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DOCKING STUDY OF EUCALYPTOL COMPOUNDS FROM *MELALEUCA CAJUPUTI* POWELL WITH SOME TARGETS RELATED WITH ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: Molecular docking has an important role in the search for new drug compounds. Docking can provide predictive information on the interaction between candidate compounds and the preferred receptor target through the value of binding affinity, and the bonds formed. This study was to determine the mechanism of interaction between eucalyptol compounds from *Melaleuca cajuputi* Powell against receptors associated with antibacterial activity. This study used 3 target receptors associated with antibacterial activity: Penicillin Binding protein 3 (PBP3), N-myristoyltransferase (NMT), and Cytochrome P450 14 alpha-sterol Demethylase (CYP51). The molecular interactions of eucalyptol were compared with the native ligands at the active site of the receptor. **Materials and Methods:** Ligand files and target receptors are obtained by downloading at https://pubchem.ncbi.nlm.nih.gov/ and https://www.rcsb.org/. The docking process begins with ligand and receptor preparations using Pyrx software, MgTool, then molecular docking processes and visualization of interactions with AutoDock Vina and Discovery Studio Visualizer. **Results:** The eucalyptol ligand binding activity score with PBP3 is -5.4 kcal/mol; -4.3 kcal/mol with CYP51, and -4.2 kcal/mol with NMT. **Conclusions**: The binding activity data from molecular docking concluded that the eucalyptol compound had less dominant antibacterial activity at the three receptors compared to each native ligand.

KEYWORDS: molecular docking, antibacterial, eucalyptol, PBP3, NMT, CYP51.

INTRODUCTION

The case of antibacterial resistance has become a serious concern for experts, because it makes treatment more difficult and also costs rise.[1] The World Health Organization (WHO) has issued a priority list of Multi-Drug Resistant (MDR) bacteria which is a reference for researchers in the development of new antimicrobials. Carbapenems resistant (CR) *Acinetobacter baumannii*, CR *Klebsiella pneumoniae*, and MDR *Pseudomonas aeruginosa* were the most dominant microorganisms causing infections in hospitals.[2,3] The condition of gram-negative MDR bacilli creates challenges in the treatment of nosocomial infections.[4] MDR infections have increased the production of -lactamases (e.g. extended spectrum -lactamases [ESBLs] enzymes, carbapenemases, and metallo-ß-lactamases), leading to third generation cephalosporins and carbapenem resistance.[5]

Molecular docking methods have an important role in the rational design of new drug compounds. Docking can predict the preferred/best orientation of a candidate compound when it binds to a specific receptor target.[6,7] This technique allows predicting the affinity and activity of a drug candidate molecule with the protein where it will act.[8]

Medicinal plants are now more profitable than modern drugs, taking into account aspects of better patient tolerance, low toxicity effects, and relatively avoidable side effects and resistance.[9] Melaleuca tree (Melaleuca cajuputi Powell) is a plant species which belongs to the Myrtaceae family, grows well at temperatures of 17-33°C.[10] Main constituents that are commonly found in melaleuca essential oils consist of alkenes, acids, alcohols, aldehydes, esters, ketones, phenols and nitrogen compounds.[11] The extraction of essential oils from *M. cajuputi* leaves may result in the yield ranging from 0.3-0.6% depending on the quality of the leaves used. Major components in the essential oil of the plant may include 1,8-cineole (eucalyptol) (46.9-57.9%) with monoterpenic alcohol (-)α-terpineol, (-)-linalool and (-)-terpinene-4-ol.[12]

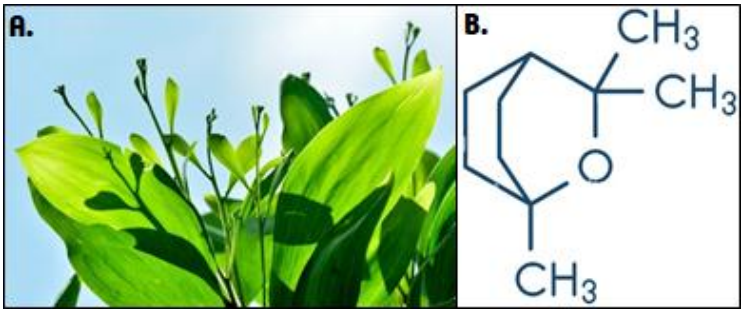

Figure 1. (A). *Melaleuca cajuputi* Powell, (B). Eucalyptol.

PBP3 (FtsI) is an essential protein of the divisome, catalyzing peptide cross-bridges between the glycan chains of the peptidoglycan. PBP3 is involved in many interactions within the divisome. It interacts directly with PBP1b which localizes at the division site during septation in a PBP3 dependent fashion.[13] PBP3 works in concert with PBP1b to incorporate the nascent glycan chain into the existing peptidoglycan.[14] The N-terminal 56 residues of PBP3 (containing a cytoplasmic peptide, the transmembrane segment and a short periplasmic peptide) interact with PBP1b in a two-hybrid assay. However, other interacting sites should be present in the periplasmic part of PBP1b and PBP3.[15] PBP3 also interacts directly with FtsW and with FtsN, which itself interacts with PBP1b and stimulates its activity.[16]

N-myristoyltransferase 1 (NMT 1: EC 2.3.1.97) is a key cellular enzyme which carries out lipid modification by facilitating the attachment of myristate to the N-terminal glycine of several protein molecules.[17] The enzyme's function is indispensible for the growth and development of many eukaryotic organisms and several rotaviruses. Myristoylation increases protein lipophilicity and is important for the full expression of biological functions of proteins. It controls the functioning of proteins by targeting them to specific localization, promoting specific protein-protein and protein-lipid interactions and ligand-induced conformational changes.[18]

Cytochrome P450 14a-sterol demethylases (CYP51) are essential enzymes in sterol biosynthesis in eukaryotes.[19] CYP51 removes the 14a-methyl group from sterol precursors such as lanosterol, obtusifoliol, dihydrolanosterol, and 24(28)-methylene-24,25-dihydrolanosterol. Inhibitors of CYP51 include triazole antifungal agents fluconazole and itraconazole drugs used in treatment of topical and systemic mycoses. The presence of two different channels, with one being open to the surface, suggests the possibility of conformationally regulated substrate-inyproduct-out openings in CYP51.[20]

MATERIALS AND METHODS

Software and Tools

Protein Data Bank (http://www.rcsb.org/pdb), PubChem (https://pubchem.ncbi.nlm.nih.gov), autoDock Vina 1.1.2, MGL tools, Pyrx, Discovery Studio Visualizer.

Ligand Preparation

Eucalyptol and various ligands (positive control) were used as ligands for docking studies were listed in Table 1.

Table 1: Ligands used in the study.

No	Ligand	Molecular Formula	References
1	Eucalyptol	$C10H_{18}O$	[12]
2	Erythromycin	$C_{37}H_{67}NO_{13}$	[11]
3	Ampotherecyn B	$C4_7H_{73}NO_{17}$	[15]
4	Fluconazole	$C_{37}H_{67}NO_{13}$	[21]

Protein Preparation

All protein target files can be obtained in the protein data bank (http://www.rcsb.org). The target receptor file was then carried out with protein preparation and converted into a file in the PDBQT format using Discovery Studio and PyRx software.

Docking Studies Using AutoDock Vina

The structure of eucalyptol and other ligands was minimized energy first by using AutoDock Vina 1.1.2. The prepared receptor files and ligands must be in PDBQT format files. The molecular docking process uses a closed receptor in a box with a distance of 1 Å. This is so that the receptors are rigid and the ligands are flexible molecules. The energy of the interaction between the ligand and the receptor is calculated and compared (kcal/mol).

Protein–Ligand Interactions

The molecular interactions that form between the ligand and the target receptor in the active binding pocket can be visualized in 2D with the Discovery studio visualizer program. This 2D visualization can explain molecular interactions that occur such as hydrogen bonds, hydropbobicity, bond distances and also the amino acids that interact through these bonds.

RESULTS AND DISCUSSION

Ligand Preparation

The structure of ligand was minimized and saved in the PDBQT file format with the PyRx program. The physicochemical properties of all the ligands are shown in Table 2.

Table 2: Physiochemical parameters of ligand.

No	Ligand	Molecular Weight (Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	XLogP3-AA	Minimize Energy
1	Eucalyptol	154.25	0	1	2.5	344.71
2	Erythromycin	733.9	5	12	2.7	2346.58
3	Ampotherecyn B	924.1	12	8	0	2824.99
4	Fluconazole	306.27	1	7	0.4	878.39

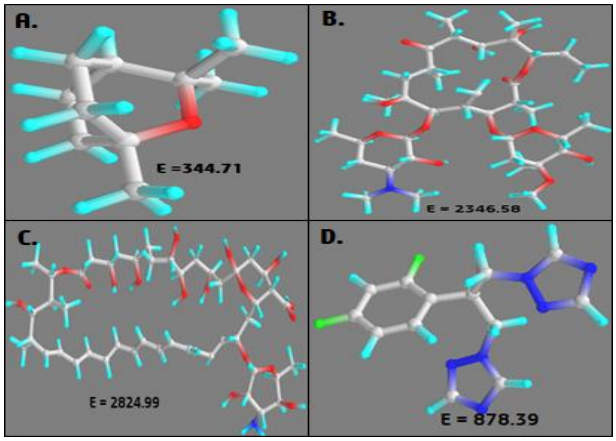

Figure 2. 3D ligand structure and energy minimized results. (A). Eucalyptol, (B). Erythromycin, (C). Ampotherecyn B, (D). Fluconazole.

Protein Preparation

Receptor targets for antibacterial are obtained through the Protein Data Bank link (http://www.rcsb.org/pdb). The file is then converted into PDBQT format using the AutoDock Tool (figure 3). The target receptors are readily available for ligand docking.

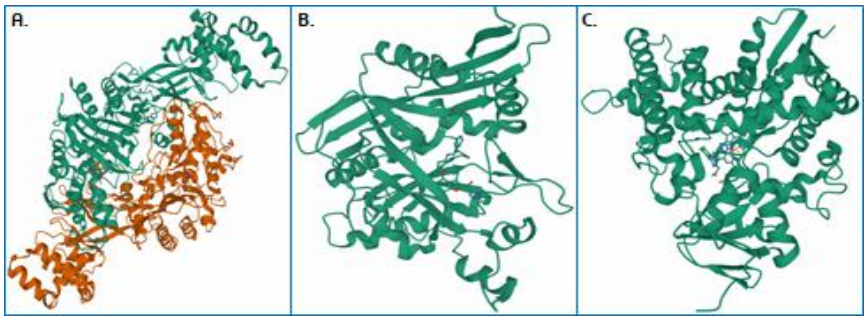

Figure 3. 3D structure of receptor, (A) PBP3; (B) NMT; (C) CYP51.

Docking Studies Using AutoDock Vina

Docking of Erythromycin with PBP3 (PDB ID:3VSL)

The docking molecule results between erythromycin and eucalyptol against the PBP3 receptor has the same amino acid that forms a bond Lys 494, Pro 500, and Tyr 278. The interaction between eucalyptol and the PBP3 receptor did not have hydrogen. In addition, Erythromycin also has hydrogen bond to the receptor Gln 548, and Gln 279. Docking analysis was performed by evaluating the binding affinity score indicating that the ligands eucalyptol -4.3 kcal/mol, and Erythromycin -6.5 kcal/mol. This provides information that eucalyptol did not have strong antibacterial activity that erythromycin through inhibitory Penicillin Binding protein 3 receptor.

Docking of Ampotherecyn B with NMT (PDB ID: 1IYL)

The molecular interactions between Eucalyptol and Ampotherecyn B ligand against the NMT receptor have similarities only to the binding of the amino acid His 307. Hydrogen bonds are formed on the interaction of the Ampotherecyn B ligand with receptor on the amino acids His 307. In addition, eucalyptol ligands also have hydrogen bond on Lys 265, Arg 371, and Glu 322. The binding activity score shows that Ampotherecyn B has a better bond affinity (-7.2 kcal/mol) versus eucalyptol (-4.2 kcal/mol). These values indicate that eucalyptol did not have a strong antibacterial activity via demic activity via N-myristoyltransferase inhibition mechanisms.

Docking of Fluconazole with CYP 51 (PDB ID: 1EA1)

The molecular docking results between the Fluconazole and eucalyptol ligands on CYP51 receptor showed did not have similarity in the amino acids. Meanwhile, the eucalyptol against the receptor did not have hydrogen bond. In addition, fluconazole has the hydrogen bond Lys 155, Cys 151, and Ala 150. The binding affinity score of eucalyptol (-3.9 kcal / mol), and fluconazole (-6.2 kcal / mol) showed that eucalyptol ligand did not have strong antibacterial activity through inhibition of the CYP 51 receptor mechanism.

Table 4: Comparative binding affinity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Penicillin Binding protein 3	Erythromycin Eucalyptol	-6.5 -3.9
2	N-myristoyltransferase	Ampotherecyn B Eucalyptol	-7.2 -4.7
3	Cytochrome P450 14 alpha-sterol Demethylase	Fluconazole Eucalyptol	-6.2 -3.9

The results of the visualization of ligand and receptor interactions with The Discovery studio visualizer program can be seen in Figure 4.

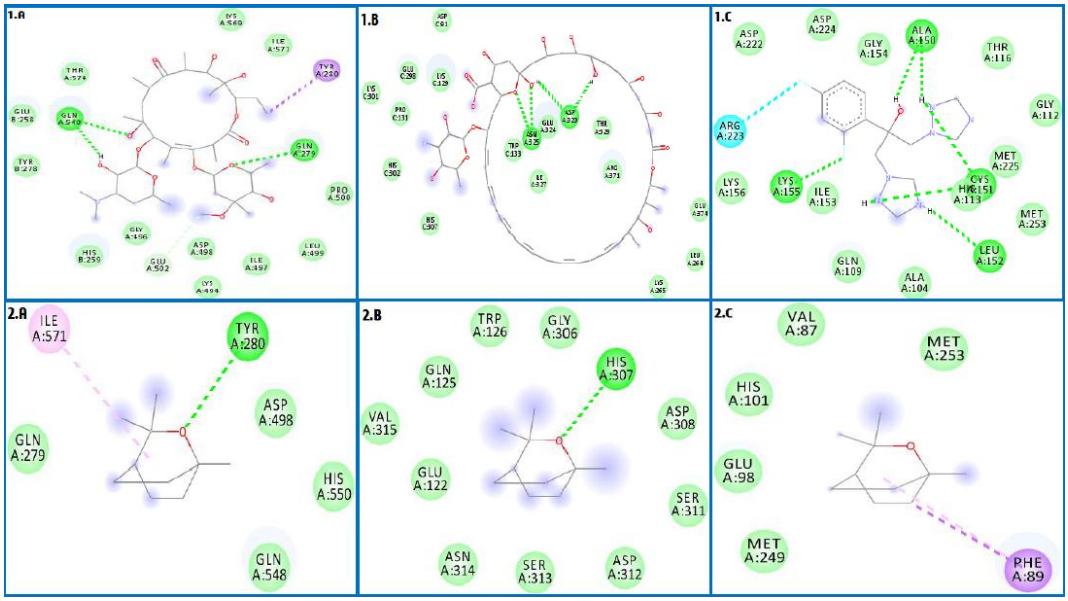

Figure 4. Interaction of ligands and target receptors. (1.A) Erythromycin bound to Penicillin Binding protein 3 receptor, (1.B) Ampotherecyn B bound to N-myristoyltransferase receptor; (1.C) Fluconazole bound to Cytochrome P450 14 alpha-sterol Demethylase receptor; (2.A) Eucalyptol bound to Penicillin Binding protein 3 receptor, (2.B) Eucalyptol bound to N-myristoyltransferase receptor, (2.C) Eucalyptol bound to Cytochrome P450 14 alpha-sterol Demethylase receptor.

CONCLUSIONS

Molecular docking studies of eucalyptol compounds from *Melaleuca cajuputi* Powell had less dominant antibacterial activity at the three receptors compared to each native ligand.

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