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Bioinformatic approach of propolis as an inhibitor of peptidoglycan glycosyltransferase to improve antibacterial agent: An *in-silico* study

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ABSTRACT

Background: *In Indonesia, the prevalence of dental and oral problems is still high at 57.6% in 2018, especially periodontitis at 74.1%. Peptidoglycan is an essential component of the bacterial cell wall. Peptidoglycan glycosyltransferase (PGT) is a protein target that plays a role in transferring lipid disaccharides II to growing glycan chains for bacterial cell wall synthesis. Propolis is a natural ingredient produced by bees and has anti-inflammatory, antibacterial, antiviral and antioxidant properties so that it has the potential to be a natural mouthwash ingredient. One of the antibacterial properties of propolis is to be able to kill and reduce the number of bacteria that cause periodontitis.* ***Purpose:*** *This study aims to investigate the potential of a specific compound of propolis as an inhibitor of protein peptidoglycan glycosyltransferase through bonding interactions.* ***Methods:*** *The method used is an in-silico test in molecular docking with computational software, namely Molegro virtual docker and Discovery Studio visualizer.* ***Results:*** *This study showed the types of bonds between the four compounds, and chlorhexidine as a control showed similar types of bonds, including hydrogen bonds, hydrophobic interactions and unfavourable bonds. The binding energy values of each of the five compounds were pinocembrin -222.166 kJ/mol, hesperetin -230.144 kJ/mol, chrysin -219.45 kJ/mol, caffeic acid phenethyl ester -266.64 kJ/mol and chlorhexidine -362.71 kJ/mol.* ***Conclusion:*** *Caffeic acid phenethyl ester (CAPE) is the most significant potential as an inhibitor of protein peptidoglycan glycosyltransferase and chlorhexidine has the highest binding affinity than the four propolis compounds, followed by caffeic acid phenethyl ester in propolis in silico.*

Keywords: *oral hygiene; peptidoglycan glycosyltransferase; propolis*

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INTRODUCTION

Oral health is an overall component of general health.[1] However, there are still many Indonesian people who do not understand the importance of maintaining oral health. It is known from the national prevalence of dental and oral problems in 2013, which reached 25.9%, with 14 provinces having a prevalence above the national figure. The prevalence increased to 57.6% in 2018.[2] One of the dental and oral diseases whose prevalence is still high in Indonesia is periodontitis. According to Basic Health Research (RISKESDAS), 2018,[2] the prevalence of periodontitis in Indonesia reached 74.1%. Periodontitis is a disease of the oral cavity, a complex infection with several etiologic factors and contributing factors. *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are pathogens that play an essential role in periodontitis.[3] Harvey explains that periodontitis is caused by a mixed bacterial infection resulting in damage to the supporting tissues of the teeth.[4]

In this study, peptidoglycan (PG) is an essential component of the bacterial cell wall. Peptidoglycan glycosyltransferase (PGT) of the 51 families is an essential enzyme for synthesizing bacterial cell wall glycan chains. This enzyme is considered a potential antibacterial target.[5] Peptidoglycan glycosyltransferase transfers lipid II disaccharides to growing glycan chains for bacterial cell wall synthesis. Lipid II (PGT substrate) is a large amphiphilic

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molecule containing a hydrophilic peptide disaccharide head and a hydrophobic undecaprenyl diphosphate tail as a barrier to the cell membrane polymerization. The PGT structure also exhibits a hydrophobic region close to the active site, most likely interacting with the substrate lipid chain upon entering the active site. PGT inhibitor with broad-spectrum antibacterial activity guards against Gram-positive and Gram-negative bacteria.[6]

A natural ingredient, namely propolis has received much attention in the medical field because it has anti-inflammatory, antibacterial, antioxidant, antifungal, antiviral, and anticancer properties.[7] Propolis has a high flavonoid content, which is 50% of the total composition in the resin.[8] Propolis enhances the immune response and has the potential as a mouthwash with minimal cytotoxic properties compared with chlorhexidine.[9,10] One of the properties of propolis is antibacterial, so it is expected to be able to kill and reduce the number of bacteria that cause periodontitis. The antibacterial properties were tested by molecular docking.

The rapid development of science and technology has encouraged various research in the health sector, one of which is an in silico-based research approach. In silico is a research term through computer simulations supported by the availability of information from a database.[11] One of the goals of in silico-based research is drug discovery and development.

Drug design aims to obtain a new type of drug with better activity and lower toxicity. Through an in-silico approach, it is possible to identify targets and compounds from the existing database to increase the efficiency of drug discovery and development. The method used in silico-based research is molecular docking, which is used for drug discovery. Furthermore, this study aims to investigate the potential of a specific compound of propolis as an inhibitor of protein peptidoglycan glycosyltransferase through bonding interactions *in silico*.

MATERIALS AND METHODS

The method used in this paper was molecular docking, an *in-silico* study in nature. The duration of the research was four months. This research was conducted online or in a network supported by hardware and software. Molecular docking is a method based on in silico studies and is widely used for drug discovery. Molecular docking allows identifying new therapeutically related compounds and predicts ligand-target interactions at the molecular level.[12] The hardware used was a set of computers or laptops with the recommended minimum specifications according to Khaerunnisa et al.,[11] namely a minimum of Core I and 2 GB RAM, operating systems (OS) Windows, Mac, and Linux, and software included Molegro virtual docker and Discovery visualizer studio version 21.1.1.

Table 1. List of propolis active compounds.

Compounds	CID
Pinocembrin	68071
Hesperitin	72281
Chrysin	5281607
Caffeic acid phenethyl ester	5281787

In determining protein and ligands, several databases, such as PubChem and Protein Data Bank (PDB), were used. The 3D structures were obtained from secondary data in the PubChem database to download compounds and the PDB to download protein structures. The target antibacterial protein is PGT with ID 3FWL. The control ligands selected were chlorhexidine with CID (CID_9552079), and the comparison ligands were four active compounds from propolis (Table 1).

Protein preparation was carried out using Molegro virtual docker software to remove solvent and native ligands attached to the protein structure. Compound or ligand preparation was done directly by entering the compound file and then clicking import.

Docking on the five target compounds was carried out using Molegro virtual docker software. The target protein cavity was identified (the native ligand-binding area) and docked in the cavity area. Docking parameters included: cavity volume 1184.77A; surface 2833.92A with Grid X 40.59; Y 78.00; Z -37.29; MolDock Score [Grid] 0.30A; number of running 10; binding pose maximum 5, and RMSD maximum 2.

Visualization of the interaction of each protein with the ligand was presented using the Discovery studio visualizer version 21.1.1 software. This device aims to determine the binding area of protein with a specific ligand along with the constituent atoms of the compound, the amino acids that interact with the ligand and the type of bond. Binding energy is obtained by summing the MolDock score, MolDock Grid score and Rerank score, which is averaged from 5 replications.

RESULTS

The 3D view shows pinocembrin, hesperetin, chrysin, caffeic acid phenethyl ester (CAPE), and chlorhexidine compounds bound to protein transglycosylase at various amino acid residues (Table 2). The benefits of propolis components are listed in Table 3**.** The types between the four compounds and chlorhexidine as a control show similar hydrogen and hydrophobic interactions. In addition, it also shows poor binding to the fifth complex ligand – PGT protein. Pinocembrin binds to peptidoglycan glycosyltransferase protein via hydrogen at amino acid residues LEU584, TYR517, ALA520, LEU521, LEU565, THR577 and also binds to LEU580, GLY581 and ALA583 with an unfavourable bond (Figure 1). The energy of pinocembrin is -222.166 kJ/mol. Hesperetin has an energy value of -230.144 kJ/mol at amino acid residues GLN675,

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TYR517, GLY697, SER572 LYS698, TYR517, LEU671, ILE713, PHE625, PRO514, LEU569, VAL677, LYS698, ALA568, LEU565, VAL566, ALA568, LEU569 and GLY697. The types between hesperetin and PGT proteins are hydrogen, hydrophobic and are unfavorable (Figure 2). Chrysin interacts with PGT protein at the amino acid residues LEU584, ALA520, TYR527, TYR517, ILE533, LEU584, LEU521, LEU565, THR577, LEU580, GLY581 and LEU584 with an energy of -219.45 kJ/mol. The type between chrysin and PGT protein is hydrogen and hydrophobic (Figure 3). CAPE has the lowest energy compared with the other three active compounds, -266.64 kJ/mol. Caffeic acid phenethyl ester binds to PGT protein via hydrogen at amino acid residues GLY697 and LEU671, hydrophobic acid at amino residues LEU521, LEU529, VAL566, LEU569, PRO514, VAL677, ALA696, and

Table 2. Interaction of pinocembrin, hesperetin, chrysin, caffeic acid phenethyl ester and chlorhexidine compounds on protein transglycosylase

Ligands/ Binding Energy (kJ/mol)	Interactions	Category	Types
Pinocembrin/ -222.166	LEU584, TYR517, ALA520, LEU521, LEU565, THR577	Hydrogen Bond	Conventional Hydrogen Bond
	LEU580, GLY581, ALA583	Unfavorable	Unfavorable Bump
Hesperitin/ -230.144	GLN675, TYR517, GLY697, SER572 LYS698	Hydrogen Bond	Conventional Hydrogen Bond
	TYR517, LEU671, ILE713, PHE625, PRO514, LEU569, VAL677, LYS698, ALA568	Hydrophobic	Pi-Pi Stacked
	LEU565, VAL566, ALA568, LEU569, GLY697	Unfavorable	Unfavorable Bump
Chrysin/ -219.45	LEU584	Hydrogen Bond	Conventional Hydrogen Bond
	ALA520, TYR527, TYR517, ILE533,	Hydrophobic	Pi-Sigma
	LEU584, LEU521, LEU565, THR577, LEU580, GLY581, LEU584	Hydrophobic	Pi-Alkyl
Caffeic acid phenethyl ester (CAPE)/ -266.64	GLY697, LEU671	Hydrogen Bond	Conventional Hydrogen Bond
	LEU521, LEU529, VAL566, LEU569, PRO514, VAL677, ALA696, LYS698	Hydrophobic	Pi-Alkyl
	LEU565, VAL566, LEU569, GLN675	Unfavorable	Unfavorable Bump
Chlorhexidine/ -362.71	VAL566, THR570, TYR517	Hydrogen Bond	Conventional Hydrogen Bond
	VAL563, LYS513, PRO514 ALA568 ILE533 TYR517 TRP532, LEU565 ALA568	Hydrophobic	Pi-Sigma
	VAL566, LEU565, ASP567 ALA568 LEU569 THR570 PRO576 ARG571 SER572	Unfavorable	Unfavorable Bump

Table 3. Benefits of propolis components

Component	Benefit
Caffeic acid phenethyl esters (CAPE)	- HO1 regulation can counteract oxidative stress and inflammation involving the P38 MAPK signal.[13] - Inhibits NF-kβ p65 subunit so that it can control anti-inflammatory activity in periodontitis.[14] - Inhibits bacterial RNA-polymerase.[15]other
Pinocembrin	Inhibits bacterial RNA-polymerase.[15]
Chrysin	Downregulation of pro-inflammatory cytokine expression.[16]
Hesperetin	Inhibits the ACE-II enzyme.[17]

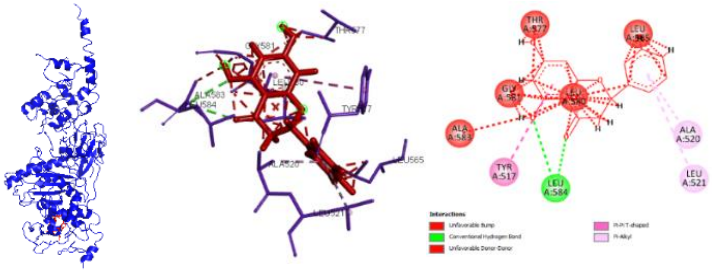

Figure 1. 3D and 2D models of docking between pinocembrin ligand and peptidoglycan glycosyltransferase protein.

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LYS698, and via unfavorable bonds in amino residues LEU565, VAL566, LEU569 and GLN675 (Figure 4). Chlorhexidine which is the control compound has the lowest energy compared with for pro-specific compounds, namely -362.71 kJ/mol and binds to amino acid residues VAL566, THR570, TYR517 via hydrogen, VAL563, LYS513, PRO514, ALA568, ILE533, TYR517, TRP532, LEU565, and ALA568 via hydrophobic bond and VAL566, LEU565, ASP567, ALA568, LEU569, THR570, PRO576, ARG571, SER572 via unfavorable bond (Figure 5).

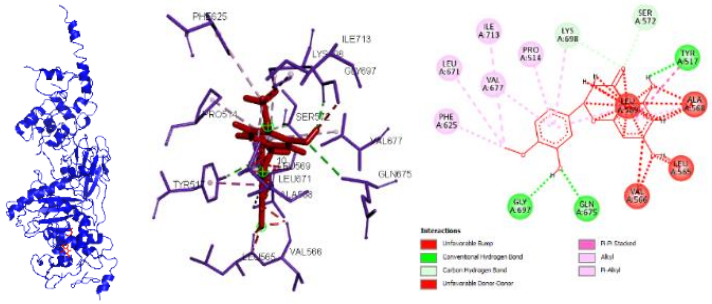

Figure 2. 3D and 2D models of docking between hesperitin ligand and peptidoglycan glycosyltransferase protein.

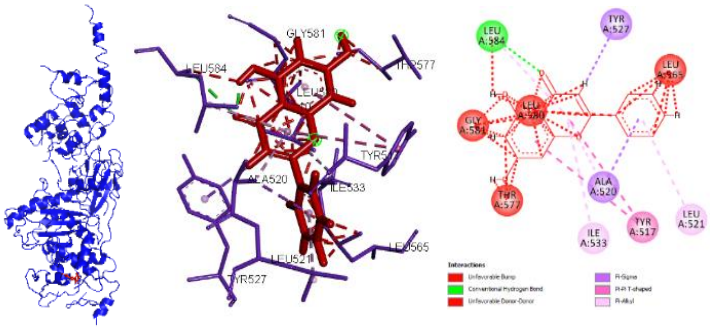

Figure 3. 3D and 2D models of docking between chrysin ligand and peptidoglycan glycosyltransferase protein.

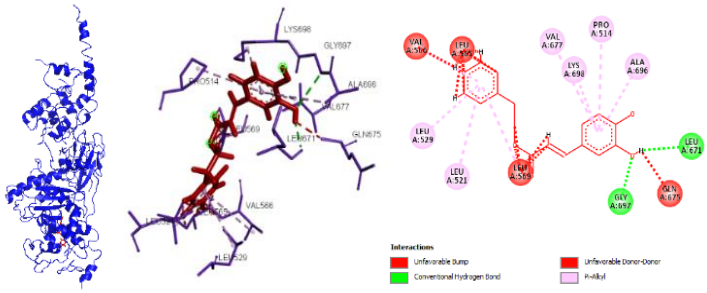

Figure 4. 3D and 2D models of docking results between CAPE ligand and peptidoglycan glycosyltransferase protein.

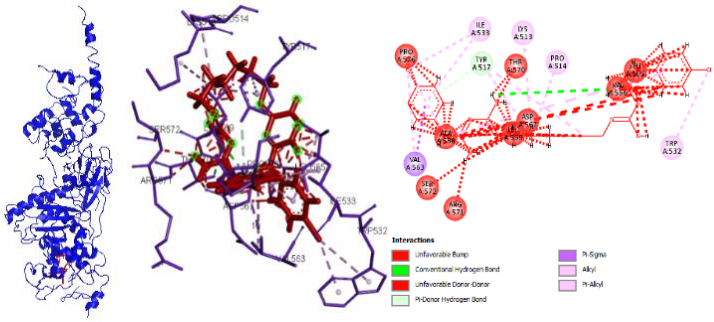

Figure 5. 3D and 2D models of docking between chlorhexidine ligand and peptidoglycan glycosyltransferase protein.

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DISCUSSION

Several residues of the active site of the four compounds identified were found on the active site of chlorhexidine as a control, including TYR517, PRO514, VAL566, LEU565, ALA568, LEU565 and LEU569. It indicates that the four compounds have inhibitory potential as antibacterial, almost the same as chlorhexidine as a control. The docking results showed that chlorhexidine had the lowest bond energy as a control, which was -362.71 kJ/mol. The lower ligand and protein interaction, the stronger bond between the ligand and protein complex. Therefore, chlorhexidine has the strongest binding to PGT protein. However, the caffeic acid phenethyl ester compound had the lowest bond energy of -266.64 kJ/mol compared with the three other specific propolis compounds, namely pinocembrin, hesperetin and chrysin. Research states that one type of propolis, namely Tetragonula sapiens, has good anti-inflammatory properties at a concentration of 120 mg/mL.[18] The cytotoxic test is safe to use in the concentration range of 6.25-200 g/mL. One of the most essential ingredients in propolis that is quite large is flavonoids. Flavonoids in propolis are stored in large amounts of resin, which is about 50% of the total content. Other propolis content is about 30% wax, 10% aromatic compounds and oils and 5% pollen.[8] The composition of propolis may vary based on the propolis-producing region, but previous studies have shown that different propolis samples contain flavonoids such as luteolin, chrysin, pinocembrin, hesperetin and rutin. The most abundant components are chrysin, hesperetin and pinocembrin. In addition, one of the phenolic bonds in propolis, namely CAPE, reaches 50% of the total components.[17]

Propolis has antibacterial properties by eliminating the permeability of *Porphyromonas gingivalis* bacterial cells by lysing bacteria through protein binding.[19] The flavonoids in propolis also inhibit the biofilm by reducing the number of polysaccharides in the biofilm and bacterial adhesion.[20] Antibacterial compounds have several mechanisms to inhibit bacterial growth. These include the destruction of cell walls by changing cell walls after they are formed, inhibiting the synthesis of new cell walls, changing the cytoplasmic membrane so that the core material inside the cell comes out, inhibition of enzyme action, inhibition of nucleic acid and protein formation, as well as changes in protein molecules [21] In molecular docking, the target protein used is peptidoglycan glycosyltransferase. Protein peptidoglycan glycosyltransferase is a protein that plays a role in transferring disaccharide peptides from lipid II to the glycan chain in bacterial wall synthesis.[6]

Based on the research conducted, it was found that the four specific propolis compounds, including pinocembrin, hesperetin, chrysin and CAPE, and the control compound chlorhexidine, could inhibit protein peptidoglycan glycosyltransferase. The inhibition of the four propolis and chlorhexidine compounds occurred at the active site for disaccharide transfer so that the disaccharide transfer activity in PGT decreased. If the transfer of disaccharides decreases, the cell wall synthesis will be disrupted and this results in the inhibition of bacterial growth.[22]

Based on this research, it can be concluded that CAPE is a propolis compound with the most significant potential as an inhibitor of protein peptidoglycan glycosyltransferase because it has the strongest bond to protein compared with the other three specific propolis compounds. However, chlorhexidine had the most robust binding to the inhibition of PGT protein. Unfortunately, this research did not show the toxicity of propolis components such as pinocembrin, hesperitin, chrysin and CAPE as an antibacterial agent, so it requires further treatment to evaluate the toxicity of each component. Further research is needed to identify and analyse the primary molecular candidates through in vitro or in vivo studies using physicochemical parameters.[23]

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