Mode action prediction of catechin from *Uncaria gambir* Roxb. against UDP-*N*-acetylenolpyruvyl-glucosamine reductase (MurB enzyme) of *Streptococcus mutans*: *In silico* study

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ABSTRACT

The prevalence of oral health problems in the global population is still high, especially dental caries, which is considered a multifactorial disease involving the role of bacteria, namely *Streptococcus mutans.* Gram-positive bacteria metabolize carbohydrates and sugars and convert them into lactic acid, causing dental caries. The peptidoglycan (PG) layer at the outer surface of the bacteria acts as protection. MurB enzyme is known for its contribution to PG biosynthesis. Gambir (*Uncaria gambir* Roxb.) is famous for many efficacies. Previous studies show that catechin from herb plants such as *U. gambir* has antibacterial activity. This study aimed to evaluate and predict the antibacterial activity of catechin from *U. gambir* against the MurB enzyme, which contributes to forming the bacteria PG, with an *in silico* approach. The structure of the MurB enzyme was collected from UniProt, and the ligands (catechin and chlorhexidine) structures were obtained from PubChem. The AutoDock software was used to dock both ligand and MurB enzyme visualized using PyMOL and analyzed using BIOVIA. The results showed that catechin has a binding affinity of more than $-$ 7 kcal/mol against the MurB enzyme, and chlorhexidine has a higher binding affinity than catechin. Both catechin and chlorhexidine have similar amino acids attachment by hydrogen bonds. The results showed that catechin has competitive antibacterial activity against chlorhexidine in inhibiting the MurB enzyme.

Key words: Catechin, dental caries, gambir, MurB enzyme, peptidoglycan

INTRODUCTION

Dental caries is the most common oral disease and is experienced by most people in the world. Therefore, it becomes a significant public health problem globally. According to the Global Burden of Disease Study 2016, dental and oral health problems, especially dental caries,

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affects almost half of the world's population (3.58 billion people). Gum disease (periodontal) ranks 11th most common disease in the world. While in Asia-Pacific, oral cancer is the 3rd most common type of cancer. Dental and oral health can reflect the overall health of the body, including if there is a lack of nutrition and symptoms of other diseases in the body. In particular, severe dental caries leads to tooth infections which can cause pain and chronic systemic infections.[1]

As a multifactorial disease, dental caries is caused by interrelated conditions. Notably, there are five factors that are showing prominent influence to the formation of caries lesions, namely retention and accumulation of plaque, frequency of carbohydrate intake, frequency of exposure to acidic foods, protective factors for pellicle and saliva, as well as fluoride and other substances that can control caries development.[2] *Streptococcus mutans* is a Gram-positive bacteria that has the ability to metabolize carbohydrates and sugars and further convert them into lactic acid (known as lactic acid bacteria), which causes tooth demineralization and leads to the formation of dental caries.[3-5] The cell wall of Gram-positive bacteria is composed of complex macromolecules. The macromolecules of which consist of peptidoglycans (PG), which resembles a sac that surrounds the cytoplasmic membrane on which other glycopolymers such as teichoic acid (TA) or polysaccharides (PSs) and proteins attached to.[4] PG consists of a glycan chain formed from *N*-acetylglucosamine (GlcNac) and *N*-acetylmuramic acid (MurNAc) with various attached amino acids.[3,4] The initial stage in the cytoplasm is the formation of *N*-acetylglucosamine-*N*-acetylmuramyl pentapeptide, which is catalyzed by several Mur (MurA–F) enzymes. UDP-*N*-acetylenolpyruvylglucosamine reductase (MurB) catalyzes the reduction of enolpyruvate to D-lactate to produce UDP-*N*-acetylmuramate, which has a role in the formation of PG.[3]

Clinically, one of the treatment modes carried out to prevent and treat caries is the Caries Management by Risk Assessment (CAMBRA) approach, introduced by Young and Featherstone.[6] CAMBRA is a tool used to assess the risk factors of dental caries for diagnosis, preventive measures, and dental care management. Caries risk assessment is carried out through history taking, bacterial tests, and caries preventive planning through several measures, such as fluoride application, chlorhexidine mouthwash, dental sealants, and use of xylitol.[6]

Chlorhexidine has been long used as an antiseptic and is commonly used as a mouthwash in the field of dentistry.[7,8] Chlorhexidine-contained mouthwash has long been used and recognized as a regimen to clean teeth mechanically.[9,10] Chlorhexidine gluconate with a concentration of 0.12% is considered to have the highest efficacy as a mouthwash in preventing dental plaque formation and has become the gold standard of antimicrobial mouthwash against *S. mutans*.[10,11] Chlorhexidine has bacteriostatic properties with low concentrations (0.02%–0.06%) and has a high concentration of bactericidal properties ($\geqq$0.12%).[11] Chlorhexidine is more effective in killing (bactericidal) Gram-positive bacteria while having a low bactericidal effect on Gram-negative bacteria. Although chlorhexidine provides several benefits, it gives some side effects, including staining of the teeth and tongue, burning sensation, mucosal irritation, and taste disturbances.[10-13] Chlorhexidine can provide a cytotoxic effect on gingival fibroblasts, periodontal ligament cells, and osteoblasts. Karpiński and Szkaradkiewicz[11] stated that chlorhexidine could stimulate apoptosis and necrotic cell death.

Paul *et al*.[14] stated that natural sources could be used as an alternative to antimicrobials, which helps to reduce side effects. In combination with natural sources, antimicrobial agents can be used as synergically in therapies or as an auxiliary to delay the development of bacterial resistance.[14] One of the medicinal plants is gambir (*Uncaria gambir* Roxb.) which has long been used by locals and used for maintaining teeth and gum health.[15] The active compound of *U. gambir* is catechin, either in form of pure catechin or catechol. Catechin had been proven in preventing the formation of extracellular glucan, which attaches *S. mutans* to the tooth surface, while catechol can inhibit the activity of the glucosyltransferase enzyme. This enzyme is related to the formation of dental plaque.[16] *U. gambir* has astringent, antibacterial, and pharmacological properties.[17] *U. gambir*, which is used as an antibacterial in mouthwash preparations, is expected to be able to terminate or inhibit the growth of bacteria that cause dental plaque. The use of gambir as a mouthwash preparation is an effort to explore the benefits of *U. gambir*.[15,17] This research aimed to observe the interaction of the active compound catechin from *U. gambir* against UDP-*N*-acetylenol pyruvylglucosamine reductase (MurB enzyme) which plays role in the formation of PG in *S. mutans* through the *in silico* approach using a molecular docking modeling.

MATERIALS AND METHODS

Materials for *in silico* analysis

UDP-*N*-acetylenolpyruvylglucosamine reductase (MurB enzyme) as the protein with code P08373 was obtained from UniProt (https://www.uniprot.org/). The ligands of *U. gambir* content were catechin and chlorhexidine, determined as control ligands, obtained from PubChem (https://www.pubchem.ncbi.nlm.nih.gov/).

In silico of Uncaria gambir Roxb compounds

The chemical structure of catechin and chlorhexidine was obtained from PubChem using the three-dimensional (3D) structure with Open Babel 3.1.1 Protein Data Bank (PDB) format program. UDP-*N*-acetylenolpyruvyl glucosamine

reductase (MurB enzyme) structure was obtained from UniProt with the PDB format. The AutoDock version 1.5.6 software (The Scripps Research Institute, La Jolla, Ca, USA) removed the H2O groups and natural ligands in the UDP-N-acetyl enol pyruvyl glucosamine reductase protein (MurB enzyme), then saved it in PDB format. The docking results were visualized using PyMOL (Schrodinger, New York, NY, USA) and then analyzed using BIOVIA (Dassault Systèmes, 10, Vélizy-Villacoublay, France), which showed the docking position and ligand-residue interactions in 3D molecules.

RESULTS

The binding affinity of ligands against the MurB enzyme was determined based on the docking results between both ligands against the MurB enzyme, and it was observed that the binding affinity of catechin was lower than chlorhexidine. The binding affinity of each ligand to the MurB enzyme is presented in Table 1. The two ligands have different interaction positions with the MurB enzyme, as shown in Figure 1. Details of amino acid interactions between ligands (catechin and chlorhexidine) and the MurB enzyme are shown in Table 2 and Figure 2.

DISCUSSION

Based on data analysis, this research discovered that catechin has a binding affinity of more than − 7 kcal/mol against the MurB enzyme, while chlorhexidine has a higher binding affinity than catechin. Chlorhexidine as a control ligand had the highest binding affinity of − 9.6 kcal/mol on the MurB enzyme. However, the position of each ligand (catechin and chlorhexidine) on the MurB enzyme was not the same, with different amino acid interactions been shown in Figure 1 and Table 2.

Hydrogen bonds are formed by an attractive intermolecular force when hydrogen atoms are strongly bonded to electronegative atoms. Hydrogen bonds consist of intramolecular and

intermolecular hydrogen bonds.[18,19] Intramolecular hydrogen bonds are bonds that are formed in a single molecule. The hydrogen donor and receiver are in one molecule, and they are both closely spaced between molecules. Atoms that act as hydrogen acceptors are electronegative atoms of a molecule or ion that consist of lone pairs of electrons that play a role in hydrogen bonds. Due to their electronegative solid charge, hydrogen donors (N, O, and F) attract the covalently bonded electron pair closer to the nucleus of the molecule and away from the hydrogen atom. The donors to hydrogen bonds are often powerfully and electronegatively charged atoms such as N, O, or F, covalently bonded with hydrogen bonds. The hydrogen atom is allowed to have a partially positive charge which forms an attraction between the hydrogen atoms and binds to the donor and the lone pair acceptor. The result of this interaction forms hydrogen bonds.[18]

An intermolecular hydrogen bond is a bond that occurs between separate molecules. This bond occurs since there is an interaction between the hydrogen donor and the hydrogen acceptor. However, hydrogen bonding cannot occur without a significant electronegative difference between hydrogen and bonding atoms.[18] The present study showed that catechin and chlorhexidine ligands form amino acid attachments with the same hydrogen bond, Ser50, Asn226, Asn51, and Ser229. The attachment of amino acids with the same hydrogen bonds between catechins and chlorhexidine indicates that catechins have antibacterial
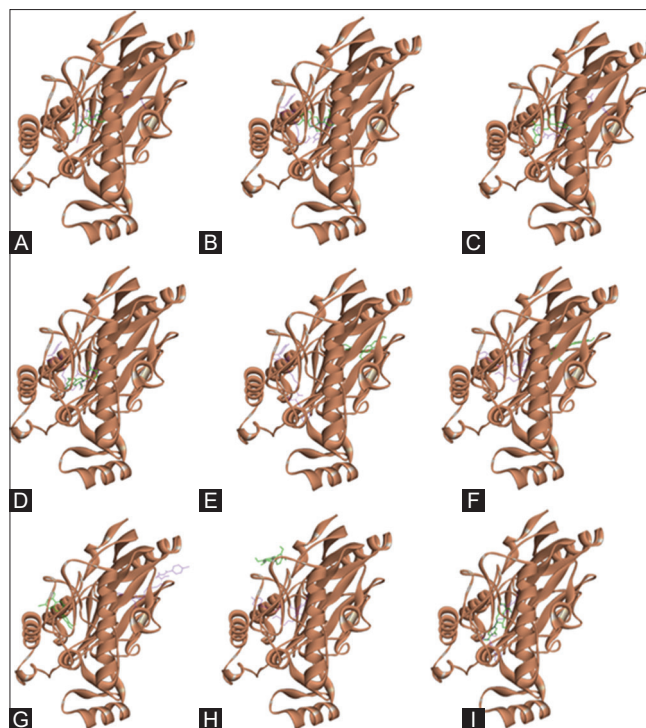

Figure 1: Positions of catechin (green chain) and chlorhexidine (purple chain) against MurB enzyme in nine positions or modes: (A) position in mode 0; (B) position in mode 1; (C) position in mode 2; (D) position in mode 3; (E) position in mode 4; (F) position in mode 5; (G) position in mode 6; (H) position in mode 7; (I) position in mode 8

Table 1: The binding affinity of catechin and chlorhexidine against the MurB enzyme

The binding affinity of ligand against the MurB enzyme			
Position in mode	Catechin	Position in mode	Chlorhexidine
0	−8.5	0	−9.6
1	−8.3	1	−9.5
2	−8.1	2	−8.9
3	−8	3	−8.9
4	−7.7	4	−8.8
5	−7.7	5	−8.7
6	−7.5	6	−8.7
7	−7.3	7	−8.6
8	−7.2	8	−8.6

Table 2: Interaction of ligand-MurB enzyme

Attachment of amino acids by hydrogen bonds			
Position in mode	Catechin	Position in mode	Chlorhexidine
0	Ser116, Ser50, Asn51	0	Pro111, Ser50
1	Ser116, Arg327, Asn226	1	Ser229, Asn226, Pro219
2	Ser50, Asn51, Arg327	2	Ser116, Ile45, Asn51
3	Ser229, Gly123	3	Ser229, Asn226, Pro219
4	Asp169	4	Gly123, Asn226, Pro219
5	Arg327, Asp169, Ile45	5	Asn226, Pro219, Pro111, Ser50
6	Asp220, Asn226, Ser229	6	Val43, Phe171, Gln168, Phe163
7	Asn224, Pro221, Asn9, Asn294	7	Ser50, Pro219
8	Ser50, Arg159, Ser229	8	Gly123, Ser50
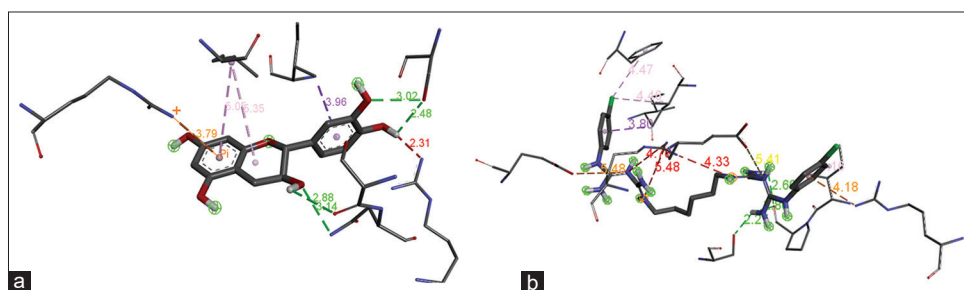
Figure 2: Ligand-MurB enzyme interaction: (a) catechin; (b) chlorhexidine

activity as an inhibitor of the MurB enzyme with a binding affinity of more than − 7 kcal/mol, which is predicted to have an antibacterial activity or similar mechanism as chlorhexidine and is competitive against chlorhexidine in inhibiting the MurB enzyme. Catechin can be used as an alternative to the MurB enzyme inhibitor.

Catechin is a complex flavonoid compound from the polyphenol group with antioxidant and antibacterial properties.[20] Previous research discovered that phenols could change cell membrane permeability, changes in an intracellular function triggered by hydrogen bonds of phenol compounds to enzymes, and changes in rigidity of cell wall with loss of cell wall integrity due to the interaction of phenol with cell membranes.[21] The docking results found in the current research indicated that catechins are competitive inhibitors of the MurB enzyme and thus can be used as an alternative material with antibacterial properties with a binding affinity value of more than − 7.0 kcal/mol which showed antibacterial activity as an inhibitor of the MurB enzyme.

Chlorhexidine has a binding affinity of more than − 8.6 kcal/mol as a control ligand. Chlorhexidine has been used to prevent dental caries and is known to inhibit the formation of dental plaque, and is bactericidal against Gram-positive bacteria, including *S. mutans*, which is the primary agent of dental caries.[11] This study is an introductory study and can be used as a reference for further research, including *in vitro* and clinical research.

CONCLUSIONS

Natural products can be the alternative sources to find an antibacterial agent. For example, the phenolic compound from *U. gambir* has antibacterial by inhibiting the MurB enzyme. This research found that *U. gambir* has similar amino acid attachments with a similar hydrogen bond to chlorhexidine, the gold standard for the antibacterial agent. However, further analysis is still needed to clarify and find the activity of compounds using *in vitro* methods for clinical implementation.

Conflicts of interest

There are no conflicts of interest.

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