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RESEARCH PAPER

Docking studies for screening antibacterial compounds of Red Jeringau (Acorus calamus L.) using Shigella flexneri protein as a model system

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Abstract: Alpha (α) and beta (β) asarone were identified as the main compounds of red Jeringau (Acorus calamus L.) and had antimicrobial properties. This study aimed to know the antibacterial mechanism and toxicity prediction of these two compounds against the PBP 2 protein and 50S Ribosomal Protein of Shigella flexneri. Molecular docking protocol using PyRx device was performed with Exhaustiveness value= 106, grid x=38.738375, y=112.645792, z=46.926417 for PBP2, and grid x=71.721251, y=47.551601, z=9.663173 for 50S Ribosomal Protein. The results of molecular docking on the α -Asarone compound obtained an affinity value of -5.7 kcal/mol for PBP2 and an affinity value of -5.6 kcal/mol for 50S Ribosomal Protein, while β-Asarone had an affinity value of -5.6 kcal/mol to PBP2 and an affinity value of -5.7 kcal/mol for 50S Ribosomal Protein. The α and β-Asarone affinity are better values than the control. Molecular docking of α and β-Asarone compounds results in ionic bonds to the TYR529 amino acid and polar bonds to the ASN552 amino acid of PBP2. However, only β-Asarone produces ionic bonds at the amino acid ILE17 and polar bonds at the amino acid GLU13 from 50S Ribosomal Protein. Based on this study, the α and β-Asarone compounds were shown to have antibacterial activity by interfering with the permeability of the bacterial cell wall. Both compounds are also predicted to have carcinogenic and mutagen effects.

Keywords: Red Jeringau (Acorus calamus L.), α and β-Asarone, Antibacterial, Autodock VINA, ProTox-II5

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INTRODUCTION

Shigellosis is an infectious digestive tract disease transmitted through food or water containing Shigella bacteria [1, 2]. Shigella is a Gram-negative, non-motile bacterium belonging to the Enterobacteriaceae family with four species: S. dysenteriae, S. flexneri, S. boydii, and S. sonnei (respectively designated as serogroups A, B, C and D) with several serotypes [3–6]. The serotype that causes diarrheal disease in developing countries is S. flexneri. Diarrhea due to S. flexneri is more familiar with most cases reported in children (28 cases/100,000 in children under four years and 25 cases/100,000 in children aged 4 to 11 years) [1, 7].

According to the World Health Organization (WHO), diarrhea is the second leading disease that causes many deaths, especially in children. About 1.7 million cases of diarrhea are found annually in the world. Many countries, such as India, Nigeria, Afghanistan, and Pakistan, have a high mortality rate caused by diarrheal disease 7.0 to 8.0. [5, 8, 9].

The primary treatment for shigellosis is medical management and includes electrolyte hydration management. The use of second-generation cephalosporins, ampicillin, and trimethoprim-sulfamethoxazole can be used for the treatment of Shigella infection. At the same time, third-generation cephalosporins are recommended in high-risk patients, including HIV-infected patients [3, 10, 11].

Permeability inhibition of the bacterial cell membrane is played by one of the crucial components of essential oils, namely β-Asarone. β-Asarone is a vital chemical compound from the Acorus calamus, which is indicated to have most of the biological activity of plants. α and β-Asarone were identified as the main compounds with antibacterial properties from A. calamus in all parts of the plant, roots, rhizomes, and essential oils. The essential oil from the tetraploid form of A. calamus rhizome contains about 95-96% β-Aaron as a component mainly [12, 13].

A molecular docking study with is a branch of science that uses a computational approach to predict a relationship between a drug compound and a receptor [14–16]. One of the docking programs in the AutoDock Suite, Vina is undoubtedly one of the more popular ones due to its open-source nature, relative simplicity, and speed compared to other docking programs inside and outside the suite. Simple and sophisticated docking simulations can be designed and carried out more easily with the help of AutoDock Vina 1.2.0. The updated version offers Python bindings, making it simpler to script for complex applications like virtual screening. In order to speed up high-throughput virtual screens, we also incorporated batch processing and simultaneous multiple-ligand docking against a single target structure [17].

Increasing computational predictive ability is an opportunity to develop simulations and calculations in designing drugs. Computers offer in silico methods as a complement to the in vitro and in vivo methods commonly used in drug discovery. Molecular docking is carried out by linking active compounds or also called ligands, and linked to the target protein. This study aimed to obtain a docking score between the test compound and the targeted protein and the interaction between the ligand and the receptor.

PBP 2, or Penicillin Binding Protein 2, is a protein in bacteria that becomes a receptor for active compounds that work in the bacterial cell wall synthesis process [16]. PBP 2 has a mechanism of action with cephalosporin group compounds [18,19]. PBP 2 can be viewed and downloaded via the website: https://www.rcsb.org with the structural code 7RCW. 50S Ribosomal Protein is used as a comparison protein with a different mechanism of action than PBP 2 [20]. With the Vina application, we conducted a study to determine the antibacterial ability of α and β-asarone compounds on PBP 2 and 50S ribosomal receptors.

MATERIALS AND METHODS

Instrument. The instrument used in this study are hardware with Intel-Core-i3 specifications, Celeron(R), and NVIDIA GT 520M. The software used consists of Discovery Studio 2021 Client (DS) for visualization, Autodock Vina (Version 4.2, updated for version 4.2.6), and ChemDraw 2D (Version 19.1). All software is in trial form except Autodock Vina.

Material

The materials used in the study were the three-dimensional structure of the α and β-Asarone ligands from ChemDraw (Version 19.1) in pdb format, the three-dimensional structure of the Shigella flexneri receptor downloaded from the Protein Data Bank (http://www.rcsb. org/pdb/) in pdb format.

Methods

ligand preparation

The ligands used were downloaded first from http://PubChem.ncbi.nlm.nih.gov in .sdf format. The ligands used in this study were α and β-Asarone compounds as test ligands, ZZ7 as the natural ligand of PBP2 with ceforanide and ampicillin compounds as positive controls, on 50S Ribosomal protein using Tetracycline as control. Discovery Studio is used to open the .sdt file. Ligands are given a torque or rotary force to the compound to work in the molecule docking process. Next, the energy is added to the ligand with add polarly and stored in .pdb format. The ligands are then processed in the PyRx application to minimize energy and change the .pdb ligand format to .pdbqt format to carry out the molecular docking process.

Protein Preparation

Penicillin Binding Protein 2 (PBP2) and 50S Ribosomal Protein from the Protein Data Bank (http://www.rcsb.org/pdb/) downloaded in .pdb format. The selected protein codes are 7RCW and 1HNW. The protein was then prepared using the Discovery Studio program to determine the ligand grid in the protein, separate the natural ligands present in the protein, remove water residues, and provide energy in an add polar manner, after which it was brought to the PyRx application to change the protein macromolecule data format from .pdb to .pdbqt [21, 22].

The water residue in the protein has to be removed so the molecular docking process runs optimally without any interference from residues scattered on the target protein. The protein is then minimized in an add-polar way to activate the macromolecule to proceed with molecular docking. Add Polar also aims to adjust the docking environment and

mediate the relationship between the ligand and the target protein [21].

Molecular Docking with AutoDock Vina

Compounds that have been prepared will then be docked with previously prepared and validated proteins. The validated proteins were selected based on the RMSD value—the protocol docking using AutoDock Vina, linked to the PyRx application [17, 21]. The docking protocol sets the ligand grid on the PyRx application according to the XYZ grid obtained. Gridbox size is 25x25x25, and gridbox positions can be based on existing ligands from X-Ray crystallographic data. The gridbox on the protein with the code 7RCW shows x=38.738375, y=112.645792, z=46.926417, while the protein code 1HNW shows x=71.721251, y=47.551601, z=9.663173. The XYZ grid represents the position where the ligand compound will act on the target protein. The molecular docking process with the PyRx docking protocol uses a value of 106 Exhaustiveness. The exhaustiveness value will determine the molecular docking process to produce the best results for docking data. The greater the Exhaustiveness value, the longer AutoDock Vina works, but the more molecular docking data can be obtained, and the data accuracy of the docking process increases [23]

Data Analysis. The research data were expressed as mean ± standard error of mean (SEM) and the experiment was carried out in at least three replications.

RESULTS AND DISCUSSION

Validation is carried out to ensure that the protein to be used is valid. The valid protein is the result of the re-docking of the tested protein with an RMSD value of < 2. The validation results obtained an RMSD value of 0.7624 Å (figure 1). The RMSD validation value below 2 A indicates that the selected protein is valid and can use for the next step [15, 16].

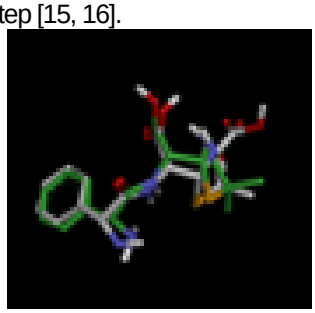


Figure 1. Visualization of the protein validation results

The molecular docking results in this study were seen from the Root Mean Square Deviation (RMSD) values, ΔGbind, and the interaction of the ligand with protein residues. RMSD is said to be good if <2 Å. The greater the deviation, the greater the error in the prediction of the interaction of the ligand with the protein. The conformation of each docked ligand is ranked based on the ΔGbind value from the smallest to the largest [24, 25]. The ΔGbind results of molecular docking can be seen in Table I.

Table I. ΔGbind Ligand Values from Molecular Docking

Protein	Native Ligand	α-asarone	β-asarone	Cefo-ranide	Ampicillin	Tetracycline
ΔGbind (*kcal*/mol) PBP2	-7,9	-5,7	-5,6	-8,4	-7,8	-
ΔGbind (*kcal*/mol) 50SRP	-	-5,6	-5,7	-	-	-6,8

The more negative ΔGbind value indicates that the conformation formed is stable, while a large ΔGbind value indicates a less stable complex formed [24, 25]. The α-asarone compound has an affinity value of -5.7 kcal/mol for PBP 2 and -5.6 kcal/mol on 50S Ribosomal Protein. In comparison, β-asarone has an affinity value of -5.6 kcal/mol for PBP 2 and -5.7 kcal/mol for 50S Ribosomal Protein. ZZ7 or ((2R,4S)-2-[(R)-{[(2R)-2-amino-2-phenylacetyl] amino} (carboxy) methyl]-5,5-dimethyl-1, 3-thiazolidine-4-carboxylic acid) as a native ligand has an affinity value of -7.9 kcal/mol. The drug control on PBP 2, namely Ceforanide, obtained an affinity value of -8.4 kcal/mol, and ampicillin received an affinity value of -7.8 kcal/mol. In comparison, the drug control on 50S Ribosomal Protein in Tetracycline received an affinity value of -6.8 kcal /mol. Compared to a docking study of an essential oil compound called Copaene against *Shigella flexneri* bacteria, it proves the antibacterial activity of *Shigella flexneri*. The docking test of the copaene compound obtained a bond energy value of -6.4 kcal/mol and could inhibit the growth of *Shigella flexneri*. A docking test was also carried out on gram-negative bacteria, namely *E. coli*. Peptidoglycan consists of glycan strands polymerized from beta-1,4 linked cross-linked N-acetyl-glucosamine-N-acetyl-muramic acid, which in *E. coli* this reaction is carried out by penicillin-binding proteins (PBPs) PBP5 *E. coli* participates significantly in the construction and maintenance of bacterial cell walls. When PBP 5 is disturbed, it will cause the bacterial wall to become unstable [26].

The difference in the ΔGbind value is due to the difference in the molecular formula of each compound. A more complex molecular formula causes a more varied and robust bonding interaction between the amino acid and the target protein[27]. Visualization of the interaction resulting from the docking of α and β-Asarone compounds as the primary assay compound in this study is shown in Figure 2 and 3.

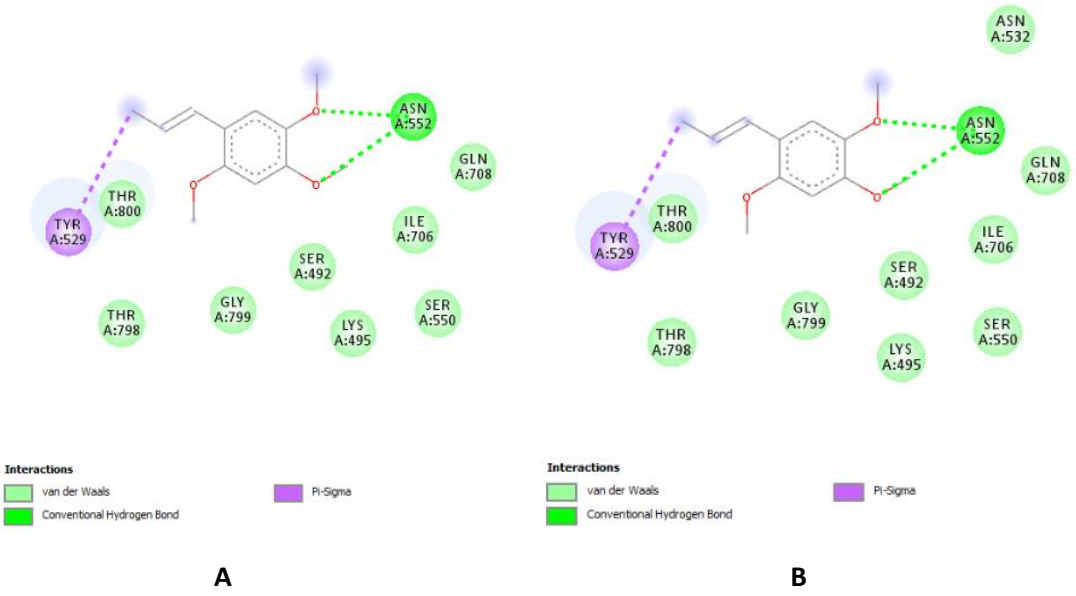


Figure 2. Interaction of PBP 2 residues against ligand test (A) α-Asarone compound (B) β-Asarone compound

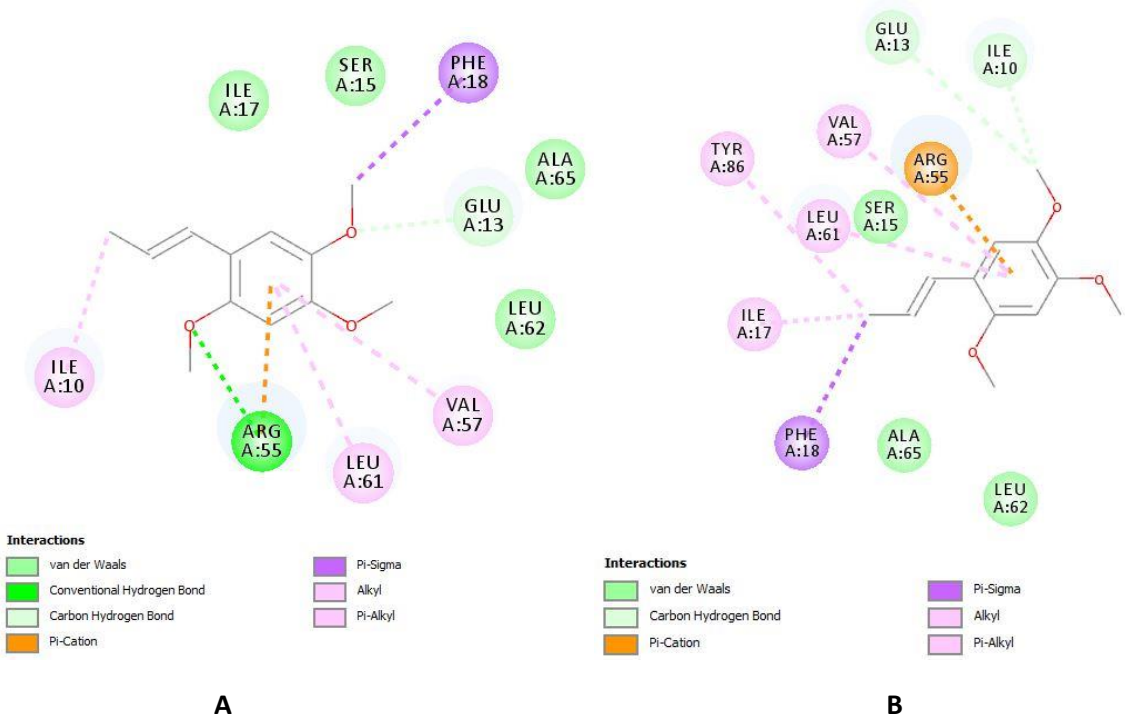


Figure 3. Interaction of 50S Ribosomal Protein residues against ligand test (A) α-Asarone compound (B) β-Asarone compound

Visualization of the results of docking studies shows various interactions of ligands with protein residues that can occur due to molecular docking. Compounds with more complex and bulky molecular formulas result in more ligand interactions with amino acid residues. A recapitulation of the interactions of ligands with protein residues from molecular docking can be seen in Table 2 and Table 3. The interactions on amino acid residues are grouped into four bond groups based on their amino acid structure, namely ionic, hydrophilic, aromatic, and aliphatic. Each interaction of protein residues affects the value of ΔGbind. Ionic residues contributed the most in determining the value of ΔGbind, followed by hydrophilic, aromatic, and aliphatic residues, respectively [28, 29].

Table 2. Ligand interactions with PBP 2

	Native Ligand	α-Asarone	β-Asarone	Ceforanide	Ampicillin
Ionic	TYR529	TYR529	TYR529	GLN708, ASN552, TYR529	TYR529
Polar	THR800, GLN708, ASN552, SER550, LYS495	ASN552	ASN552	THR800, GLN708, ASN552, SER550, GLU549, ASN532, TYR529, GLY527, SER492	THR800, GLN708, ASN552
Aromatic	VAL530, TYR529	-	-	GLU549, VAL530, TYR529	-
Aliphatic	LYS797, THR782, ILE706, GLU549, HIS538, ASP528, GLY527, SER492	THR800, GLY799, THR798, GLN708, ILE706, SER550, LYS495, SER492,	THR800, GLY799, THR798, GLN708, ILE706, ASN532, SER550, LYS495, SER492	LYS784, THR782, ILE706, HIS538, ASP528, LYS495, SER492	SER945, GLY799, THR798, LYS797, THR782, ILE706, ASN552, SER550, GLU549, HIS533,

Table 3. Ligand interactions with 50S Ribosomal

	Tetracycline	α-Asarone	β-Asarone
Ionic	ILE17	PHE18, ILE10	TYR86, PHE18, ILE17
Polar	ARG53, ASP50, LYS20, GLU13	ARG55, GLU13	GLU13, ILE10
Aromatic	GLU16	LEU61, VAL57, ARG55	LEU61, VAL57, ARG55,
Aliphatic	THR88, TYR86, GLY51, LYS12	ALA65, LEU62, SER15, ILE17	ALA65, LEU62, SER15

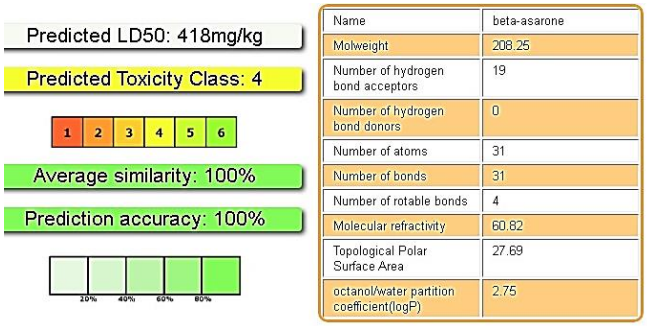
The molecular docking results in Table 2 show that ZZ7, as a native ligand, shows the activity of the amino acids TYR529 and ASN552 in the PBP2 protein. The α and β-Asarone ligands have protein residue interactions similar to ZZ7 on the primary amino acid, namely TYR529, with the bond that occurs in the form of an ionic bond, followed by ASN552 amino acid with its hydrophilic bond. The ionic bond to the TYR529 amino acid and the hydrophilic bond to the ASN552 amino acid are the primary keys to the interaction of PBP2 protein residues on the α and β-Asarone ligands because it is also proven by the positive controls, namely ceforanide and ampicillin which have ionic bonds on the TYR529 amino acid and hydrophilic bonds on the ASN552 amino acid. In several previous docking studies, it was shown that the atomic interaction of the test ligand with similar amino acid residues showed pharmacological activity with the exact mechanism of action [16]. The binding site (binding site) is the area of binding of the protein to the ligand, which will affect the conformation and function of the protein. Binding sites show residues of amino acids that play an essential role in forming interactions between macromolecules and ligands, such as hydrogen bonds, hydrophobic bonds, and electrostatic bonds.

By comparing the results of the interaction of α-Asarone and β-Asarone with Ceforanide and Ampicillin based on the visualization results showed some similarities in amino acid residues (table 2). The binding positions are almost similar, involving identical residues, so α-Asarone and β-Asarone can have inhibitory activity on PBP 2. However, their inhibitory activity/affinity value is less potent than Ceforanide and Ampicillin.

The results in Table 3 show that the tetracycline compound forms ionic bonds at the amino acid ILE17 and polar bonds at the amino acid GLU13 residue. Tetracycline inhibits aminoacyl tRNA from binding to the ribosome. The α-Asarone compounds do not form ionic bonds but form polar bonds to the amino acid GLU13. The β-Asarone compound succeeded in forming ionic bonds at the amino acid ILE17 and polar bonds at the amino acid GLU13. The bond formed indicates that the β-Asarone compound can inhibit aminoacyl tRNA so that it does not bind to the ribosome and causes the failure of bacterial DNA replication.

After the docking study was carried out, the α and β-Asarone compounds were also carried out for toxicity studies using the ProTox-II application as information on their safety [31]. The results of checking the level of toxicity on ProTox-II showed that the α and β-Asarone compounds were in class 4 (Figure 4). Class 4 indicates that α and β-Asarone compounds are acceptable with minimal risk. The risk can be determined from the effects that the test compound can cause. The effect can be seen in Figure 5. α and β-Asarone compounds have a carcinogenic effect with a probability of occurrence of 56% and a mutagen effect with a probability of occurrence of 92%. This result is in line with previous studies stating that the metabolism of asarone compounds causes cancer in overdose range. However, if it is consumed in a therapeutic dose range, the incidence of cancer caused by the consumption of asaron is not found [32, 33].

Based on this study, it is necessary to conduct a laboratory assay for the Asarone compound, especially in determining its effective dose. The effective dose obtained will then be tested for its toxicity to determine the safety of the asarone compound.



Name	beta-asarone
Molweight	208.25
Number of hydrogen bond acceptors	19
Number of hydrogen bond donors	0
Number of atoms	31
Number of bonds	31
Number of rotable bonds	4
Molecular refractivity	60.82
Topological Polar Surface Area	27.69
octanol/water partition coefficient(logP)	2.75

Figure 4. Toxicity class of α and β-Asarone compound

Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.63
Toxicity end points	Carcinogenicity	carcino	Active	0.56
Toxicity end points	Immunotoxicity	immuno	Inactive	0.67
Toxicity end points	Mutagenicity	mutagen	Active	0.92
Toxicity end points	Cytotoxicity	cyto	Inactive	0.55
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.97
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.97
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.90
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.90
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.97
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.97
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.97

Figure 5. Toxicity model report α and β-Asarone compound

CONCLUSION

It can be concluded from the research that the α and β-Asarone ligands have an affinity value of -5.65kcal/mol for *Shigella flexneri*. The α and β-Asarone compounds interact at identical amino acid residues as the native ligands and control drugs used through ionic, polar, and aliphatic bonds. The results of the toxicity prediction study show that the asarone group can cause mutagenic effects. Further studies need to be carried out with molecular biology studies and toxicity assay for further evidence.

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