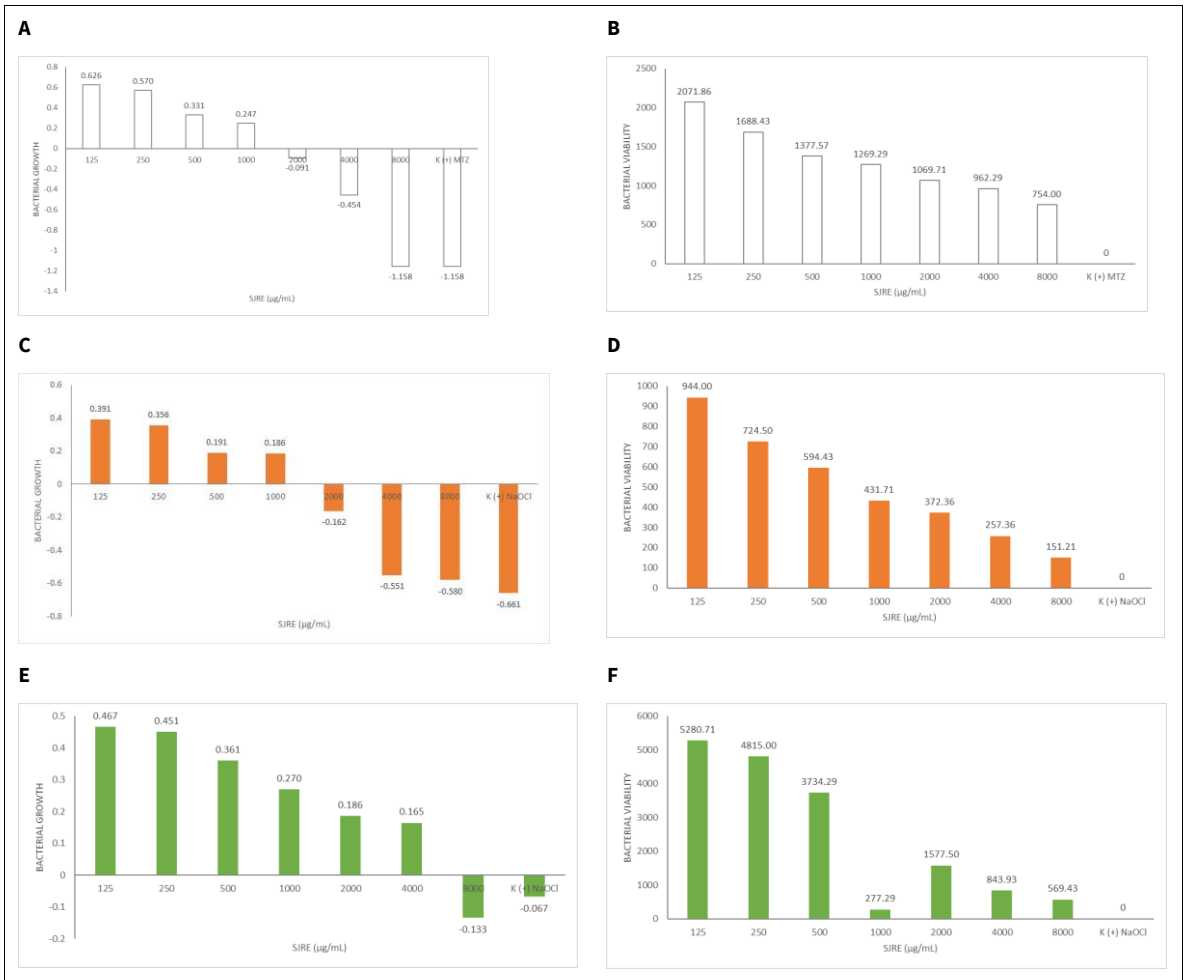


Figure 2. Antibacterial activity of *S. jamaicensis* roots extract (SJRE).

(A) MIC of *Aggregatibacter actinomycetemcomitans*; **(B)** MBC of *Aggregatibacter actinomycetemcomitans*; **(C)** MIC of *Enterococcus faecalis;* **(D)** MBC of *Enterococcus faecalis*; **(E)** MIC and MBC of *Actinomyces* spp.; **(F)** MBC of *Actinomyces* spp.

Table 3. Protein target from database.

No.	Name	Visualization method	Uniprot ID	PDB ID	Resolution (Å)	Weight (kDa)	Sequence length (mer)	Chain
1	TNF-α	X-ray diffraction	P01375	1TNF	2.60	52.11	157	A/B/C
2	NF-κB	NMR	P19838	2DBF	-	10.62	100	A
3	RANKL-RANK	X-ray diffraction	A3RF19	3URF	2.70	38.38	162	A
4	IL-6	NMR	P05231	1IL6	-	21.01	185	A
5	IL-10	X-ray diffraction	Q13651	1INR	2.00	18.67	160	A
6	Peptidoglycan	X-ray diffraction	P46022	2OQO	2.10	23.77	200	A
7	Flagellin	X-ray diffraction	P02968	6GOW	2.10	47.81	304	A
8	Dectin	X-ray diffraction	Q6QLQ4	2bph	2.20	32.50	120	A/B

Table 4. ADMET analysis of luvangetin and xanthyletin.

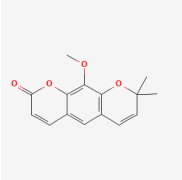
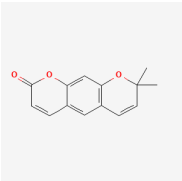
Compounds	Physicochemical properties	Water solubility	Drug-likeness	Pharmacokinetics
Luvangetin ID: 343582 	Formula: $C_{15}H_{14}O_4$ Weight: 258.27 g/mol Num. heavy atoms: 19 Num. arom. heavy atoms: 10 Fraction Csp3: 0.27 Num. rotatable bonds: 1 Num. H-bond acceptors: 4 Num. H-bond donors: 0 Molar Refractivity: 73.10 TPSA: 48.67 Å²	Log S (ESOL): -3.53 Class: Soluble Log S (Ali): -3.48 Class: Soluble Log S (SILICOS-IT): -4.61 Class: Moderately soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability: 0.55	Predicted LD_{50}: 2.391 mol/kg Hepatotoxicity: No AMES toxicity: No
8,8-dimethyl-2H,8H-pyrano[3,2-g]chromen-2-one (xanthyletin) ID: 65188 	Formula: $C_{14}H_{12}O_3$ Weight: 228.24 g/mol Num. heavy atoms: 17 Num. arom. heavy atoms: 10 Fraction Csp3: 0.21 Num. rotatable bonds: 0 Num. H-bond acceptors: 3 Num. H-bond donors: 0 Molar Refractivity: 66.61 TPSA 39.44 Å²	Log S (ESOL): -3.47 Class: Soluble Log S (Ali): -3.32 Class: Soluble Log S (SILICOS-IT): -4.48 Class: Soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability: 0.55	Predicted LD_{50}: 2.307 mol/kg Hepatotoxicity: No AMES toxicity: No

Table 5. The molecular docking result in a molecule target.

Protein	Autogrid						Binding affinity (kcal/mol)	
	Center (Å)			Dimensions (Å)			Luvangetin	Xanthyletin
	X	Y	Z	X	Y	Z		
TNF-α	19.968	49.6750	39.9300	62.7286	63.8888	61.3590	-7.8	-7.9
NF-κB	-0.7426	-3.3354	-0.1610	36.1752	39.1602	31.3259	-6.0	-5.8
RANKL-RANK	-3.0065	-3.6827	23.7825	73.9681	82.8082	75.0812	-7.5	-7.2
IL-6	-0.2005	0.3333	0.2940	53.9946	35.2560	39.6528	-6.6	-7.4
IL-10	13.0255	21.3802	4.3991	45.5835	38.5491	66.3735	-7.2	-7.4
Peptidoglycan	37.6467	37.7330	21.9306	49.9397	45.1712	45.0019	-6.3	-6.9
Flagellin	14.1973	89.2737	152.1143	46.6384	84.8978	60.8079	-7.0	-7.2
Dectin	28.9906	33.4845	37.7241	79.2648	46.4051	36.8908	-6.7	-7.4

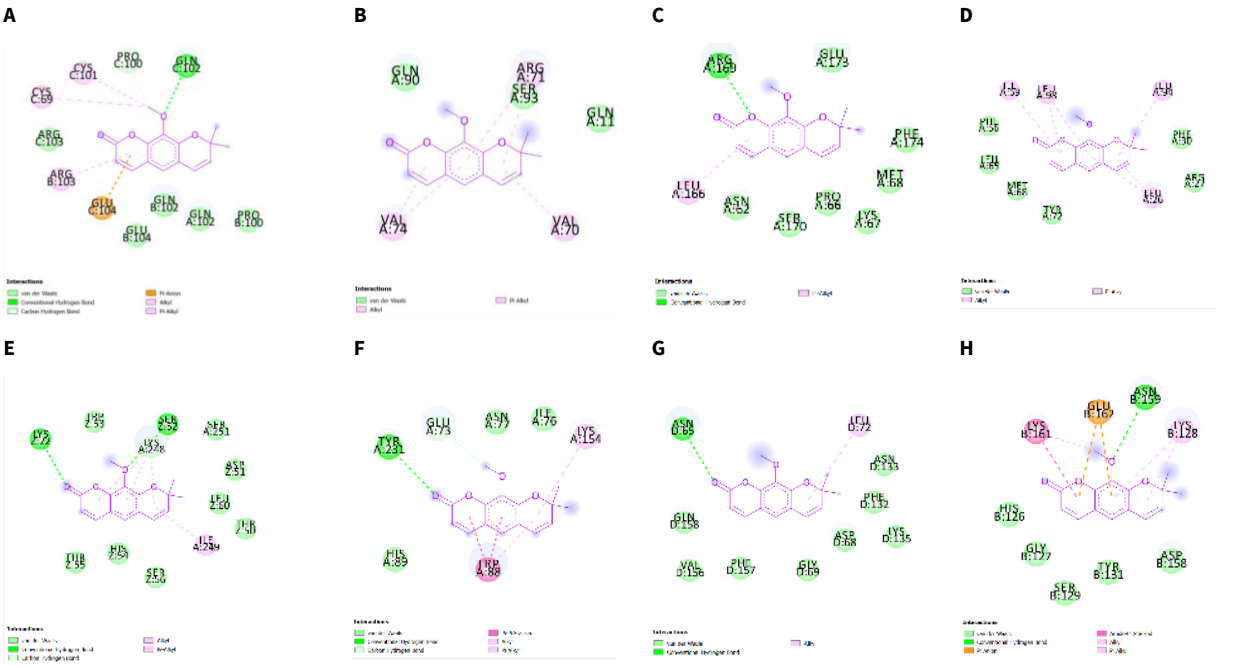


Figure 3. Molecular complex from docking simulation in 2D picture.

(A) TNF-α-Luvangetin; **(B)** NF-κB-Luvangetin; **(C)** IL-6-Luvangetin; **(D)** IL-10-Luvangetin; **(E)** RANKL-RANK-Luvangetin; **(F)** Peptidoglycan-Luvangetin; **(G)** Flagellin-Luvangetin; **(H)** Dectin-Luvangetin.

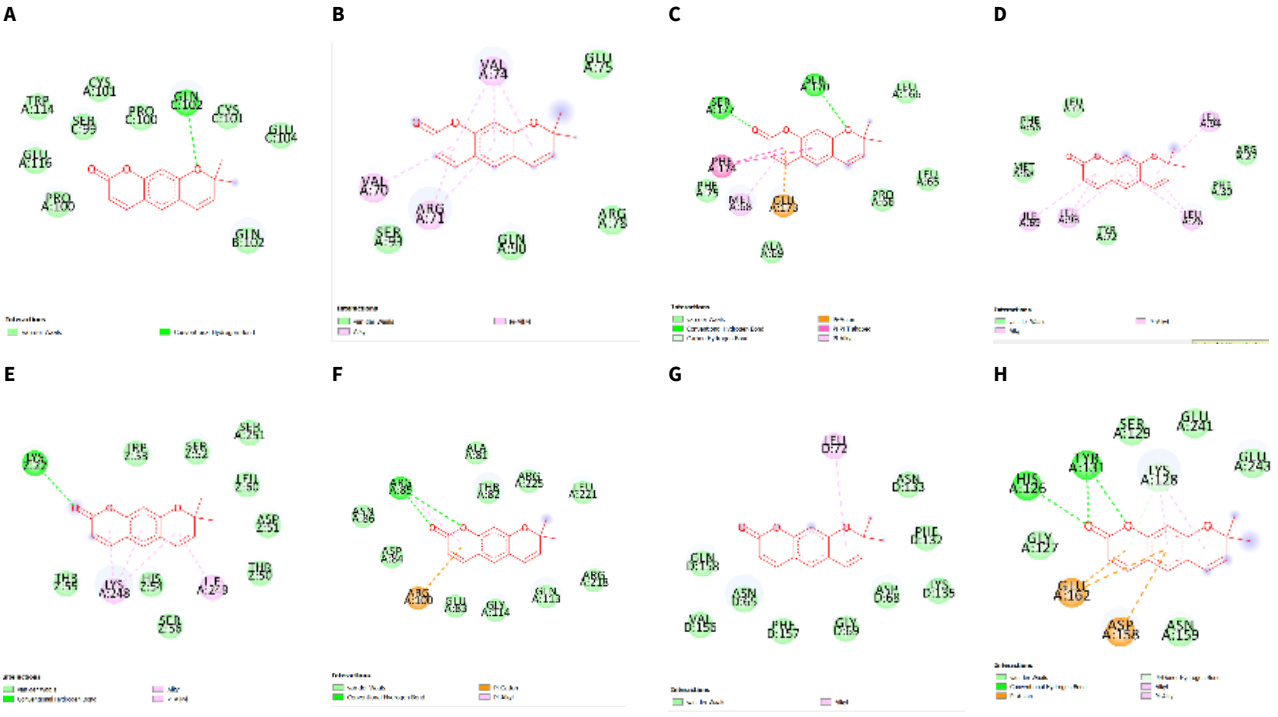


Figure 4. Molecular complex from docking simulation in 2D picture.

(A) TNF-α-Xanthyletin; **(B)** NF-κB-Xanthyletin; **(C)** IL-6-Xanthyletin; **(D)** IL-10-Xanthyletin; **(E)** RANKL-RANK-Xanthyletin; **(F)** Peptidoglycan-Xanthyletin; **(G)** Flagellin-Xanthyletin; **(H)** Dectin-Xanthyletin.

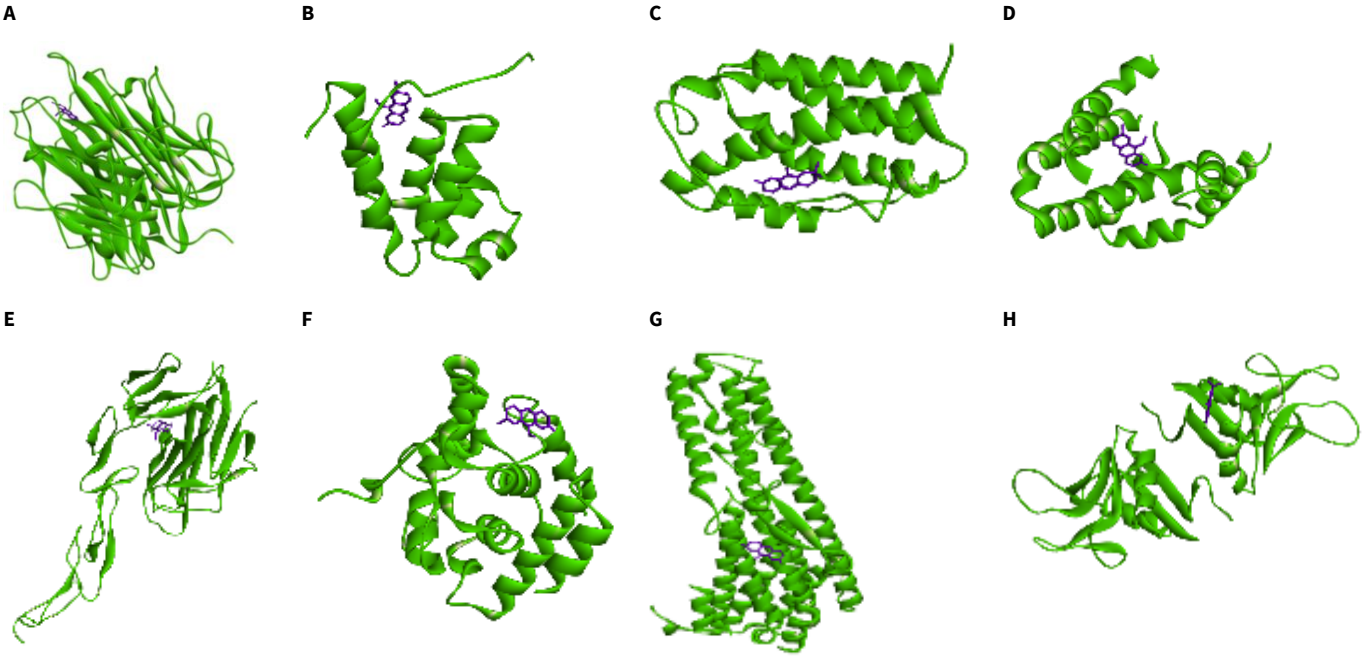


Figure 5. Molecular complex from docking simulation in 3D picture.

(A) TNF-α-Luvangetin; **(B)** NF-κB-Luvangetin; **(C)** IL-6-Luvangetin; **(D)** IL-10-Luvangetin; **(E)** RANKL-RANK-Luvangetin; **(F)** Peptidoglycan-Luvangetin; **(G)** Flagellin-Luvangetin; **(H)** Dectin-Luvangetin.

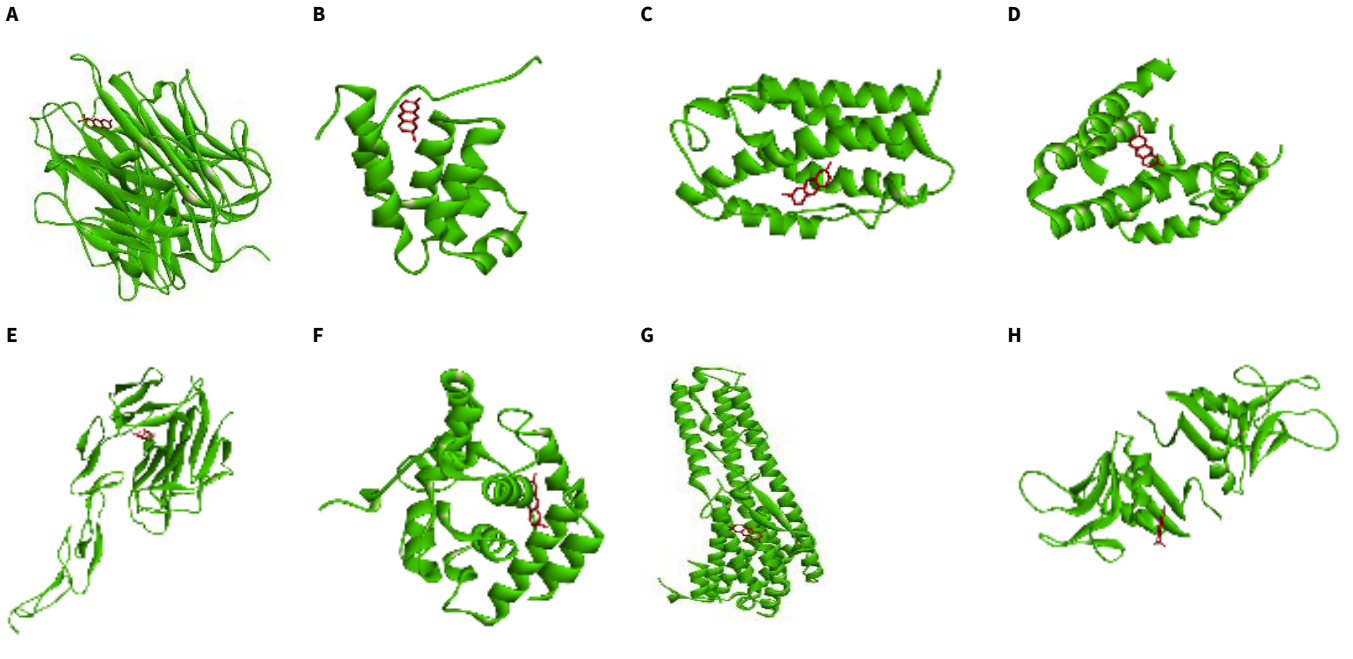


Figure 5. Molecular complex from docking simulation in 3D picture.

(A) TNF-α-Xanthyletin; **(B)** NF-κB-Xanthyletin; **(C)** IL-6-Xanthyletin; **(D)** IL-10-Xanthyletin; **(E)** RANKL-RANK-Xanthyletin; **(F)** Peptidoglycan-Xanthyletin; **(G)** Flagellin-Xanthyletin; **(H)** Dectin-Xanthyletin.

Table 6. Results of molecular interaction analysis.

Ligand-Protein	Chemical interaction	Ligand-Protein	Chemical interaction
Luvangetin-TNF-α	**Hydrogen:** Gln102 **Van der Waals:** Pro100 **Pi:** Glu104, Cys101, Cys69, Arg103	Luvangetin-RANKL-RANK	**Hydrogen:** Ser52 **Van der Waals:** Lys248 **Pi:**Ile249
Xanthyletin-TNF-α	**Hydrogen:** Gln102	Xanthyletin-RANKL-RANK	**Hydrogen:** Lys22 **Pi:** Ile249, Lys248
Luvangetin-NF-κB	**Van der Waals:** Ser93 **Pi:** Val70, Val74, Arg71	Luvangetin-Peptidoglycan	**Hydrogen:** Arg85 **Pi:** Arg85, Arg100
Xanthyletin-NF-κB	**Pi:** Val70, Val74, Arg71	Xanthyletin-Peptidoglycan	**Hydrogen:** Thr198, His214, Arg215, Ala216 **Pi:** Arg215
Luvangetin-IL-6	**Hydrogen:** Arg169 **Pi:** Leu166	Luvangetin-Flagellin	**Hydrogen:** Asn65 **Pi:** Leu72
Xanthyletin-IL-6	**Hydrogen:** Ser170, Ser177 **Pi:** Glu173, Met68, Phe174	Xanthyletin-Flagellin	**Pi:** Leu72
Luvangetin-IL-10	**Pi:** Leu26, Leu94, Leu98, Ile69	Luvangetin-Dectin	**Hydrogen:** Asn159 **Pi:** Lys161, Glu162, Lys128
Xanthyletin-IL-10	**Pi:** Leu26, Leu94, Leu98, Ile69	Xanthyletin-Dectin	**Hydrogen:** Tyr131, His126 **Van der Waals:** Lys128 **Pi:** Lys128, Glu162, Asp158

Phenol compounds act as toxins in protoplasm, denature bacterial cell proteins, inhibit cell wall synthesis, and damage cell membranes (Bourab-Chibane et al., 2019). Tannins work by inactivating bacterial cell adhesion, inactivating enzymes, inactivating the function of genetic material, and attacking cell wall polypeptides to interfere with cell permeability and cause growth retardation and even death (Maisetta et al., 2019; Vu et al., 2017). Terpenoids react with porins on the outer membrane of bacterial cells and reduce the permeability of the bacterial cell wall. This mechanism causes bacterial cells to lack nutrients and causes bacterial growth to be inhibited or bacterial lysis (Guimarães et al., 2019; Wang et al., 2019; Yang et al., 2020).

In addition, coumarins are active compounds found in SJRE content. Flavonoids, tannins, coumarins, terpenoids and steroids, alkaloids, and anthraquinones were detected in *Stachytarpheta cayenensis* ethanol extract (Onofre et al., 2015). According to LC-HRMS results, luvangetin and xanthyletin are coumarin compounds detected as major compound peaks. The impressive biological properties such as luvangetin and xanthyletin can act as antiulcer, antibacterial effects, and antifungal activity (Erst et al., 2022; Tatsimo and Lamshöft, 2015). Luvangetin inhibited the NO and PGE_2 production in LPS-stimulated BV2 cells (Tuan Anh et al., 2017). Based *on in silico,* xanthyletin has a better binding affinity score than luvangetin and interferes with the component of peptidoglycan, flagellin, and dectin to inhibit bacterial activity.

The positive control group with metronidazole showed significant inhibition and killing power in this study against *A. actinomycetemcomitans*. Metronidazole gel works by inhibiting the synthesis of nucleic acids, damaging the DNA helix structure, and causing the DNA chain to break. The impact on DNA synthesis is inhibited and causes bacterial cells to die (Shafquat et al., 2019). The inhibitory ability and killing power of 2.5% sodium hypochlorite as positive control can release the active ingredient in the form of chlorine resulting from the neutralization reaction. Chlorine in contact with DNA causes a decrease in amino acids. It produces an antibacterial effect by inhibiting the nucleic acid synthesis and impairing DNA synthesis, and this mechanism is similar to chlorhexidine (Ruksakiet et al., 2020; Zhou and Nanayakkara, 2021).

Molecular docking simulations are used to predict the mechanism of binding of luvangetin and xanthyletin to proteins. These two compounds were chosen because they have the highest maximum area and major compound peak based on LC-HRMS results. The simulation seeks to create negative energy by determining the level of binding ability of a ligand to a protein domain based on the binding affinity value of the ligand-protein stable complex (Prahasanti

et al., 2021). When a protein interacts with a ligand, binding affinity is established. According to thermodynamic rules, this energy is created by a reversible reaction at constant temperature and pressure (Pinzi and Rastelli, 2019). The grid in the docking simulation aids in the direction of ligand binding to the target protein (Kumar et al., 2018).

The activity of luvangetin and xanthyletin compounds in SJRE allows it to be anti-inflammatory through inhibition of regulation or decrease in the activity of proinflammatory proteins such as TNF-α, NF-κB, RANKL-RANK and IL-6, which can then trigger upregulation of anti-inflammatory proteins such as IL-10. The activity of these compounds can inhibit peptidoglycan, flagellin, and dectin activity. Hydrogen bonds, hydrophobicity, Van der Waals, and pi all play a role in the docking complex's weak bond interactions, which help to initiate the creation of specific biological activities (Kharisma et al., 2021). Overall, weak binding interactions can help build stable ligand-protein complexes and trigger activity responses on target proteins, including enhancement and inhibition.

SJRE may be promising phytotherapy with anti-inflammatory, anti-periodontitis, and antibacterial abilities. In addition, SJRE can be a potential therapeutic candidate to prevent periodontal disease. Therefore, this study result is limited to *in silico* and *in vitro* studies using ATCC bacteria related to periodontal disease. Further study is still needed to investigate the exact mechanism of periodontal disease after administering SJRE *in vivo* with various experimental methods.

CONCLUSION

Based on the findings of this investigation, it can be inferred that SJRE has an active compound that may be effective against some mouth pathogen bacteria *in vitro*. In addition, an in-silico analysis found that luvangetin and xanthyletin in SJRE have antibacterial and anti-inflammatory properties. Further research on the potential of xanthyletin as the best active compound from SJRE in the periodontal disease model *in vivo* is urgently needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Utami JP	Diana S	Arifin R	Taufiqurrahman I	Nugraha KA	Sari MW	Wardana RY
Concepts or ideas	x						
Design	x						
Definition of intellectual content	x	x	x	x			
Literature search	x	x	x	x			
Experimental studies	x				x	x	x
Data acquisition	x				x	x	x
Data analysis	x				x	x	x
Statistical analysis					x	x	x
Manuscript preparation	x				x	x	x
Manuscript editing	x						
Manuscript review	x	x	x	x	x	x	x

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