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Christinin molecular mechanism identification from Arabian bidara leaves (*Ziziphus spina-christi*, L.) on female genital problem-caused microorganism through computational approaches

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ABSTRACT

Arabian *bidara* leaves (*Ziziphus spina-christi*, L.) are known to have strong antimicrobial activity against microorganisms that cause infection in the female genital area, namely *Staphylococcus aureus* bacteria and *Candida albicans* fungi. They contain main secondary metabolites such as flavonoids, alkaloids, and saponins. Christinin is a saponin glycoside derivative compound. It consists of four types: christinin-A, B, C, and D. The role of computational studies in the discovery of new drugs is crucial and interesting nowadays because it is relatively cheap, effective, fast, and precise with a reliable level of accuracy. This computational study result will later be used to confirm in vitro test results carried out using experimental microbiological testing methods in the laboratory. This study identified, evaluated, and explored the interactions between christinin-A, B, C, and D compounds with Penicillin Binding Protein (PBP) from *Staphylococcus aureus* and Dihydrofolate Reductase from the fungus *Candida albicans* using the molecular docking computational study method. The christinin-A, B, C, and D compounds were modeled into 3D conformation using GaussView 5.0.8 and Gaussian09 software. The best conformation was selected for molecular interaction studies on Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from *Candida albicans* using MGLTools 1.5.6 software with AutoDock 4.2. The molecular interactions that occurred were further observed using the BIOVIA Discovery Studio 2020 software. Based on the molecular docking results, the christinin-B compound had the highest affinity for Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria, with a binding-free energy value of −7.67 kcal/mol. Meanwhile, the christinin-A compound has the highest affinity for Dihydrofolate Reductase from the fungus *Candida albicans*, with a binding-free energy value of −8.38 kcal/mol. Thus, it is predicted that christinin compounds can be chosen as the main component in feminine hygiene preparations to maintain the female genital area's health.

***Keywords**:* Arabian bidara leaves, christinin, *Staphylococcus aureus*, *Candida albicans*, feminine hygiene, Computational study

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INTRODUCTION

Maintaining the female genital area's health is very important, especially after menstruation or childbirth because of the humid conditions and the use of tampons, which can cause infection by the *Staphylococcus aureus* bacteria and also the fungus *Candida albicans*. Arabian *bidara* leaf has Latin names as *Ziziphus spina-christi* (L.) Willd. (*Rhamnaceae*). It is one of the plants mentioned in the Qur'an, which has a profound activity in inhibiting and even killing microorganisms (Elbashir et al., 2018; Kadioglu et al., 2016). It is proven that the ethanol extract of Arabian *bidara* leaves contains secondary metabolites such as alkaloids, flavonoids, polyphenols, tannins, and saponins (Asgarpanah, 2012; Kalayou et al., 2012). In the isolated butanol extract of Arabian *bidara* leaves, four types of saponin glycosides are named christinin-A, B, C, and D (Amin et al., 2020; Niamat et al., 2012).

Several secondary metabolites contained in Arabian *bidara* leaves have also been known to have effective antibacterial activity against *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typimurum*, *Proteus merabilis*, *Klebsiella pneumonia*, *Enterobacter* spp., *Acinetobacter*, *Serratia* spp., *Staphylococcus aureus*, *Staphylococcus aureus saprophytycus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* (Jebur, 2020; El-Ansary et al., 2018; Abalaka et al., 2010). This plant has antifungal activity against *Candida albicans*, *Trichophyton rubrum*, *Trichophyton mentagaphytes*, *Microsporum canis*, and *Aspergillus fumigatus* (Adamu et al., 2006; Pirbalouti et al., 2010). Based on some scientific evidence, it can be predicted that Arabic *bidara* can be used as the main component, especially to formulate antiseptic preparations to maintain the female genital area's health.

In the Al-Qur'an and Hadith, it is proven that the Arabian *bidara* plant is a plant that is in heaven and has many benefits for life. The Prophet Muhammad SAW advised humankind about Arabian *bidara* leaves' importance as a mixture in purifying or cleansing the body. This is contained in one of the hadiths from Umm ʻAthiyyah r.a, which explains that Rasulullah SAW recommended the use of *bidara* leaves in maintaining the health of the female genital area.

The role of computational studies in new drug discovery is crucial and exciting nowadays because it is relatively cheap, effective, fast, and precise with a reliable accuracy level. These computational study results can later be used to confirm the results of in vitro tests carried out using experimental microbiological testing methods in the laboratory (Leelananda and Lindert, 2016; Sliwoski et al., 2014). Thus, this study aims to model the christinin-A, B, C, and D from Arabian *bidara* leaves. Moreover, identification, evaluation, and exploration of the molecular interactions between these compounds against the target protein in *Staphylococcus aureus* and *Candida albicans* fungi were carried out using the molecular docking method. This research hopes that christinin compounds' characteristics will be obtained to develop antiseptics for maintaining the female genital area's health.

MATERIALS AND METHOD

Target protein macromolecules. The target protein macromolecules used in this study were the crystal structure of Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from the fungus *Candida albicans*. The target protein macromolecules were obtained from the Protein Data Bank web (http://www.rcsb.org/pdb) with PDB codes 3VSL with a resolution of 2.40 Å and 4HOE with a resolution of 1.76 Å, respectively (Figure 1) (Yoshida et al., 2012; G-Dayanandan et al., 2014).

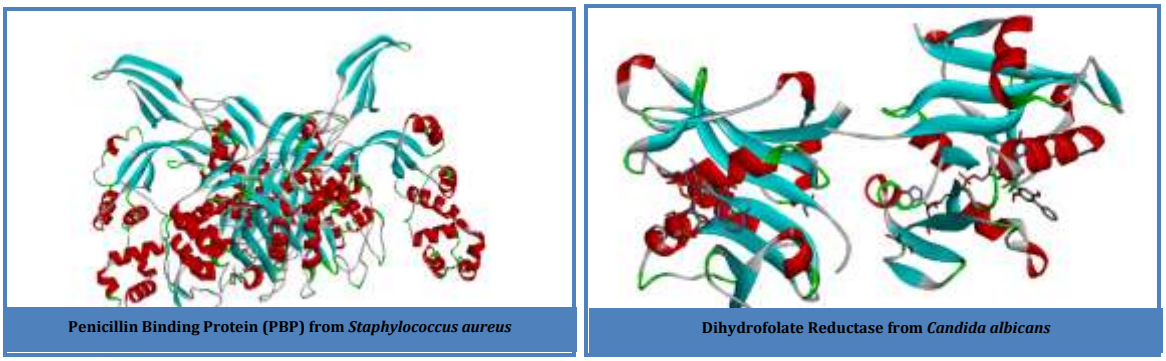

Figure 1. The crystal structure of the target protein macromolecules that have formed complexes with natural ligands

Test compound molecule. The test compound molecule used in this study is a saponin derivative that has activity against *Staphylococcus aureus* and *Candida albicans* fungi and has been proven in previous studies. The test compounds' molecules are christinin-A, B, C, and D derived from Arabian *bidara* leaves (Figure 2).

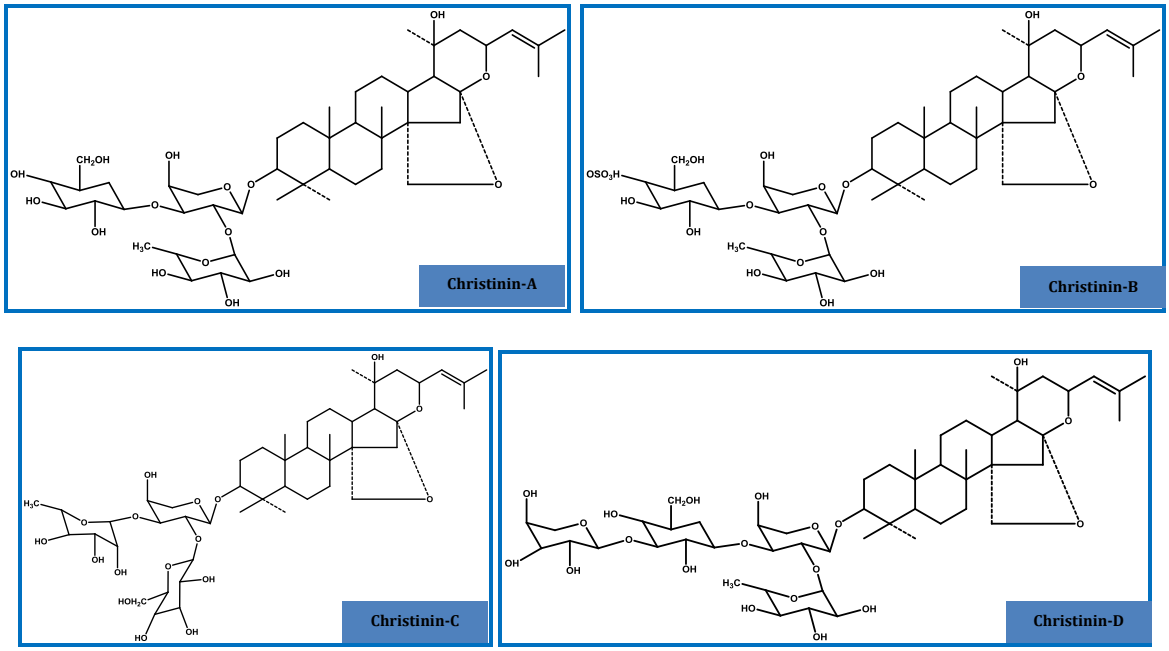

Figure 2. The molecular structure of the christinin compound derived from Arabian *bidara* leaves

Target protein macromolecule preparation. The crystal structure of target protein macromolecules that has been downloaded from the Protein Data Bank web is then prepared first using MGLTools 1.5.6 software with AutoDock 4.2. The macromolecule preparation stage for this target protein is carried out by removing water molecules and natural ligands, adding polar hydrogen atoms, and calculating Gasteiger's partial charge (Fakih, 2020).

Modeling of the test compound molecules. Molecular modeling of the test compound was carried out using GaussView 5.0.8 and Gaussian09 software. The chosen method at this modeling stage was the semi-empirical method based on the AM1 basis set. The test compound molecule, which had been modeled and its partial charge data modified, was used as input for the molecular docking simulation (Frisch et al., 2016).

Molecular docking method validation. The validation of the molecular docking method was carried out using MGLTools 1.5.6 software with AutoDock 4.2 to determine several parameters to be used in the molecular docking simulation. The method validation of molecular docking parameters was carried out using the re-docking method by limiting the maximum radius of the Root Mean Square Deviation (RMSD) value to 2 Å (Gaillard, 2018).

Molecular docking simulation. Molecular docking simulations were carried out using MGLTools 1.5.6 software with AutoDock 4.2 to observe interactions between Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from *Candida albicans* fungi with christinin-A, B, C, and D compounds. The macromolecule's surface distance of the target protein and the test compound was limited to a maximum radius of 2 Å. The parameters used in this simulation were based on the representation of the molecular shape, the active part of the target protein binding, and the selection and assessment. This molecular docking simulation was carried out efficiently in rigid molecular bonds (Fakih and Dewi, 2020).

Analysis of molecular docking simulation results. Interactions between Penicillin Binding Protein (PBP) from *Staphylococcus aureus* and Dihydrofolate Reductase from the fungus *Candida albicans* with christinin-A, B, C, and D compounds were then identified and evaluated based on the value of the free energy binding. Further observations were made of the amino acid residues responsible for protein-ligand molecular interactions using the BIOVIA Discovery Studio 2020 software (Biovia, 2016).

RESULT AND DISCUSSION

Previous research has proven that isolated n-butanol extract of Arabian *bidara* (Ziziphus spina-christi L.) leaves contains four triterpenoid saponins glycosides: christinin-A. Christinin-A is the main saponin compound with the core structure of steroids and further derived into several compounds such as christinin-B, C, and D (Elbashir et al., 2018; Kadioglu et al., 2016). This Arabian *bidara* leaf has been widely used as a natural antiseptic solution in preventing infections in the female genital area. Through this research, further predictions will be made regarding the affinity and molecular interactions formed between the christinin-A, B, C, and D compounds against the target receptors on Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from the fungus *Candida albicans* (Yoshida et al., 2012; G-Dayanandan et al., 2014).

The macromolecular structure of Penicillin Binding Protein (PBP) from *Staphylococcus aureus* and Dihydrofolate Reductase from the fungus *Candida albicans* are used as target proteins for christinin-A, B, C, and D. Then, polar hydrogen atoms are added. Finally, Gasteiger's partial charge is calculated using MGLTools 1.5.6 software with AutoDock 4.2. This target protein macromolecule preparation aims to ensure that the interaction between Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from the fungus *Candida albicans* with the christinin-A, B, C, and D compounds can form a stable conformation. Several antimicrobials on the market were used as a comparison to observe the high affinity and interactions. The antimicrobials used were cefadroxil and fluconazole.

The three-dimensional structural modeling of the christinin-A, B, C, and D compounds was carried out using GaussView 5.0.8 and Gaussian09 software. The best confirmation of the christinin compound from modeling was selected based on the total energy value. The total energy integrated into the GaussView 5.0.8 and Gaussian09 software confirms that the christinin compound structure that has been modeled is close to its original state. It is expected to produce a stable interaction with the active site of the target protein macromolecule. Based on the results of christinin compounds modeling contained in Table 1, it can be predicted that these compounds will be able to interact well on the active site of the binding. The active binding site is the Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from the fungus *Candida albicans*.

Table 1. The total energy value of the optimization result of the geometry of christinin compounds

Christinin Compounds	Total Energy (au)
Christinin-A	−1.21023344
Christinin-B	−1.42436566
Christinin-C	−1.28240292
Christinin-D	−1.52824472

The target protein macromolecules of Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from *Candida albicans* fungi that have previously been prepared, then enter the re-docking stage or simulation of molecular docking between the target protein macromolecules and natural ligand molecules. This process aims to validate several parameters used in the molecular docking simulation of christinin-A, B, C, and D compounds. Based on this method's validation results, the grid box size is 94 x 90 x 90, and the Lamarckian Genetic Algorithm method is used with 100 confirmations. In this re-docking process, the value of Root Mean Square Deviation (RMSD) is limited to a maximum radius of 2 Å, and the type of complex is selected by the protein-ligand method.

Table 2. The value of binding-free energy resulting from molecular docking

Target Protein Macromolecules	Test Compounds	Binding Free Energy (kcal/mol)
Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria	Cefadroxil	−7.25
	Christinin-A	−7.20
	Christinin-B	−7.67
	Christinin-C	−7.11
	Christinin-D	−7.59
Dihydrofolate Reductase from the fungus *Candida albicans*	Fluconazole	−6.76
	Christinin-A	−8.38
	Christinin-B	−6.90
	Christinin-C	−7.77
	Christinin-D	−6.67

A computational study utilizing the molecular docking method using MGLTools 1.5.6 software with AutoDock 4.2 was carried out. It aims to observe the high affinity among several christinin compounds' molecules and observe the interactions involved with the target protein macromolecules of Penicillin Binding Protein (PBP) from bacteria *Staphylococcus aureus* and Dihydrofolate Reductase from the fungus *Candida albicans*. The protein-ligand complex system with the best conformation resulting from molecular docking is selected and compared based on the binding-free energy value. The molecular docking results in Table 2 show that the christinin-B compound has a higher affinity for the Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria compared to christinin-A, C, D, and cefadroxil compounds, with a binding-free energy value of −7.67 kcal/mol. Meanwhile, different results were shown when the christinin compound interacted with Dihydrofolate Reductase from the fungus *Candida albicans*; the christinin-A compound has a higher affinity than christinin-B, C, D, and fluconazole, with a binding-free energy value of −8.38 kcal/mol.

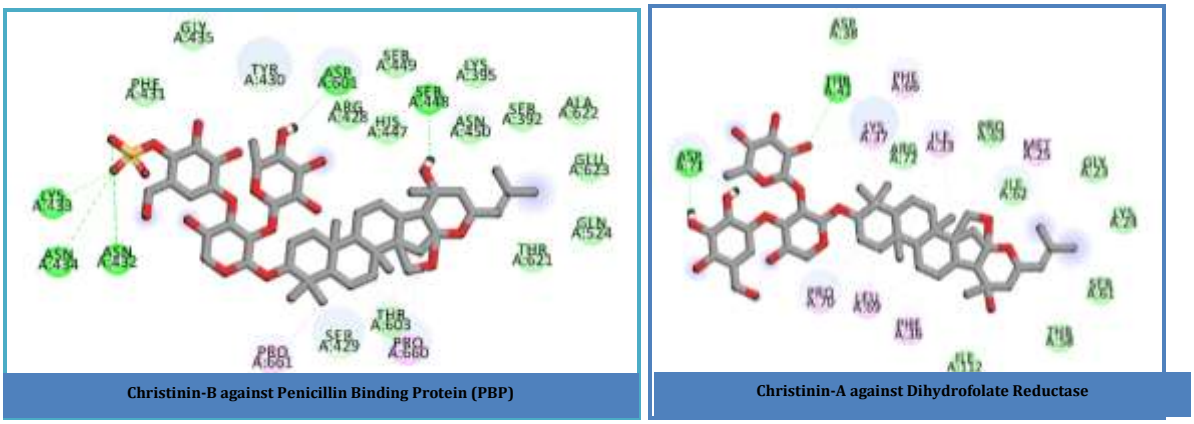

Figure 3. The interaction between the christinin compounds and the active site of the target protein macromolecules

Further observations were made to visualize the complexes of christinin compounds and target protein macromolecules of Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria and Dihydrofolate Reductase from the fungus *Candida albicans*. Based on Figure 3, it can be observed that if the interaction comparison is carried out, the christinin-B compounds have more interactions than christinin-A, C, D, and cefadroxil compounds against Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria. The interactions formed include 8 hydrogen bonds (with Ser429, Tyr430, Asn432, Lys433, Asn434, Ser448, and Asp601) and 5 hydrophobic interactions (with Pro660 and Pro661). Meanwhile, the interactions formed between the christinin-A compound and Dihydrofolate Reductase from the fungus *Candida albicans* include 4 hydrogen bonds (with Thr41 and Asp71) and 12 hydrophobic interactions (with Met25, Ile33, Phe36, Lys37, Phe66, Leu69, and Pro70). This phenomenon can occur because most christinin compounds have relatively large molecular weights, with an average value of around 900 g/mol. Thus, these compounds can interact maximally and cover the entire active site area of each target macromolecule.

CONCLUSION

An identification, evaluation, and exploration of the molecular interactions that occur between the christinin-A, B, C, and D compounds and target protein macromolecules of Penicillin Binding Protein (PBP) from *Staphylococcus aureus* and Dihydrofolate Reductase from the fungus *Candida albicans* have been successfully determined using molecular docking methods. Based on molecular docking results, it was found that the christinin-B compound had the highest affinity for the Penicillin Binding Protein (PBP) from *Staphylococcus aureus* bacteria, with a binding-free energy value of −7.67 kcal/mol. The christinin-A compound has the highest affinity for Dihydrofolate Reductase from the fungus *Candida albicans*, with a binding-free energy value of −8.38 kcal/mol. Therefore, the christinin compound can be a significant component in feminine hygiene preparations to maintain the female genital area's health.

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