RASĀYAN *J. Chem.*
Vol. 18 | No. 3 |1229-1234| July-September | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

POTENTIAL OF THE *Spatholobus littoralis* AS AN ANTIBACTERIAL AND PREDICTION OF THE MECHANISM OF ACTIVITY AGAINST PBP3 RECEPTOR MODEL OF *Staphylococcus aureus*

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ABSTRACT

Spatholobus littoralis is a typical plant of Kalimantan, which is ethnopharmacologically used in herbal medicine. The purpose of this study was to test the activity of *S. littoralis* root extract against *Staphylococcus aureus* bacteria in vitro and to predict the mechanism of its interaction with *Staphylococcus aureus* penicillin-binding protein-3 (PBP-3) in silico. Extraction of *S. littoralis* using ethanol solvent, and continued with sequential fractionation using *n*-hexane, chloroform, and ethyl acetate. Antibacterial activity test against *Staphylococcus aureus* was carried out by agar diffusion using the Kirby-Bauer test method. The prediction of activity mechanism against the 3VSL receptor using ligands from compounds that have been found in the genus *Spatholobus*. Extracts and fractions of *S. littoralis* root have antibacterial activity against *Staphylococcus aureus*. Several compounds were found in the genus *Spatholobus.* It showed stronger binding energy than natural ligands. The compound 6-methoxyeriodictyol shows the most stable binding energy to the 3VSL receptor; the binding site of the compound is at the amino acid residues Tyr636, Lys395, and Asn 450. Thus, the roots of the *S. littoralis* plant have the potential to be developed as a natural antibacterial.

Keywords: Antibacterial, in Vitro, in Silico, Bindingsite, *Spatholobus littoralis*, *Staphylococcus aureus.*

RASĀYAN *J. Chem.*, Vol. 18, No. 3, 2025

INTRODUCTION

Staphylococcus aureus is a gram-positive bacterial that can infect humans.[1] *Staphylococcus aureus* is easy to reproduce because it can grow at an optimal temperature of around 30°C.[2] This infection can cause pneumonia, wounds, pneumonia, and endocarditis or sepsis.[3] Serious infections due to *Staphylococcus aureus* can occur when the immune system is weakened.[4] The various effects caused by these bacteria require drugs that can overcome them without more serious side effects. One of them is using natural medicines that have minimal side effects. *Spatholobus littoralis* is known locally as Bajakah tempala, is found in many Kalimantan forests and is commonly used as an herbal medicine. Previous studies have shown the presence of phenolic content, which has antioxidant and cytotoxic properties, in the ethanol extract of *S. littoralis* wood.[5-6] Plants from the genus *Spatholobus* are reported to contain phenolic compounds from the flavone, flavanone, chalcone, isoflavan, and isoflavone groups, and show interesting biological activities such as anti-inflammatory, antioxidant, and anticancer.[6-9] Nowadays, in-silico assays are widely used to understand the interactions between compounds and molecular targets, which are easier and can test all interactions that are difficult to do experimentally.[10] The mechanism of interaction between penicillin binding protein-3 (PBP-3) *Staphylococcus aureus* with several compounds that have been found in plants of the genus *Spatholobus* can be studied using the molecular docking method, which is a computational system in biological screening. The in-silico research method used is a molecular docking study through modeling the mechanism of action of active chemical compounds with an enzyme/target protein with a computational approach by assessing the strength of the affinity of the bond between the two, which is indicated by the Gibbs free energy value (ΔG).[11] To complement the research results on *S.*

http://doi.org/10.31788/RJC.2025.1839167

littoralis, it is necessary to conduct antibacterial activity tests, especially against bacteria that are abundant on human skin, namely *Staphylococcus aureus*. The purpose of this study was to test the antibacterial activity of extracts and fractions of *S. littoralis* roots against *Staphylococcus aureus* bacteria in vitro, and to predict the molecular binding activity mechanism of compounds that have been found in plants from the genus *Spatholobus* against the PBP3 receptor of *Staphylococcus aureus* in silico. Information from this study is expected to provide scientific information about the use of *S. littoralis* in the treatment of infectious diseases.

EXPERIMENTAL

Apparatus and Reagents

The materials used include root wood of *S. littoralis* plant, ethanol, methanol, chloramphenical, *Staphylococcus aureus* ATCC 25923 bacterial oasis, Muller Hinton Agar (MHA), Lactose Broth (LB), paper disc (disc diameter of 6 mm), filter paper, and distilled water. This study used several equipment, including a digital analytical balance, Rotavapor R114, a refrigerator, an incubator, a set of glassware, a computer with Intel Xeon CPU specifications, 32 GB RAM, 10 cores, 500 GB SSD. The software used included Autodock Tool 1.5.7, pymol, ligPlus V-2.2.9, GIM 2.1, and Avogadro 1.94.0. *Staphylococcus aureus* PBP3 receptor was downloaded from the RCSB PDB Database page (www.rcsb.org).

Preparation of *S. littoralis* Extract

S. littoralis was obtained from a traditional market in Pontianak, Indonesia. Further identification of the leaves and stems of the plant was carried out by staff from the Faculty of Biology, Gadjah Mada University, Indonesia. A total of 3 kg of *S. littoralis* root wood was collected, dried, and ground to form a fine powder. The root wood powder was then macerated with technical ethanol and left for 24 hours. The filtrate was filtered and re-macerated twice. The filtrate obtained was collected and evaporated using a vacuum evaporator to a volume of 1/3. The ethanol extract of *S. littoralis* was then fractionated sequentially using *n*-hexane, chloroform, and ethyl acetate solvents. Each extract and fraction was then concentrated and used for further testing.

Antibacterial Activity Test

In vitro activity tests were conducted on *S. littoralis* root wood extract at various concentrations using *Staphylococcus aureus* ATCC 25923 bacterial cultures. Antibacterial testing was carried out using the agar diffusion method with slight modifications.[12] Sterile MHA solution was poured into a 100 x 15 mm glass petri dish and allowed to harden. *S. aureus* bacteria were spread on the agar media in the glass petri dish. Each sample was dissolved in methanol at a concentration of 5%. Methanol was used as a negative control, and chloramphenicol was used as a positive control. Each disc that had been soaked in the sample solution, chloramphenicol, and methanol, was arranged on a medium that had been grown with bacteria. Measurements were carried out in three repetitions. The diameter of the inhibition zone of each sample was measured at incubation times of 3; 6; 9; 12; 15; 18; and 21 hours.

The Mechanism of Activity of Compounds from the genus *Spatholobus* against the PBP3 Receptor Model of *Staphylococcus aureus*

The analysis stages are carried out as follows: (1) Preparation of the target molecular structure, to the target penicillin-binding protein 3 (PBP3) receptor model of *Staphylococcus aureus* downloaded from the RCSB PDB Database page (www.rcsb.org). Then, the macromolecules are optimized using Pymol software, and the location of the molecular attachment is determined. (2) Preparation of three-dimensional ligand structure. The ligands used are compounds that have been reported to be found in the genus *Spatholobus,* which are downloaded via PubChem (http://PubChem.ncbi.blm.nih.gov).

All compounds were then minimized in energy using the Avogadro application. (3) Molecular docking simulations were carried out using the AutoDock Tool 1.5.7 software. The best docking conformation obtained from the natural ligand redocking results was used for analysis of ligand compounds found in the *Spatholobus* plant previously. In this study, only 14 phenolic compounds were selected that were most commonly found in the *Spathollobus*. Furthermore, the interaction between ligands and receptors was visualized using the Pymol program, ligplus (ligand-receptor interaction), and GIMB 2.10 to determine the interaction between ligands and receptors.

RESULTS AND DISCUSSION

A total of 3 kg of *S. littoralis* root samples were extracted using ethanol solvent, evaporated to produce a thick ethanol extract of 420 g. Fractionation of the ethanol extract using solvents with different polarities sequentially produced 13.63 g n-hexane, 153.57 g ethyl acetate, and 104.26 g chloroform fractions. Antibacterial activity test of each extract and fraction obtained using the agar diffusion method. The results of measuring the diameter of the inhibition zone of each extract and fraction at incubation times of 3, 6, 9, 12, 15, 18, and 21 hours are obtained as in Fig.-1. These data show that optimal activity was obtained at the 9th hour of incubation.

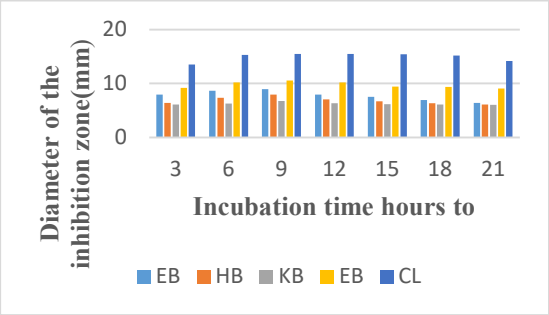

Fig.-1: The Diameter of the inhibition zone in each sample against *Staphylococcus aureus* at incubation times of 3; 6; 9; 12; 15; 18; and 21 hours (EB = Ethanol Extract of S. littoralis; HB = *n*-Hexane Fraction; KB = chloroform Fraction; EB = Ethyl Acetate Fraction; CL = Chloramphenicol (Positive Control), each at a concentration of 5%

Ethanol extract and each fraction of *S. littoralis* root wood showed antibacterial activity against *Staphylococcus aureus* with medium criteria. The highest activity was found at 9 hours of incubation. The fraction that showed the highest antibacterial activity was the ethyl acetate fraction. Previous studies have shown that *S. littoralis* root wood extract has a high phenolic content.[6] Several studies have shown that phenolic compounds play a role in destroying the cell membrane of bacteria.[13] The activity of phenolic compounds is related to several mechanisms such as inhibition of microbial cell wall biosynthesis, protein synthesis, nucleic acid synthesis, metabolic pathways, and disruption of cell membrane integrity.[14] Ethanol extract of *S. littoralis* root wood contains various phenolic compounds. Several studies have shown that phenolic compounds found in abundance in the genus *Spatholobus* are flavones, flavanones, chalcones, isoflavones, and isoflavanes.[7-9] Several flavonoid compounds have also been reported from the *S. littoralis* plant.[15] The structures of 14 phenolic compounds frequently found in the genus *Spatholobus* that are used as ligands are shown in Fig.-2.

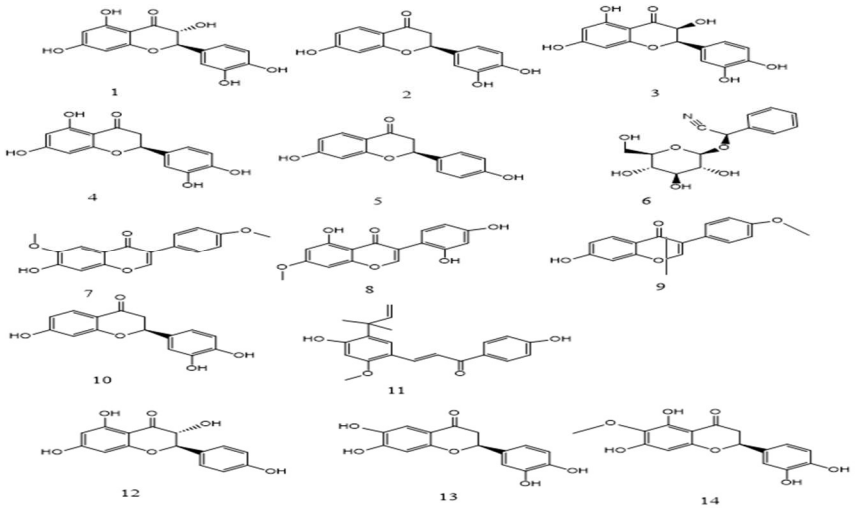

Fig.-2: Phenolic Compounds from the genus of *Spatholobus*

Prediction of the interaction mechanisms of phenolic compounds from the *Spatholobus* genus against the penicillin binding protein 3 (PBP3) receptor *from Staphylococcus aureus* using 3VSL. The receptor binds to the ligand cefotaxime (CEF)(C_{14} H_{15} N_5O_5 S_2). Furthermore, validation of the method was carried out by redocking the 3VSL receptor. The redocking results produced a grid box with a center x: 8.924; y: -47.894; z: 25.316, with a box size x = y = z = 22 Å, and had a root-mean-square deviation (RMSD) value of 0.687 Å. Therefore, the RMSD value <2 Å, it can be stated that the method used is valid.[16] Furthermore, using the obtained grid book configuration positions, docking was performed with ligands from 14 phenolic compounds of the *Spatholobus* genus. The results of the molecular docking analysis are shown in Table-1. The data shows that there are three phenolic compounds that have the highest binding energy on the 3VSL receptor, namely 6-methoxyeriodictyol, licochalcone-A, and plathymenin. Furthermore, using the Pymol program, the binding sites on the 3VSL receptor of the three phenolic compounds that show the most stable binding energy can be analyzed and explained. The binding site is a hollow part or pocket of the drug target where small molecules or ligands bind to amino acid residues that are specific to each type of ligands.[17-19] The three phenolic compounds had the most stable binding to 3VSL receptors compared to other phenolic compounds have specific binding sites. The compound 6-methoxyeriodictyol has binding sites at amino acid residues Tyr636, Lys395, and Asn 450; Licochalcone-A shows binding sites at Ser429, Thr621; while plathymenin has binding sites at Asn450, Ser448, and Lys395 as shown in Fig.-3. This compound is a flavonoid, which has been widely evaluated for its antibacterial properties that can inhibit the growth of various pathogenic microorganisms, including multidrug-resistant bacteria.[20] From the results of antibacterial analysis of the 3VSL receptor, which is the PB3 receptor of *Staphylococcus aureus* in silico, it was shown that several compounds that were previously found in the genus *Spatholobus* have higher activity than natural ligands (CEF). The molecular docking analysis was conducted in this study.

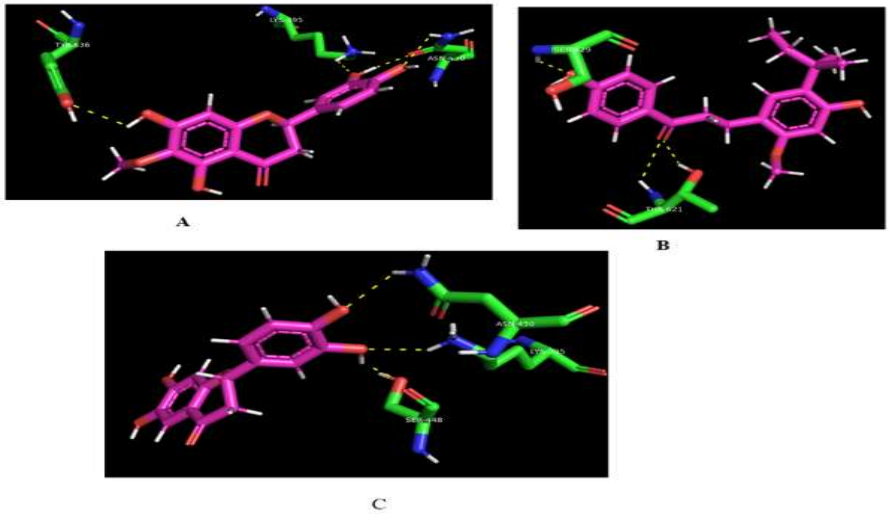

Fig.-3: Binding Site of 6-Methoxyeriodictyol (A); Licochalcone-A (B); and Plathymenin (C) on 3VSL Receptor

Table-1: In Silico Analysis Data of Phenolic Compounds from the Genus *Spatholobus* and Natural Ligands (CEF) against the 3VSL Receptor

No	Ligands	3VSL receptor		
		Binding energy (kcal/mol)	Hydrogen bonds	Hydrophobic interactions
1	Dihydroquercetin	-9.2	His447; Asn450 Ser392; Thr621	Tyr430, Thr603, Ser429
2	Butin	-8.9	Thr621; Ser392; Ser448; Asn450; Lys395;	Pro660, Thr603, Tyr636, Thr619
3	Epicatechin	-8.7	Ser392; Ser429 His447; Asn450 Thr621	Asp601, Tyr430, Thr603, Ser448

4	Eriodictyol	-9.2	Asn450; Lys395; Ser448 Thr621;	Pro660, Thr603, Tyr636, Thr619, Ser392
5	Liquiritigenin	-7.9	Thr619	Asp601, Tyr430, Ser429, Ser448, Ser392, Thr621, Thr603, Gly620, His447
6	Prunasin	-8.7	Asn450; Thr621; Gln524 Glu623	Ser448, Ser429, Ile522, Arg428, Ala622, Pro660
7	Afrormosin	-7.9	Ser392; Asn450 Ser448	Ser429, Pro659, Pro660, Thr621, His447, Val658, Glu623
8	Cajanin	-8.3	Ser329; Ser429	Tyr430, His447, Thr603, Thr621, Ser448, Glu623, Gln524, Asn450
9	Formononetin	-8.0	Thr621	Ser429, Ser448, Asn450, His447, Pro660, Val606, Thr664, Thr619, Thr603, Leu663, Tyr636
10	3',4',7'-Trihydroxyflavone	-8.9	His447; Ser392; Ser448 Lys395; Asn450	Asp601, Tyr430, Ser429
11	licochalcone A	-9.6	Ser429;	Asn450, Ser392, Ser448, Asp601, Leu663, Val606, Thr603, Thr621, Thr619, Pro660, Gly620
12	Dihydrokaempferol	-8.7	Ser448; Thr619; Thr621; Tyr636; Leu663	Pro660, Thr603, Tyr430, Ser429, His447, Gly620
13	Plathymenin	-9.3	Asn450; Ser392; Ser448; Thr621; Tyr636; Leu663	Pro660, Thr603, Thr619, Val606
14	6-Methoxyeriodictyol	-10.1	Asn450; Ser448; Lys395; Tyr636	Ser392, Thr 603, Thr 619, Thr621, Pro660, Val606
15	CEF (Natural ligand)	-7.4	Ser392; Ser448; Gln524; Thr621; Glu623	His447, Gly620, Ser429, Pro660, Asn450

CONCLUSION

S. littoralis contains active compounds that are antibacterial against *Staphylococcus aureus*. Several compounds found in the genus *Spatholobus* show a stronger affinity than natural ligands. The compound 6-methoxyeriodictyol shows the most stable binding energy to the 3VSL receptor; the binding site of the compound is at the amino acid residues Tyr636, Lys395, and Asn 450. Thus, the root of the *S. littoralis* plant has the potential to be developed as a natural antibacterial.

ACKNOWLEDGMENTS

This research was financially supported by the Research Assignment of Professors and Structural Lecturers, Universitas Negeri Yogyakarta, with contract number 13.1/UN34/II/2024, dated February 1, 2024. The author is very grateful for the research grant.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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