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Molecular Docking Application for the Potential use of Palmitic Acid as an AcrB, an Efflux Pump Protein, Inhibitor

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Abstract: Studies continue every day with the increase in multi-drug resistance in bacteria. In this study, the effect of potential inhibitors against the efflux pump with natural components in extracts obtained from some macrofungus to increase the effectiveness of antibiotics was investigated with an *in silico* study. Molecular docking was performed on the inhibition effect of palmitic acid against the AcrB (4DX5) protein of the RND pump. As a result of the modelling, it was determined that it had an inhibitory effect, and the binding affinity was -2.14 kcal mol^{-1}. Although palmitic acid may be a promising RND efflux pump inhibitor against multi-drug resistance *Escherichia coli*, further supportive studies are required.

Keywords: Efflux pumps, inhibitor, *E. coli*, antibiotic resistance, multi-drug resistance, molecular docking, RND

Introduction: The efflux pump, one of the antibiotic resistance mechanisms, has different superfamilies. The RND pump is just one of them (Altınöz & Altuner, 2019; 2022). The research on the Resistance-nodulation-division (RND) family in this study is due to the MDR *E. coli* strains used in the *in vitro* study. RND pump is one of the pump types found in gram-negative bacteria (Ashtiani et al., 2023; Colclough et al., 2020). The RND pump is a characterized efflux pump. It contains the AcrAB-TolC complex. AcrA is a periplasmic adapter protein, AcrB is an inner membrane-embedded protein, and TolC is an outer membrane factor protein (Du et al., 2014; Szal et al., 2023). This study targeted the AcrB (4DX5) protein (Samreen et al., 2023). Apart from the 4DX5 protein, there are also various proteins in different subtypes, such as 2D2R (Yilmaz et al., 2015), 1T9Y, and 1T9U (Rafiq et al., 2018), which belong to the AcrB protein. Before the *in silico* study, *in vitro* studies of this study were performed. In the *in vitro* part of the study, MDR *E. coli* strains with active multidrug-resistant efflux pumps were used (Altınöz & Altuner, 2019, 2020). A combined study with antibiotics was conducted using *Fomes fomentarius* and *Phellinus hartigii* macrofungi extracts. As a result of the *in vitro* study, inhibition was observed. Afterwards, the components were determined by GC/MS analysis of the extracts. The study continued in an *in silico* environment to observe the inhibition effect of the components reflected in the results on the efflux pump. Some of these components were included in the study by applying molecular docking. Palmitic acid is one of them. A study was conducted to observe the effect of palmitic acid inhibition on the RND pump. Since *E. coli* strains were used in the study, the effects of metabolites determined in the fungi extracts on the RND pump were investigated.

Palmitic acid is a common, saturated-free fatty acid. It is also found in the human body as free fatty acid. It is known to be included in traditional Chinese medicine and many foods. In cell experiments regarding palmitic acid, it has been reported that palmitic acid has toxic side effects on organs such as the heart, kidney, liver, and lung. However, there are no reports of this situation in studies conducted on animal models, and it is known that the uncertainty of the toxic mechanism continues (Lv et al., 2023; Pascual et al., 2021; Yuan et al., 2021). When the mechanism of action of palmitic acid is examined, it is stated that the target organelle may be the cell membrane of a virus, fungus, parasite, or bacteria. Palmitic acid has detergent permeable properties. Therefore, the cell membrane may deteriorate, and cell organelles may lose their functionality. Additionally, it is an advantage that palmitic acid has amphipathic properties and can interact with the cell membrane and cell wall. Thus, tiny pores are formed along the cell membrane. As a result, foreign substances cannot enter the organelles of the microorganism and are limited. For this reason, substances cannot be regularly moved into or out of the cell, resulting in osmotic shock. Palmitic acid can also use processes such as enzyme activity inhibition, toxic peroxidation, cell lysis, nutrient uptake, and release to achieve antimicrobial activity (Desbois & Smith, 2010; Johnson & Daniel, 2014; Pohl et al., 2011).

Molecular docking study was carried out using the Autodock 1.5.6 program. The AcrB protein MDR *E. coli* (Protein Data Bank: 4DX5, close monomer) was used to dock with palmitic acid. The 3D crystal AcrB protein structure was downloaded from the protein database in .pdb format (https://www.rcsb.org/structure/4DX5). Palmitic Acid 3D conformation (CID: 985) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in sdf format and converted into pdb file using Open Babel GUI. Ligand and water residues were removed from the receptor protein, and docking analysis was performed. Post-analysis figures were created using Biovia Discovery Studio.

Research Results: Considering the data obtained in the *in vitro* section, extract and antibiotic combinations against the MDR *E. coli* strain causing inhibition of the bacteria were applied.

Therefore, it is predicted that the extracts contain components for potential efflux pump inhibitors. Of course, detailed *in vitro* studies on these components will continue. However, as is known, working in an *in silico* environment saves time for experiments to be carried out in an *in vitro* environment. In this study, firstly, molecular docking analysis of palmitic acid, which is thought to have an effect, was performed, and it was studied how the applied compound binds to the AcrB (4DX5) protein of the relevant RND pump as a ligand. Also, GC/MS % data of palmitic acid macrofungus extract results are as follows so; *F.fomentarius* methanol 4.91%, *F.fomentarius* aeetone 1.99%, *F.fomentarius* ethyl acetate 1.33%, *P. hartigii* methanol 4.00%, *P. hartigii* asetone 1.00%. Molecular docking results are given in Figure 1, and Figure 2.

Figure 1

3D Image Depicting The Interacting Residues For Palmitic Acid-AcrB Interaction

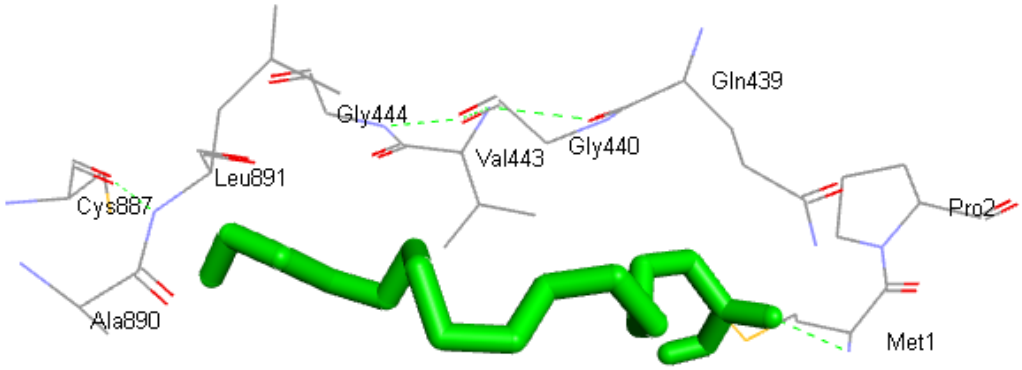
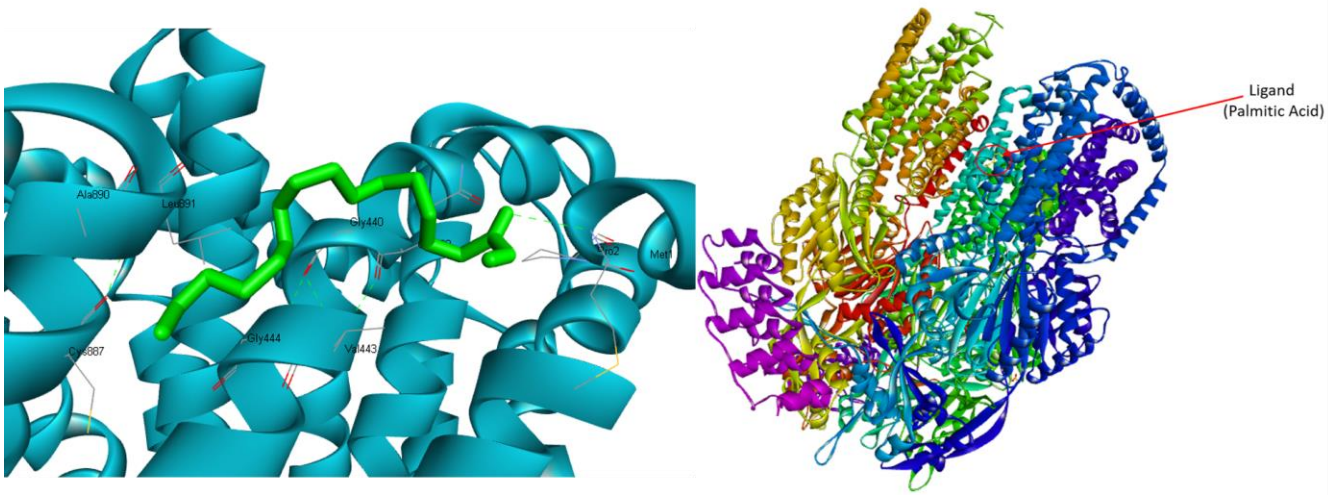


Figure 2

Molecular Docked Complex of Palmitic Acid with AcrB (PDB: 4DX5) and general view of AcrB protein (PDB: 4DX5) and Molecular Docked Complex



Conclusions: Molecular docking study of the palmitic acid ligand with AcrB (PDB: 4DX5) protein. Although it may be a promising RND efflux pump potential inhibitor against *E. coli*, it should taken into account that this is a potentially toxic component. Further studies are needed to continue to achieve the goal of re-entering the palmitic acid into the cell and restoring the amount of the drug whose concentration has decreased in the cell.

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