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**IN SILICO STUDY OF *Stachytarpheta jamaicensis* ACTIVE COMPOUNDS
AS ANTIBACTERIAL MATERIAL**

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ABSTRACT

Background: *Stachytarpheta jamaicensis* is a wild plant from the Verbenaceae family that grows in tropical areas such as Indonesia. *S. jamaicensis* extract was proven to contain secondary metabolite compounds such as flavonoids, phenols, saponins, tannins and terpenoids. The active compound of *S. jamaicensis* can be used as a drug candidate in the medicine field, especially as an antibacterial compound. One of the first steps in predicting the effectiveness of these compounds can be done through in-Silico studies with molecular docking. **Objective:** This study aims to evaluate the interaction of active compound *S. jamaicensis* with bacterial proteins through an in silico study. **Methods:** Using in silico method with computational docking analysis on seven active compounds of *S. jamaicensis* namely apigenin, luteolin, chlorogenic acid, gamma butyric acid, dopamine, ipolamide and geraniol, as well as two antibacterial drugs (metronidazole and chlorhexidine) as comparisons bound with bacterial cell wall protein, namely Glucosamine-6-phosphate synthase (G1mS). Docking in silico uses Autodock Vina, which is integrated with PyRx 8.0 and visualized using Discovery Studio Visualizer v19.1.0.18287 (2019 version) with data presentation based on docking scores. **Conclusion:** The best binding affinity score has been the luteolin-G1mS complex with a binding affinity value of -10.8 kcal/mol and was the highest value compared to the comparison ligand binding and the binding of other active compounds of *S. jamaicensis*.

Keywords: Antibacteria, *in Silico*, *Stachytarpheta jamaicensis*

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INTRODUCTION

According to Basic Health Research in 2018, the percentage of dental and oral health problems of Indonesian population in 2013 and 2018 increased from 25.9% to 57.6%.¹ South Kalimantan province ranks 14th position as a province with highest dental and oral health problems with a percentage of 59%. The prevalence of cavities in South Kalimantan was 60%.² Cavities or caries are damage to the hard tissue of the teeth due to metabolism of bacteria in dental plaque. Bacteria cause several problems if left unchecked and will make a serious impact on dental and oral health.

Stachytarpheta jamaicensis is a wild plant of Verbenaceae family that grows in tropical areas such as Indonesia. This plant is a weed on agricultural land. Although considered a wild plant, *S. jamaicensis* can be used as a medicine for

allergies, respiratory disorders, fever, constipation and digestive complications.^{3,4} Resident in Kalimantan have been using 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 of *S. jamaicensis* have been using as a toothache therapy.^{5,6}

S. jamaicensis contains several active substances that plays an important role as an antibacterial, including saponins, tannins and flavonoids. Based on a review article by Liew (2015), *S. jamaicensis* root contains secondary metabolites such as flavonoids, phenols, saponins, tannins and terpenoids.^{3,7} *S. jamaicensis* leaves also have secondary metabolites such as alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and quinones.⁸

In a study conducted by Ololade (2017), it's known that *S. jamaicensis* leaf extract can inhibit

the growth of several bacteria, namely *E. coli*, *E. faecalis*, *M. varians*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*, *P. mirabilis*, *S. agalactiae*, *S. marcescens* and *S. typhimurium* at concentrations of 250µg/ml, 500µg/ml and 1000µg/ml with different zones of inhibition.⁹

The active compounds content of *S. jamaicensis* can be used as a drug candidate in the medicine field, especially as antibacterial compounds. First steps in predicting the effectiveness of these compounds can be conducted through in-Silico studies with molecular docking.

Molecular docking is a method to predict the interaction between ligands (small molecules) with large molecules such as proteins, enzymes and carbohydrates, while predicting the stability and spontaneity of the interaction can be seen through the small free energy.^{10,11} Molecular docking often used in finding potential compounds that can act as inhibitors of disease agents such as viruses and bacteria. Molecular docking also used to predict the binding orientation of small molecule drug candidates into their protein targets to predict the affinity and activity of small molecules. Thus, molecular docking plays an important role in drug discovery and rational drug design.¹¹

The mechanism of antibacterial inhibition was known involve the inhibition pathway of nucleic acid synthesis, inhibition of bacterial motility, inhibition of cell membrane permeability and causes death of the cells. Glucosamine-6-phosphate synthase (G1mS) is an enzyme that plays a role in building important macromolecules in formation of cell walls, such as chitin, mannoprotein and peptidoglycan in prokaryotes. G1mS catalyses the initial and limiting step in the hexosamine biosynthetic pathway, controlling the synthesis of uridine-50-diphospho-N-acetyl-D-glucosamine, a major building block for peptidoglycan formation in bacteria, chitin in fungi and glycoproteins in mammals.¹² G1mS is a target development for a better and safer preservative because of its role in the synthesis of microbial cell walls.¹³ Development of inhibitors from this enzyme has potential as an antibiotic candidate.¹⁴ Docking in silico studies can be carried out to predict the action of active compound against G1mS as a target for antibacterial therapy in gram-positive and gram-negative bacteria.^{15,16}

In this context, it is imperative to understand and elucidate the underlying role of the active compounds and its interaction with G1mS and the mode of binding to the desired protein to facilitate the discovery of new antibacterial drugs. This study aims to evaluate the interaction of the active compound *S. jamaicensis* with bacterial proteins through in silico research.

MATERIALS AND METHODS

Data Sample Preparation

The receptor preparation in the form of a 3D structure of GlcN-6-P synthase (1JXA) was taken from the Protein Data Bank (<https://www.rcsb.org/>). Preparation of the structure of the bioactive compound *S. jamaicensis* and the reference 3D structure of the ligands (Metronidazole and Chlorhexidine) were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Protein structures are downloaded in .pdb format, while ligands are downloaded in SDF format.

Ligand and Protein Preparation

The compounds used were apigenin-7-glucuronide (CID 5319484), gamma-amino butyric acid (CID 119), chlorogenic acid (CID 1794427), geraniol (CID 637566), ipolamiide (CID 442425), luteolol-7-glucuronide (CID 5280601). Molecular Docking Proteins were prepared using Discovery Studio version 16 (Dassault Systèmes BIOVIA, 2015) to remove pre-installed ligands, while ligands were prepared using Open Babel integrated in PyRx 8.0 (Dallakyan & Olson, 2015) to minimize their energy and convert them to pdb format.¹⁷

Docking Protein-Ligand and Visualizations

Molecular docking simulations were performed using Autodock Vina integrated with PyRx 8.0. Analysis and visualization of virtual predictions Protein-ligand complexes from the docking step were analysed and visualized using Discovery Studio. The interaction sites were analysed based on ligand-residue interactions and structural conformation. ClcN-6-P also docked with a reference ligand to compare the binding affinity of the 7 active compounds used in this study in accordance with previous studies.

RESULTS

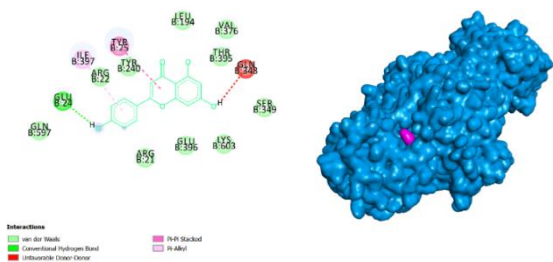
Table 1. The Binding affinity score of *S. jamaicensis* compounds and reference ligands with G1mS.

Compound	Docking score (kcal/mol)
Apigenin 7-glucuronide	-8.5
gamma-Aminobutyric acid	-4.4
chlorogenic acid	-8.8
Dopamine	-6.3
luteolol-7-glucuronide	-10.8
Geraniol	-6.1
Ipolamiide	-8.3
Metronidazol	-6.7
Chlorhexidine	-9.6

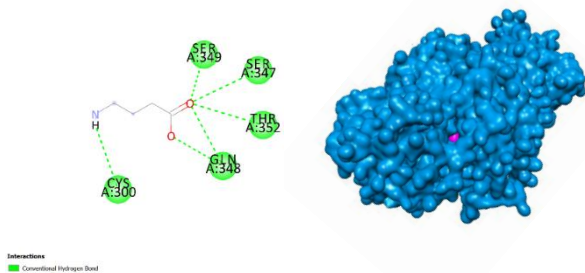
Table 1. showed the binding capacity of the bioactive compounds *S. jamaicensis* on G1mS as an enzyme associated with bacteria cell walls. The bond energy scores of the seven bioactive compounds varied from -4.4 to -10.8 kcal/mol.

Four bioactive compounds of *S. jamaecensis* bind more negatively than Metronidazole, namely apigenin, chlorogenic acid, luteolol-7-glucuronide (luteolin) and ipolamiide. Meanwhile, only luteolin has the most negative bond energy value compared to chlorhexidine. The binding interaction value has the potential to predict the interaction between ligand and receptor and this means the more negative the binding energy value, the better is binding interaction.¹⁷ The scoring function generates a score for each bond pose and the result value was used to rank different poses and ligands.¹⁸ The scoring function is derived from the angular deviation of the ideal H-bond interaction with the protein.¹⁹

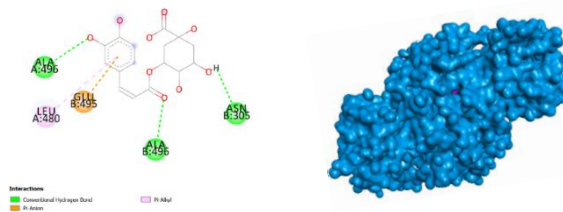
A. Apigenin – G1mS



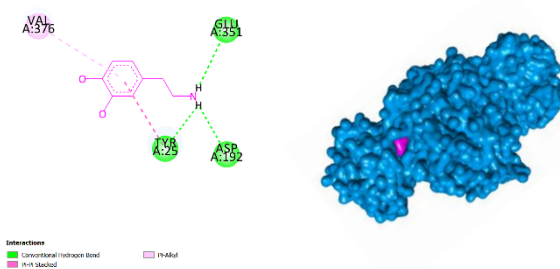
B. Gamma butyric acid- G1mS



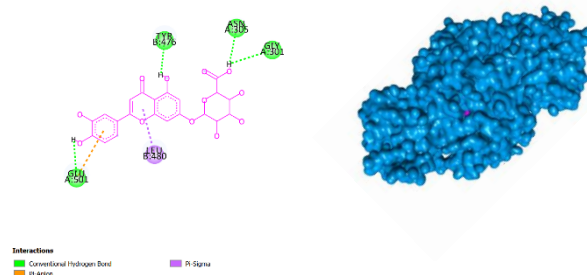
C. Chlorogenic Acid- G1mS



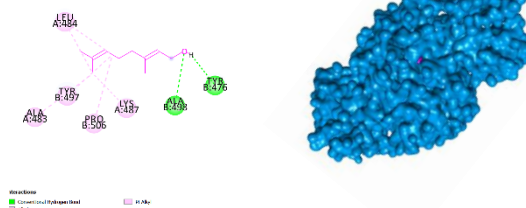
D. Dopamine – G1mS



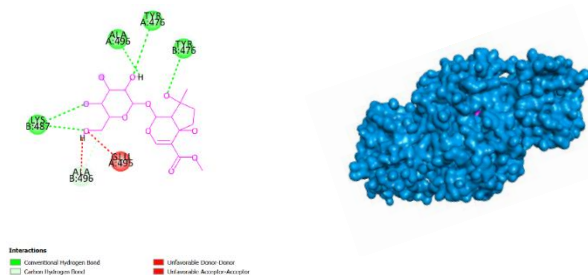
E. Luteolin- G1mS



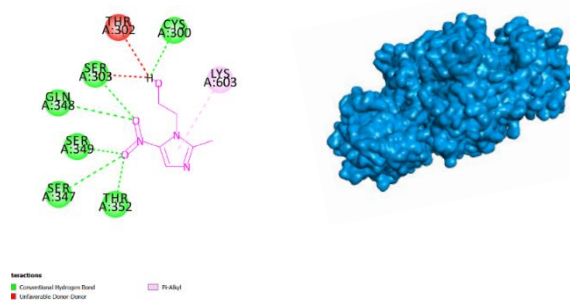
F. Geraniol – G1mS



G. Ipolamiide- G1mS



H. Metronidazol – G1mS



I. Chlorhexidine – G1mS

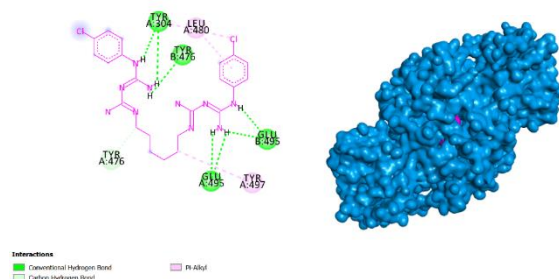


Figure 1: The 3D structure of interaction between *S. jamaicensis* compounds (purple – fuschia) with G1mS (turquoise). Right sides are visualized the 3D structure of the interaction, while code left side is 2D interaction and category bond of the complex. The molecular docking was performed using Pyrx 8.0.0 software and visualization was analyzed by Discovery Studio Visualizer v19.1.0.18287 program.

DISCUSSION

Table 1. showed the bond values of several active components of *S. jamaecensis* that was having potential to bind into G1mS, namely apigenol-7-glucuronide (CID 5319484), gamma-amino butyric acid (CID 119), chlorogenic acid (CID 1794427), geraniol (CID 637566), ipolamiide (CID 442425), luteolol-7-glucuronide (CID 5280601) and 2 comparison ligands namely metronidazole and chlorhexidine.

There are four active compounds those have binding affinity with a score more negative rather than metronidazole-G1mS binding score. Luteolin was the most negative binding affinity followed by chlorogenic acid, apigenin and ipolamide. However, from all compounds, only luteolin is higher rather than chlorhexidine and its bond energy is -9.6 kcal/mol. The more negative the binding affinity value, the better is binding interaction with the target molecules.¹⁷

The four amino acid residues in G1mS that interact directly with apigenin were TYR 240, ARG 22, GLU 24 and GLN 348 (Figure 1.A). This interaction was facilitated by van der waals, conventional hydrogen bonding and unfavorable donors. The binding energy of apigenin-G1mS was 8.5 kcal/mol. The docking energy result from the interaction between Gamma butyric acid and G1mS is -4.4 kcal/mol. The gamma butyrate-G1mS complex had the lowest number of interactions compared to other ligands (Table 1). The bonds formed in this interaction are 5 bonds (Figure 1.B). Hydrogen bonding occurs at the amino acid residues SER 349, SER 347, THR 352, GLN 348 and CYS 300.

Chlorogenic acid interacted with G1mS result in a bond energy of -8.8 kcal/mol (Fig. 1C). Based on the results of molecular docking shows ALA496, ASN305, and GLU495 are amino acid residues bound to chlorogenic acid by forming conventional hydrogen bonds and Pi Anions. Three conventional hydrogen bonds and one Pi-Alkyl bond are formed during the interaction between dopamine and G1mS (Fig. 1.D). The amino acid residues involved in the formation of hydrogen bonds are GLU351, ASP192 and TYR25. VAL 376 forms Pi-Alkyl with dopamine. The binding energy of the dopamine-G1mS complex is 6.3 kcal/mol.

The luteolin-G1mS interaction has the best binding affinity value compared to all chemical compounds to G1mS as well as the comparison drug compound, which is -10.8 kcal/mol. Luteolin is a derivative of flavonoid which is contained in *S. jamaecensis*. Previous research revealed that luteolin has antibacterial activity against *T. pyogenes* by disrupting the integrity of cell membranes and cell walls, resulting in leakage of cell contents and damage to their barrier function namely cell walls and membranes; affect protein expression and interfere with the normal processing of *T. pyogenes*; interfere with the normal metabolism of nucleic acids, which can occur through interaction with

DNA and inhibition the activity of key enzymes in nucleic acid metabolism; and reduces the ATP content in cells.²⁰

Ipolamiide, one of the verbacosides, also showed a fairly good binding affinity value of -8.3 kcal/mol. Previous studies have shown that the verbacoside content in *Stachytarpheta indica* extract is able to inhibit the growth of *Staphylococci spp.*²¹ The topology of the active binding site of G1mS with the active compound are same as well as the G1mS bond and the reference ligand (chlorhexidin), which is coated by interacting amino acids as predicted visually using Discovery. studio (Fig. 1).

Luteolin-G1mS, geraniol-G1mS and ipolamiide-G1mS has amino acid residues similar to Chlorhexidine-G1mS, namely TYR476. This means that the active compounds *S. jamaecensis* has the same antibacterial activity as chlorhexidine through inhibition of the bacterial cell wall protein, namely G1mS. These three compounds have been shown to have potential as antibacterial agents. In conclusion, based on the in-Silico test, the best binding affinity score was the luteolin-G1mS bond complex, with a binding affinity value of -10.8 kcal/mol and has the highest value compared to the binding of other active compounds *S. jamaecensis* and the comparison ligands, metronidazole and chlorhexidine.

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