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Virtual Prediction of Zerumbone Compound in Lempuyang as Anti-Bacterial Agent Through In-Silico Approach

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ABSTRACT

A native herbaceous plant of Southeast Asia, *lempuyang* is also widely distributed in Indonesia. This plant's primary bioactive component, Zerombone, has a variety of bioactivities, including an anti-bacterial effect. Typically, transglycosylase and alanine racemase were necessary proteins for forming peptidoglycan membranes and are now frequently used as anti-bacterial targets. Thus, using an in-silico approach, this work assessed the interactions between the zerumbone and the protein targets. The PubChem database was searched for the zerumbone compound (CID 5470187). Transglycosylase (PDB code: 1SLY), alanine racemase (PDB code: 4WR3), and the control protein target were also obtained from the PDB database. The Molegro Virtual Docker 5.0 version and the Discovery Studio application 21.1.1 versions were used to interface with and visualize the protein target and zerumbone compound. The result demonstrated that, like the control, the zerumbone compound in *lempuyang* blocks the active sites of the transglycosylase and the alanine racemase. The zerumbone-alanine racemase complexes additionally showed lower energy binding than other complexes. In conclusion, zerumbone in *lempuyang* can be a new candidate for anti-bacterial agents by inhibiting the synthesis of membrane peptidoglycan via suppressing transglycosylase and alanine racemase activities.

Keywords: alanine racemase, in-silico, *lempuyang*, transglycosylase, zerumbone

INTRODUCTION

As a tropical country, Indonesia is rich in mega-biodiversity, such as a medicinal plant. According to the Medicinal Herb Index, about 5,000 medicinal plants are found in Indonesia (Arozal *et al.*, 2020). One of the medicinal plants found in Indonesia is *lempuyang*, scientifically known as *Zingiber zerumbet*, which is a native rhizomatous herbaceous from Southeast Asia and belongs to a member of the Zingiberaceae family (Yob *et al.*, 2011). Based on the Statistics of Horticulture, the production of *lempuyang* in Indonesia reached 7219608 kg in 2022 (Irjayanti *et al.*, 2022). Commonly in Indonesia, *lempuyang* and its extracts are utilized as a traditional herbal medicine (Padalia *et al.*, 2018; Widyastiwi and Roseno, 2022), as a spice, as vegetables (Silalahi, 2018), or as ornamental plants.

According to Koga *et al.* (2016), terpenes and polyphenols are the primary chemical components found to dominate in *lempuyang*, with the main bioactive compound being zerumbone. Zerumbone is a valuable compound

with various biological functions, including anti-bacterial capabilities (Veena *et al.*, 2020; Ibáñez *et al.*, 2023). Previous studies reported that the zerumbone compound has excellent anti-bacterial inhibitory properties in gram-negative and gram-positive microorganisms, such as *Escherichia coli* (Moreira Da Silva *et al.*, 2018). The zerumbone compound in *lempuyang* exhibits anti-bacterial activity primarily through two main ways: imitation of anti-bacterial resistance mechanisms or chemical interference. Anti-bacterial agents frequently target the following processes in bacteria: (a) bacterial DNA replication and repair, (b) bacterial cell wall formation, (c) bacterial protein biosynthesis, (d) bacterial cell membrane destruction, or (e) metabolic pathway inhibition (Khameneh *et al.*, 2019). Alanine racemase and transglycosylase are the two examples of proteins responsible for prokaryote cell wall biosynthesis (Sauvage *et al.*, 2008; Wei *et al.*, 2016; Martinez-Bond *et al.*, 2022).

Alanine racemase is a PLP (pyridoxal-5′-phosphate) enzyme that functions to catalyze the conversion of L-alanine to D-alanine, a crucial building block in the formation of the peptidoglycan layer in bacterial cell walls (Asojo *et al.*, 2014; Wei *et al.*, 2016). This enzyme is frequently used to develop new anti-bacterial candidates because it is abundant in all bacteria but absent in higher species (Azam and Jayaram, 2016). Moreover, transglycosylases, such as alanine racemase, are required for the polymerization of glycan strands and the formation of peptidoglycan, which is required for the structural integrity of the cell wall (Galley *et al.*, 2014) and are now recognized as an intriguing target for anti-bacterial drugs and therapy (Chen *et al.*, 2019; Martinez-Bond *et al.*, 2022). As a result, as explained above, this work focused on the anti-bacterial activity of the zerumbone compound in *lempuyang* by triggering alanine racemase and transglycosylase utilizing in silico approach.

MATERIALS AND METHODS

Ligand, Protein Structure Retrieval, and Binding Cavities Prediction

The 3-D structure of the zerumbone compound was downloaded from the PubChem National Center for Biotechnology Information (NCBI) database (https://pubchem.ncbi.nlm.nih.gov/) with CID 5470187. The target protein structures are transglycosylase (PDB code: 1SLY) (Chen *et al.*, 2019) and alanine racemase (PDB code: 4WR3) (Soo *et al.*, 2016) and downloaded from the PDB (Protein Data Bank) database (https://www.rcsb.org/). The protein target structures were predicted for their active sites using the Molegro Virtual Docker 5.0 version program with binding cavities parameters: van der Waals and maximum cavities of 5 (Bitencourt-Ferreira and de Azevedo, 2019; Bare *et al.*, 2022; Sari *et al.*, 2023).

Docking Simulation and Analysis

The active sites (binding cavities) of transglycosylase proteins were docked using the Molegro Virtual Docker 5.0 programs with specific protein grids, notably X = 12.77; Y = 49.69; Z = 49.06; Radius 13, and the protein grid docking of alanine racemase is X = 44.0; Y = 2.34; Z = 2.97; Radius 1. The docking parameters were Score Function Moldock Score [Grid], grid resolution of 0.30, algorithm MolDock SE, 10x number iterations, max iterations of 1500, max population size of 50, pose generation energy threshold of 100, tries of 10 – 30, simplex evolution max steps of 300, neighbor distance factor of 1.00, multiple poses the number of poses of 5, energy threshold of 0.00, cluster similar poses RMSD threshold of 1. The protein target was docked with anti-bacterial control as a validation of the docking results of the zerombone compound, such as Bulgecin A (PDB code: 1SLY) for transglycosylase and Pyridoxal-5'-Phosphate (PDB code: 4WR3) for alanine racemase. Then, the docking results were analyzed by PyMol software version 2.2 and docking visualization to display 3D and 2D views and their interactions with the Discovery Studio program

21.1.1. version (de Azevedo Jr, 2019; Sari *et al*., 2021; Permatasari *et al*., 2022).

RESULTS AND DISCUSSION

Table 1 listed the interaction of transglycosylase as a receptor with the ligands, which are the zerumbone compound in *lempuyang* and bulgecin A as a control. The control interacted with the transglycosylase via SER487, MET498, THR501, TYR533, TYR552, GLU478, ASN553, GLY555, ALA554, and GLN496 amino acid residues through the hydrogen bonds, with low energy binding of -339.8 kJ/mol, as revealed in Table 1. Furthermore, the zerumbone compound in *lempuyang* also binds to the transglycosylase on the ILE497 amino acid residue by hydrogen bonds. Moreover, ALA491, MET498, ILE497, and TYR533 amino acid residues bind to zerumbone compounds via hydrophobic interactions. The binding energy of zerumbone-transglycosilase complexes is -207.6 kJ/mol (Table 1). Interestingly, the zerumbone compound in *lempuyang* and the control transglycosylase inhibitor (Bulgecin A) have the same active site interaction with the transglycosylase, as shown in Figure 1.

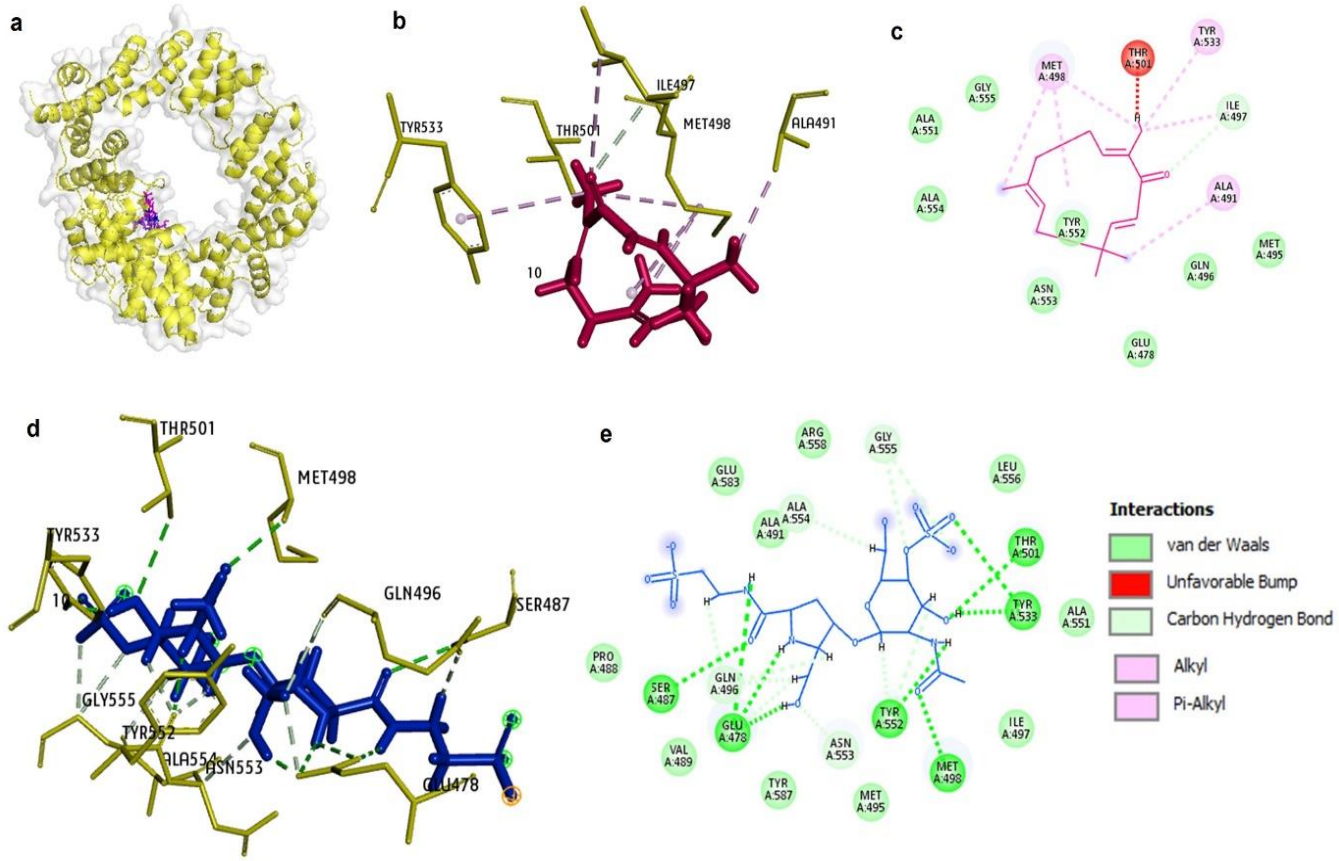

Figure 1. Interaction between zerumbone compound and protein transglycosylase. (a) 3D view of zerumbone and Bulgecin A complexes with protein transglycosylase, (b–c). 3D and 2D structures of the zerumbone–transglycosylase complex, and (d–e) 3D and 2D structures of the Bulgecin A–transglycosylase complex. The yellow ribbon structure represented transglycosylase, the pink stick structure represented Bulgecin A, and the blue stick structure represented the zerumbone compound.

Table 1. Interaction between zerumbone compound and protein transglycosylase

Compound	Bonding Energy (kJ/mol)	Interaction	Distance (A)	Category	Type
Zerumbone	-207.6	A:ILE497:CA - :10:O1	3.37108	Hydrogen Bonds	Carbon Hydrogen Bonds
		A:ALA491 - :10:C3	3.61299	Hydrophobic	Alkyl
		A:MET498 - :10	4.92497	Hydrophobic	Alkyl
		:10:C11 - A:MET498	3.54956	Hydrophobic	Alkyl
		:10:C15 - A:ILE497	5.40494	Hydrophobic	Alkyl
		:10:C15 - A:MET498	4.73121	Hydrophobic	Alkyl
		A:TYR533 - :10:C15	4.83215	Hydrophobic	Pi-Alkyl
		A:THR501:OG1 - :10:H20	1.3682	Unfavorable	Unfavorable Bump
Bulgecin A	-339.8	A:SER487:OG - :10:O10	2.91741	Hydrogen Bonds	Conventional Hydrogen Bonds
		A:MET498:N - :10:O8	2.64165	Hydrogen Bonds	Conventional Hydrogen Bonds
		A:THR501:OG1 - :10:O7	3.18807	Hydrogen Bonds	Conventional Hydrogen Bonds
		A:TYR533:OH - :10:O6	3.03518	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H9 - A:TYR533:OH	1.78291	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H10 - A:TYR552:O	2.19595	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H16 - A:GLU478:OE1	2.70192	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H16 - A:GLU478:OE2	2.96171	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H20 - A:GLU478:OE1	3.01712	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H27 - A:GLU478:OE2	1.81172	Hydrogen Bonds	Conventional Hydrogen Bonds
		A:ASN553:CA - :10:O14	3.22772	Hydrogen Bonds	Carbon Hydrogen Bonds
		A:GLY555:CA - :10:O3	3.03077	Hydrogen Bonds	Carbon Hydrogen Bonds
		A:GLY555:CA - :10:O4	3.24334	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H1 - A:TYR552:O	2.18658	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H3 - A:TYR552:O	2.59697	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H6 - A:ALA554:O	2.95363	Hydrogen Bonds	Carbon Hydrogen Bonds

Compound	Bonding Energy (kJ/mol)	Interaction	Distance (A)	Category	Type
		:10:H15 - A:GLN496:O	2.23126	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H21 - A:GLN496:OE1	2.48205	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H26 - A:GLU478:OE2	2.66473	Hydrogen Bonds	Carbon Hydrogen Bonds
		:10:H26 - A:GLN496:O	2.9668	Hydrogen Bonds	Carbon Hydrogen Bonds

The interaction between the zerumbone compound in *lempuyang* with the alanine racemase by the spending of energy binding of -243.4 kJ/mol (Table 2) amino acid residue via ALA163, ALA193, HIS159, TYR343, and TYR255 amino acid residues through seven hydrophobic interactions. Moreover, the alanine racemase control, the Pyridoxal-5'-Phosphate compound, interacted similarly with the zerumbone compound, as shown in Figure 2 and Table 2. The binding energy of Pyridoxal-5'-Phosphate-alanine racemase complexes was -288 kJ/mol, as listed in Table 2. The Pyridoxal-5'-Phosphate bound the alanine racemase via LYS34, ARG129, TYR38, TYR255, ARG280, TYR343, and PHE274 by hydrophobic interactions. Interestingly, the zerumbone compound and the control have the same amino acid residues in bounding of transglycosylase, which was TYR255 and TYR343, which indicated that the zerumbone compound in *lempuyang* had the same inhibition mechanism as the control, which is the Pyridoxal-5'-Phosphate. In earlier studies, Permatasari *et al.* (2022) also reported that the bioactive compounds of *Syzygium myrtifolium* interacted with alanine racemase via ALA193, TYR255, and TYR343. In addition, Soo *et al*. (2016) and Thunnissen *et al*. (1995) identified TYR255 as the catalytic base. In this study, the zerombone compound also binds to TYR255, which means the zerumbone compound inhibits the alanine racemase in the catalytic base, indicating a high chance of interfering with bacterial cell wall biosynthesis.

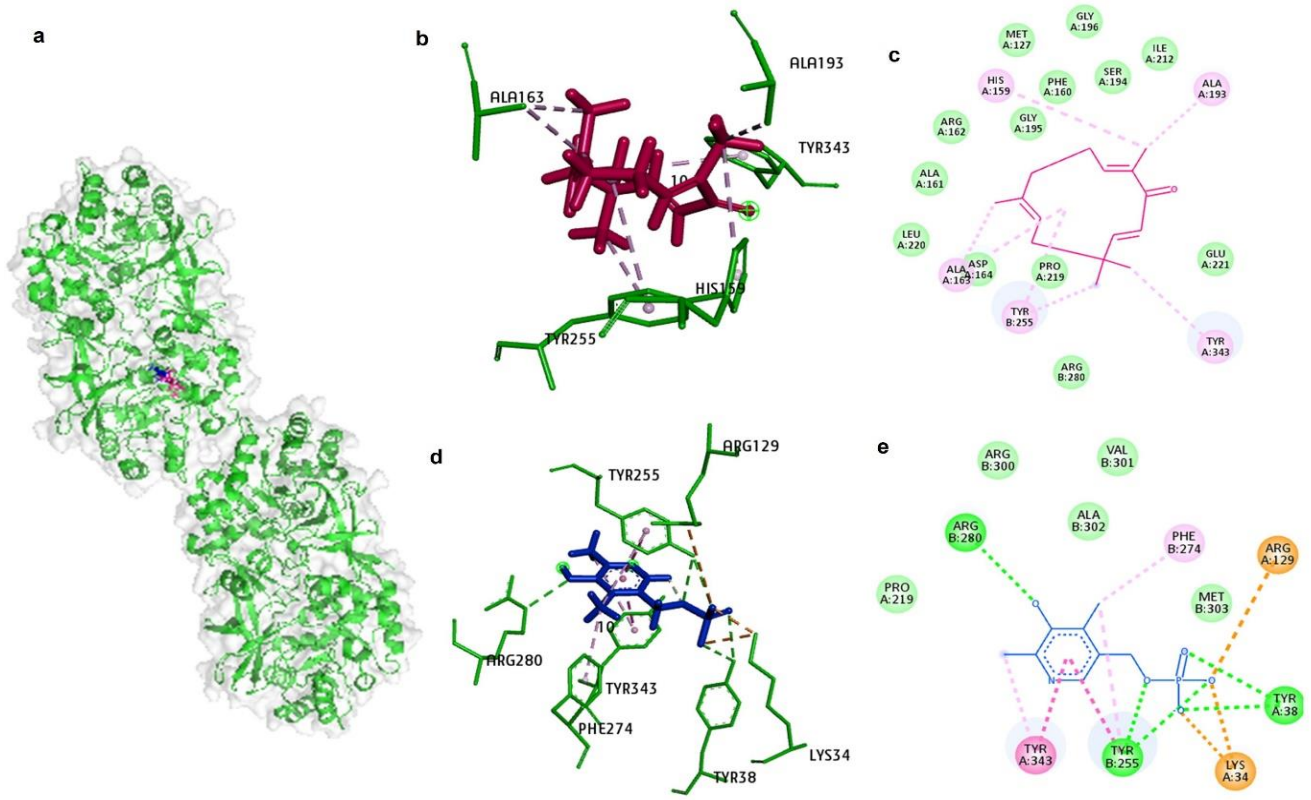

Figure 2. The interaction between the zerumbone compound and the protein alanine racemase (a) 3D view of zerumbone and Pyridoxal-5'-Phosphate complexes with alanine racemase protein, (b – c) 3D and 2D structures of the zerumbone–alanine racemase complex, and (d–e) 3D and 2D structures of the Pyridoxal-5'-Phosphate – alanine racemase complex. The green ribbon structure represented the alanine racemase, the pink stick structure represented the Pyridoxal-5'-Phosphate, and the blue stick structure represented the zerumbone compound.

Table 2. The interaction between the zerumbone compound and the alanine racemase protein

Compound	Bond Energy (kJ/mol)	Interaction	Distance (A)	Category	Type
Zerumbone	-243.4	A:ALA163 - :10	5.00505	Hydrophobic	Alkyl
		A:ALA163 - :10:C11	3.63808	Hydrophobic	Alkyl
		A:ALA193 - :10:C15	3.37588	Hydrophobic	Alkyl
		A:HIS159 - :10:C15	5.39814	Hydrophobic	Pi-Alkyl
		A:TYR343 - :10:C3	4.41259	Hydrophobic	Pi-Alkyl
		B:TYR255 - :10	4.67889	Hydrophobic	Pi-Alkyl
		B:TYR255 - :10:C4	3.6123	Hydrophobic	Pi-Alkyl
Pyridoxal-5'-Phosphate	-288,0	A:LYS34:NZ - :10:O3	2.63535	electrostatic	Attractive Charge
		A:LYS34:NZ - :10:O5	3.18835	electrostatic	Attractive Charge
		A:ARG129:NH2 - :10:O3	5.34412	electrostatic	Attractive Charge
		A:TYR38:OH -	3.1018	Hydrogen	Conventional

Compound	Bond Energy (kJ/mol)	Interaction	Distance (A)	Category	Type
		:10:O4		Bonds	Hydrogen Bonds
		A:TYR38:OH :10:O5	- 2.92902	Hydrogen Bonds	Conventional Hydrogen Bonds
		B:TYR255:OH :10:O2	- 3.07194	Hydrogen Bonds	Conventional Hydrogen Bonds
		B:TYR255:OH :10:O3	- 3.04982	Hydrogen Bonds	Conventional Hydrogen Bonds
		B:ARG280:NE :10:O1	- 3.10718	Hydrogen Bonds	Conventional Hydrogen Bonds
		:10:H8 - :10:O2	2.21946	Hydrogen Bonds	Carbon Hydrogen Bonds
		A:TYR343 - :10	3.75177	Hydrophobic	Pi-Pi Stacked
		B:TYR255 - :10	3.7622	Hydrophobic	Pi-Pi Stacked
		A:TYR343 - :10:C2	5.16787	Hydrophobic	Pi-Alkyl
		B:TYR255 - :10:C5	4.79817	Hydrophobic	Pi-Alkyl
		B:PHE274 - :10:C5	4.29921	Hydrophobic	Pi-Alkyl

The binding energy of zerumbone-alanine racemase complexes is lower than that of the zerumbone-transglycosylase complexes due to the bonds in zerumbone-alanine racemase dominated by hydrophobic interaction. Meanwhile, the zerumbone-transglycosylase complexes also had varied bonds, such as hydrogen bonds, hydrophobic interaction, and also unfavorable. Previous reports revealed that the number and interaction bonds affected the energy binding in protein-ligand interaction (Bare *et al.*, 2021; Sari *et al.*, 2021). The hydrogen bonds, unfavorable bonds, and hydrophobic interactions contributed to the tightness and binding energy. The varied interaction of the ligands-proteins complex encouraged low binding energy and tighter interaction (Permatasari *et al.*, 2022; Sari *et al.*, 2023).

CONCLUSION

In summary, the alanine racemase and transglycosylase activities on the peptidoglycan cell wall were successfully inhibited by the zerumbone compound in *lempuyang*. In future research, molecular dynamics was enjoyable to perform.

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