

Bacterial Cell Wall Synthesis Inhibition Of Clerodendrum Squamatum Vahl. Phytoconstituents: A Molecular Insight

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Abstract

Bacterial Cell Wall (BCW) is an essential part of bacteria. Defects in BCW may lead to bacterial death due to failure in maintaining pressure and cell integrity or shape. Penicillin-Binding Protein (PBP) 2a, 1, 2, 3, and 4 are proteins responsible for Methicillin-Resistant *Staphylococcus aureus* (MRSA) cell wall synthesis. MRSA is a hard-to-treat bacteria due to its antibiotic resistance. *Clerodendrum squamatum* Vahl. leaves (CSVL) are used by North Sulawesi-Indonesian people as a medicinal plant to treat inflammation and to heal wounds. So, the present study aims to explore CSVL potential as inhibitors of MRSA cell wall synthesis using in silico approach. CSVL phytoconstituents were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS), and the chemical structures were retrieved from PubChem and docked to PBP2a, PBP1, PBP2, PBP3, and PBP4 using Pyrx-Vina. The toxicity profile of the phytoconstituents was also calculated. The results showed that CSVL interacts with PBPs with a variety of types of bonding, dominated by hydrogen bonds. The toxicity prediction showed that CSVL has a safe toxicity profile. CSVL can be a good anti-MRSA candidate due to its molecular interactions with BCW synthesis proteins.

Keywords: MRSA, antibiotic resistance, medicinal plant, PBPs

Introduction

MRSA, a hard to treat bacteria, has been a major problem in health care settings. It causes skin and systemic problem. Its infection limits the treatment options and can prolong hospital stays due to antibiotic resistance (Thimmappa, et al., 2021). It has spread in Indonesia and worldwide (Santosaningsih, et al., 2017).

Medicinal plants had been widely used to treat infections. CSVL were empirically used to treat inflammation and to heal wounds. It was shown to have a wide spectrum of antibacterial activities to treat *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* (Kumakauw, et al., 2020). So, the present study aims to explore CSVL potential as inhibitors of MRSA cell wall synthesis using in silico approach.

Method

Ligand preparations

CSVL collected from North Sulawesi province in Indonesia were extracted using

methanol solvent and then analyzed using GC-MS. Identified phytoconstituents structures were retrieved from PubChem and further used as ligands.

Protein preparations

PBP2a (PDB ID: 3ZFZ), PBP4 (PDB ID: 5TW8), PBP3 (PDB ID: 3VSL), PBP2 (PDB ID: 2OLV), PBP1 (PDB ID: 5TRO) were used as protein target. The protein structures were saved in PDB format. It was cleared from water and the native ligand using Biovia discovery studio visualizer and then refined. Pymol, modbase modloop were used to add the missing amino acids, and autodock tools were used to repair the missing atoms, to add polar hydrogens, and add Kollman charges.

Molecular docking protocols

The docking protocols were conducted using Pyrx-Vina, where the proteins were made

Table 1. Docking grid box settings

Protein	Center	Dimensions	Target Domain
PBP2a (PDB ID: 3ZFZ)	X:27.4699 Y:39.7027 Z:87.9937	X:20.0Å Y:24.0Å Z:14.0Å	Transpeptidase
PBP4 (PDB ID: 5TW8)	X:22.3153 Y:-48.6732 Z:23.4581	X:22.0Å Y:14.0Å Z:20.0Å	Transpeptidase
PBP3 (PDB ID: 3VSL)	X:20.1072 Y:39.7027 Z:87.9937	X:20.0Å Y:14.0Å Z:22.0Å	Transpeptidase
PBP2 (PDB ID: 2OLV)	X:-18.9370 Y:86.2685 Z:-19.8550	X:22.0Å Y:28.0Å Z:18.0Å	Transglycosylase
PBP1 (PDB ID: 5TRO)	X:27.6481 Y:-24.6340 Z:-28.4674	X:28.0Å Y:28.0Å Z:28.0Å	Transpeptidase

macromolecules and the ligand structures were imported, minimalized, and made as PDBQT format. The grid box was set in accordance with **table 1**. Ligands with the most negative binding energy (BE) were visualized and analyzed using Biovia Discovery Studio Visualizer.

Structure-based toxicity calculation

CSVL phytoconstituents with the lowest BE were calculated for its toxicity. The toxicity calculations were performed using Protox-II, with LD50, hepatotoxicity, and immunotoxicity as the calculated parameters.

Results and Discussion

There are 24 possible phytoconstituents were identified using GC-MS (**table 2**). The identified phytocompounds are volatile and low molecular weight compounds. GC-MS is a reliable tool to use as phytoconstituents identification due to its good sensitivity, reproducibility, resolution, and cost-effectiveness compared to other tools such as LC_MS (liquid chromatography-mass spectrometry) and NMR (Nuclear Magnetic Resonance) spectroscopy (Khodadadi and Pourfarzam, 2020). The chromatogram is presented in **figure 1**.

The protein data used in this study were missing some residues. K604, M605, K606, Q607, G608, E609, T610 were added to the PBP2a. N293 and L294 were added to the PBP4. N627 and G628 were added to the PBP3. N136, L137, T138, G139, G140, F141, G142, S143,

E144 were added to PBP2. S210, K211, G212, S213, L214, R215, Y216, I217, H218, D219, I220, W221, G222, Y223, I224, A225, P226, N227, T228, K229, K230, E231, K232, Q233, P234 were added to PBP1. The refined structures are present in **figure 2**. Protein models available in Protein Data Bank are playing significant role in drug discovery. However validation, re-examination, and re-refinement of the structure models are needed to prevent false results. In another way, the refinement can enhance the docking result in terms of reproducibility and validity (Wlodawer, et al., 2020).

The ligand-protein complex along with interacting residues of the PBPs are presented in **figure 2**. There re notable numbers of interaction/bondings formed in CSVL-PBPs complex with hydrogen bonds dominated the interactions. The more the hydrogen bonds formed with the amino acid residue, the stronger the bonds. (Tumilaar, et al., 2021). Ser403, an amino acid in PBP2a, is an active amino acid that mediates the peptidoglycan formation as a nucleophilic attacker (Otero, et al., 2013). Ser75 and Ser392 are active amino acids in PBP4, and PBP3, respectively. The serine amino acid in the transpeptidase domain is critical in the formation of BCW. Therefore, interactions with these amino acids propose great inhibition characteristics of antibacterial agents (Alexander, et al., 2018; Yoshida, et al., 2012). The active amino acid in PBP2 and PBP1 are not yet known, however the catalytic site (transpeptidase and transglycosylase domain) can be identified.

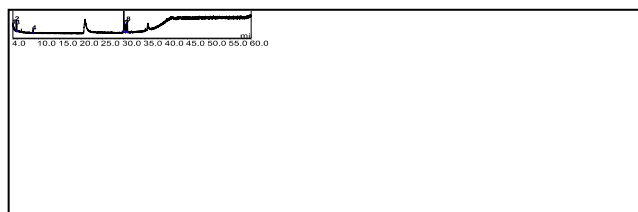


Figure 1. GC-MS chromatogram of methanol extract of CSVL

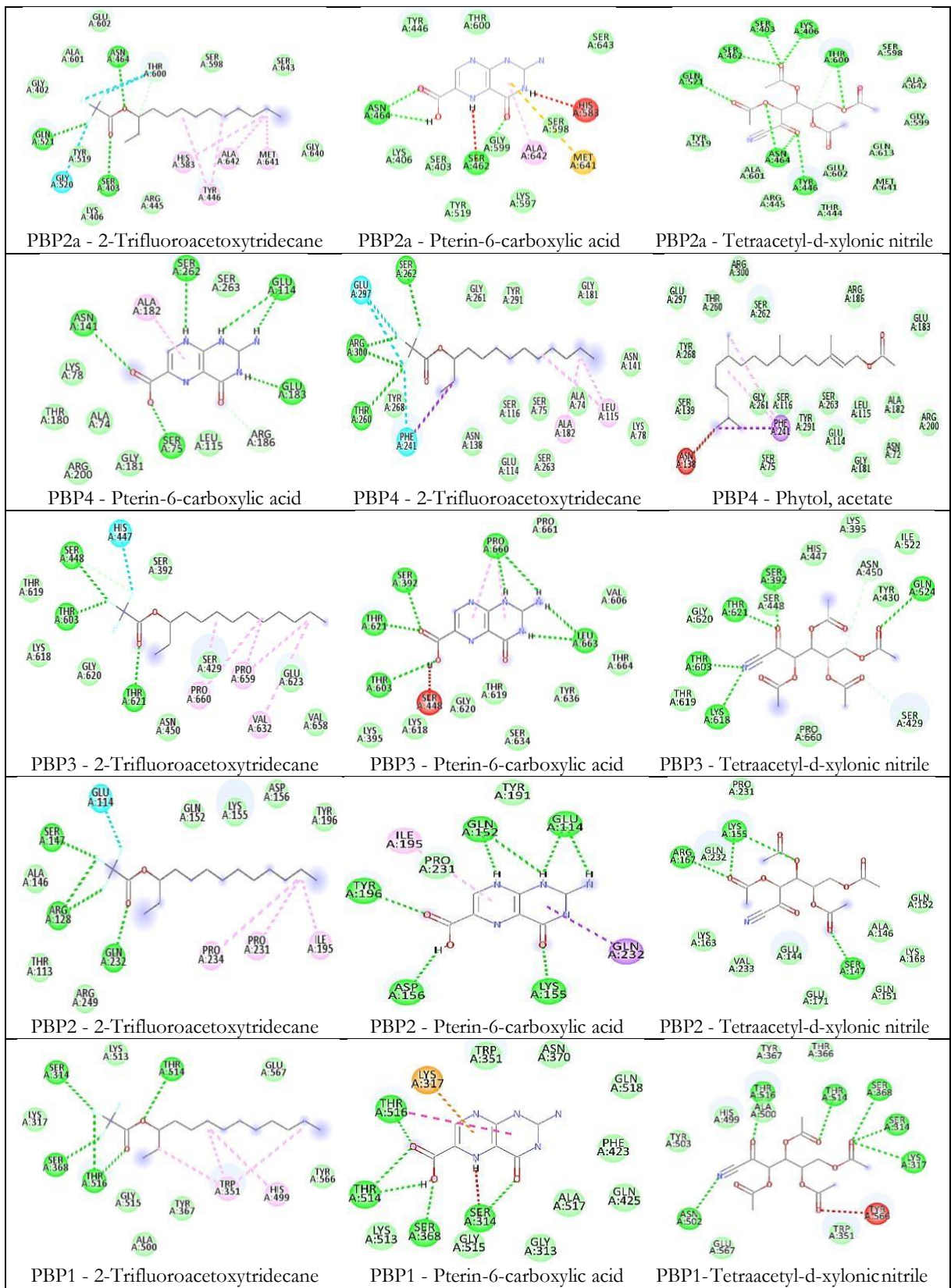


Figure 3. CSVL phytoconstituents interaction with PBPs

Table 2. Identified phytoconstituents from methanol extract of CSVL

Peak	RT	Hit# 1	Hit# 2	Hit# 3	RA (%)
1	4,01	Tetraacetyl-d-xylonic nitrile	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidiny)-2-butynyl]-	Pterin-6-carboxylic acid	2,90
2	4,78	1,3,5-Pentanetriol, methyl-	3-1,3,3-Trimethoxybutane	Propane, 1,1-dipropoxy-	9,61
3	4,92	Undecanoic acid, hydroxy-, methyl ester	3-Hexadecanoic acid, hydroxy-, methyl ester	1,3-Dioxolane-4-methanol, 2-ethyl-	6,25
4	8,76	Decane	3-Trifluoroacetoxydodecane	3-(Prop-2-enyloxy)dodecane	4,07
5	30,07	Neophytadiene	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol, acetate	47,77
6	30,21	2-Trifluoroacetoxytridecane	3-Trifluoroacetoxytridecane	4-Trifluoroacetoxy-pentadecane	7,12
7	30,58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Neophytadiene	1,4-Eicosadiene	8,26
8	30,94	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Z-4-Nonadecen-1-ol acetate	Neophytadiene	14,02

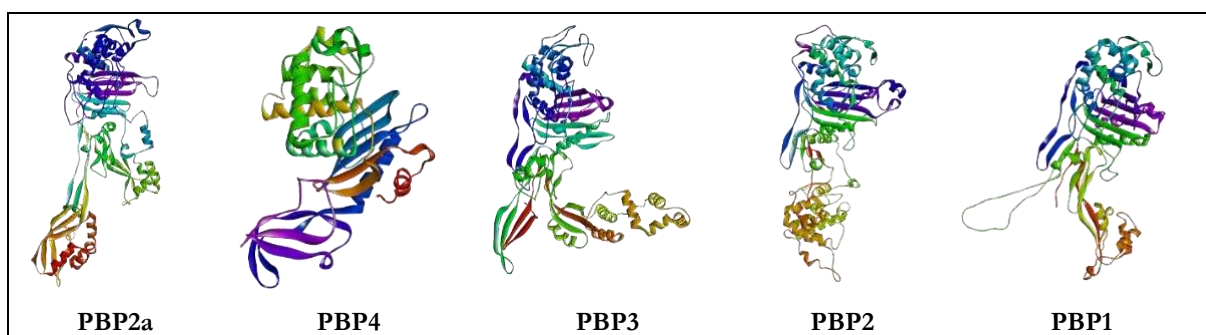


Figure 2. Refined protein-target structures

Table 3. Structure-based toxicity calculations result

Bioactive compounds	LD50 (mg/kg)	Hepa-toxicity	Immuno-toxicity
2-Trifluoro acetoxytridecane	5000	inactive	inactive
Pterin-6-carboxylic acid	1500	inactive	inactive
Tetraacetyl-d-xylonic nitrile	7000	inactive	inactive
Phytol, acetate	8000	inactive	inactive

The toxicity prediction showed that CSVL has a safe toxicity profile. The binding energy (BE) were also calculated. The lower the binding energy means the better or the more stable the interactions due to its high affinity. Table 4, 5, 6, 7, and 8 present the binding energy of CSVL phytoconstituents to PBP2a, PBP4, PBP3, PBP2, and PBP1, respectively.

Table 4. BE of CSVL-PBP2a complex

Phytoconstituents	Pubchem CID	BE
Native ligand	-	-8.2
2-Trifluoroacetoxytridecane	536345	-8.2
Pterin-6-carboxylic acid	135403803	-7.1
Tetraacetyl-d-xylonic nitrile	541568	-6.5

Table 5. BE of CSVL-PBP4 complex

Phytoconstituents	Pubchem CID	BE
Native ligand	-	-7.4
Pterin-6-carboxylic acid	135403803	-7.0
2-Trifluoroacetoxytridecane	536345	-7.0
Phytol, acetate	6428538	-5.7

Table 6. BE of CSVL-PBP3 complex

Phytoconstituents	Pubchem CID	BE
Native ligand	-	-7.0
2-Trifluoroacetoxytridecane	536345	-7.3
Pterin-6-carboxylic acid	135403803	-7.2
Tetraacetyl-d-xylonic nitrile	541568	-6.4

Table 7. BE of CSVL-PBP2 complex

Phytoconstituents	Pubchem CID	BE
Native ligand	-	-8.4
2-Trifluoroacetoxytridecane	536345	-6.4
Pterin-6-carboxylic acid	135403803	-5.7
Tetraacetyl-d-xylonic nitrile	541568	-5.2

Table 8. BE of CSVL-PBP1 complex

Phytoconstituents	Pubchem CID	BE
Native ligand	-	-
2-Trifluoroacetoxytridecane	536345	-7.7
Pterin-6-carboxylic acid	135403803	-7.6
Tetraacetyl-d-xylonic nitrile	541568	-6.8

Conclusions

CSVL can be a good anti-MRSA candidate due to its molecular interactions with BCW synthesis proteins. It interacts with the PBPs active amino acid and active site, therefore not enabling the PBPs to maintain pressure and cell integrity or shape and further lead to bacterial death.

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