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Potential of Oyster Mushroom (*Pleurotus ostreatus*) secondary metabolites on KRAS protein in-silico liver cancer

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Abstract

Liver Cell Carcinoma (KSH) currently ranks as the fourth most common cause of death due to cancer worldwide and is the sixth most common cancer in the world. HCC (Hepatocellular Carcinoma) is caused by environmental factors, viral infections, and certain genetic changes. Ras is a very important oncogene and is hypermutated in various types of cancer, including HCC. The doxorubicin compound is a curative agent that is widely used for chemotherapy treatment of various types of cancer, however, administration of the kura agent with high cytotoxic activity can cause several side effects. Secondary metabolite compounds have anti-oxidant capabilities including breaking free radical chains and chelating redox-active metal ions which cause lipid peroxidation. These properties also help prevent cancer. *Pleurotus otstreatus* bioactive compounds that have antioxidant potential, flavonol. Due to the potential cytotoxic activity of these secondary metabolites, a bioinformatics approach is needed in this research to determine the results of predicting the interaction activity of flavonol compounds on the KRAS protein in silico and to determine the amino acids and bonds involved in the interaction. between these compounds and the KRAS protein, as a reference for future HCC drugs. The method used is using the PyRx device, Biovia Discovery Studio, Autodock for the docking process and validating the docking results. The results of this research are that flavonol compounds have a docking value of -7.1 kcal/mol. This compound has the potential as a candidate compound that can be used in liver cancer therapy, because it's have high values and close to the positive control value, namely doxorubicin, of -7.6 kcal/mol.

Keywords: Carcinoma cell; Docking; Fungi; Secondary Metabolites

1. Introduction

Liver Cell Carcinoma (KSH) currently ranks as the fourth most common cause of death due to cancer worldwide and is the sixth most common cancer in the world. HCC (Hepatocellular Carcinoma) can be caused by environmental factors, viral infections, and certain genetic changes, Ras protein is a small molecular sequence regulated by guanosine triphosphate, which conveys signals from the cell membrane to the nucleus, and activates several molecular pathways involved in proliferation, transformation, and development of cancer. The Ras protein family includes H-Ras, N-Ras, and K-Ras [1]. The Ras/Mek/Erk pathway is also highly active in 50-100% of HCC [2], higher than Hras and Nras transmission [3]. Ras is a very important oncogene and is hypermutated in various types of cancer, including HCC [2].

The doxorubicin compound is a curative agent that is widely used for the chemotherapy treatment of various types of cancer such as bone sarcoma, breast cancer, ovarian cancer, acute leukemia, Hodgkin lymphoma and small cell lung cancer [4]-[7]. However, treatment with curative agents with high cytotoxic activity can cause several side effects such as hematological toxicity and cancer recurrence, so alternative compounds are needed with high cytotoxic activity against cancer cells with lower toxicity on normal cells [8].

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Secondary metabolites are non-essential compounds having various anti-inflammatory and anti-oxidant activities. These properties also help prevent cancer [9]. One of them is the white oyster mushroom, oyster mushroom (*Pleurotus ostreatus*) which is known to have properties and benefits, including being able to improve the immune system, namely being an immunomodulator. According to [10] and [11], the bioactive compounds possessed by oyster mushrooms (*Pleurotus ostreatus*) are antioxidants, namely phenol (Gallic acid) with a content of 302.95-586.60 mg/g dry weight, flavonoids with a content of 50.79- 77.54 mg/g, sterol according to Souilem et al, 2017 followed by its metabolites namely ergosta-7-enol, ergosta-5,7-dienol, ergosta-7,22-dienol (12.7%, 7.6%, 6%, respectively), ergosterol (20 mg/100 g dry weight) [12].

Due to the potential cytotoxic activity of secondary metabolites in *Pleurotus ostreatus*, a bioinformatics approach is needed in this research because in addition to accelerating analytical results and saving costs, the bioinformatics approach (using PyRx software) provides results with an accuracy of close to 90% on interactions between ligands and proteins [13]. Therefore, in this research, molecular docking will be carried out to determine the potential interaction between metabolite compounds in *Pleurotus ostreatus* and the KRAS protein. So, it can become a reference for future HCC drugs.

2. Methods

2.1. Materials

The equipment used in this research is HP laptop hardware with an Intel Celeron 4205U (1.8 GHz base frequency, 2MB cache, 2 cores) with memory 4 GB DDR4-2133 SDRAM (1x4 GB), Windows 11 pro as the operating system and software Autodock Tools, Biovia Discovery Studio, and PyRx.

2.2. Protein Preparation

The protein used is KRAS (6t5u). The 3D structure of the protein was downloaded in .PDB format from the Protein Data Bank for the KRAS protein. Proteins were further simplified with Biovia Discovery Studio software. Next, select the 'Open' menu and select the protein to be prepared. Protein data is simplified by removing water molecules, then selecting the desired protein chain and removing the native ligand bound to the protein. Then, save it in .pdb format on a computer in the same folder as the previously downloaded ligand. The final step is to change the receptor format to .pdbqt using PyRx software. The receptor is opened by clicking the 'File' menu then 'Load Molecule'. If it is open, then right click on the name of the receptor, then select 'Autodock' then 'Make Makromolecule'

2.3. Ligand Preparation

The ligand preparations used are doxorubicin and flavonol. The 3D structures of doxorubicin and flavonols were obtained from the PubChem page. After finding the desired ligand, click the download button. Ligand data is downloaded in .sdf format.

Ligand preparation begins by importing the ligand compound structure file into the PyRx software and opening the Open Babel software. Add ligands by clicking the plus sign and selecting ligands in .sdf format. Next, right-click on the initial display that shows the ligand and select the "minimize selected" menu to reduce the energy value of the ligand. Then the 'Edit Properties' box appears, click OK. Next, right-click again on the ligand name then select the 'Convert Selected to Autodock (pdbqt)' menu to change the ligand format to .pbdqt.

2.4. Docking Process

The Molecular docking process was carried out with PyRx software. Initially add the prepared protein and ligand data by selecting file then load molecule . Protein data and ligand data that have been prepared are used as input. The docking location (grid box) is adjusted to the size of the ligand that has been prepared by running autogrid and setting it by clicking the 'maximize' option. Next, select 'exhaustiveness' from Vina and enter the number 24 times. Next, run the docking process by selecting the forward option. The results of docking come out in structure predictions and Gibbs free energy values (-) as well as RMSD values. The ligand structure data resulting from docking is then saved in (.pdbqt) format.

2.5. Validation of Docking Results

Validation of docking results is carried out to determine how much the shape of the protein and ligand changes until they can finally form a ligand and protein complex. Validation is carried out by separating the protein structure from

the native ligand that has been bound to the protein, then carrying out a docking process to determine the RMSD value. The RMSD value was obtained from calculations using PyRx software at the end of the docking process.

2.6. Visualization of Docking Results

Visualization of the model resulting from docking of the KRAS protein receptor with the ligand was carried out using Biovia Discovery Studio software to obtain a 3D model of the interaction between the ligand and the receptor and to show the position of the ligand relative to the receptor. Initially, enter the docked protein and ligand files by clicking the 'Open' menu on the 'File' toolbar. Next, right-click on the protein and ligand files resulting from the docking, then select 'Copy' to be transferred to the receptor file. Then right click on the receptor file and select 'Paste'. In the 'view interaction' section, select 'ligand interaction' to view the 3D visualization so that an interaction image appears. In the next step, select 'Show 2D' to see the 2D diagram visualization. All visualizations, both 2D and 3D, are then saved on a computer using the screenshot feature or snipping tool.

3. Results and discussion

3.1. Results of data collection on KRAS protein structures, doxorubicin and flavonols.

KRAS protein data collection with PDB code: 6T5U was carried out by visiting the website www.rcsb.org. Doxorubicin structure with PubChem code: 32874, flavonol compound with PubChem code: 11349 accessed via the website www .pubchem.ncbi.nlm.nih.gov.

3.2. Receptor Preparation

Receptor preparation was successfully carried out using Discovery Studio 2020 software. Receptor preparation was carried out by removing water molecules, ions and ligands, so that these molecules would not interfere with the interaction between target molecules and proteins in AutoDock [14]. The prepared file is then saved in .pdb format. Next, the .pdb format was changed to .pdbqt using PyRx software as a special software format used for molecular docking [15]. The following is the structure of the prepared KRAS protein

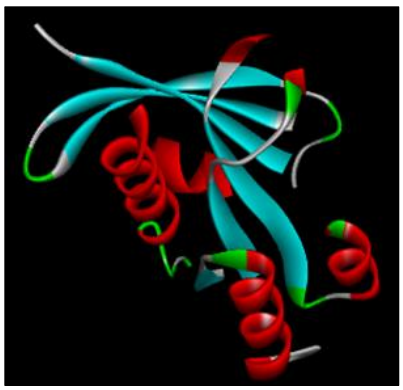

Figure 1 KRAS structure with PDB code 6T5U which has been prepared

3.3. Ligand Preparation

Ligand preparation was successfully carried out by opening the PyRx software then selecting the ligand to be prepared, then selecting minimize all to reduce the energy of the ligand. Reducing the ligand energy serves to provide structural stability to the ligand, because ligand stability is related to low interaction energy [16].

3.4. Molecular Docking with Autodock Vina

Molecular Docking has become an important aspect of in-silico drug development in recent years. This technique involves predicting interactions between small molecules and proteins at the atomic level [17]. The use of the molecular docking method as a screening tool for drug candidate compounds aims to determine the position of the ligand relative to the protein, as well as to predict the strength of the bond between the ligand and the protein. Later, the strength of the bond between the protein and the ligand will be represented by the Gibbs value [18]. Vina provides a parameter called “Exhaustiveness” to change the amount of computational effort used during docking experiments. The default

completeness value is 8; increasing it to around 24 will provide more consistent docking results [19]. The following are the results of molecular docking.

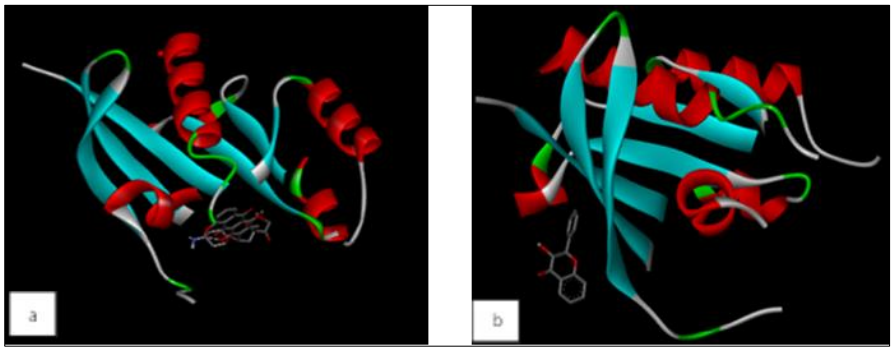

Figure 2 Visualization of docking results a) doxorubicin b) flavonol

Visualization and interaction analysis of docking results were carried out to see the docking results between the reference ligand and the test ligand used [20]. Calculation of protein-ligand binding affinity is very important for the discovery and optimization of compounds based on protein structure [21]. The stability parameter observed is Gibbs free energy (ΔG) or binding affinity. The more negative the ΔG value indicates a good level of stability between the ligand and receptor, so that the bond formed will be stronger [22]. The following are the results of the Gibbs free energy values or binding affinity docking between the KRAS protein and doxorubicin, flavonols.

Table 1 Binding Affinity Result

Binding Affinity Result		
Konformation	**Doxorubicin**	**Flavonol**
1	-7.6	-7.1
2	-7.0	-6.5
3	-6.8	-6.3
4	-6.8	-6.3
5	-6.6	-6.2
6	-6.5	-6.2
7	-6.5	-6.1
8	-6.5	-6.1
9	-6.4	-5.8

Table 2 Selection of the Best Docking Results on the Kras Protein

No.	**Ligand**	**Binding Affinity Result**
1	Doxorubicin	-7.6 Kcal/mol
2	Flavonol	-7.1 Kcal/mol

Based on these results, flavonols have Gibbs free energy or binding affinity values close to the positive control doxorubicin. Therefore, it can be said that flavonol and ergosterol compounds have the potential as alternative compounds in overcoming hepatocellular carcinoma (HCC) because of their ability to bind KRAS protein. The more negative the ΔG value indicates a good level of stability between the ligand and the receptor, and that the bond formed

will be stronger [22]. In addition, the lower the docking score of a compound, the easier the interaction between the compound and the ligand will be achieved and will form a more stable protein and ligand complex [23].

3.5. Visualization of Docking Results using Biovia Discovery Studio 2020

Visualization of docking results was performed using Biovia Discovery Studio 2020 software. The results of the visualization obtained were in the form of interactions between ligands (compounds) and amino acids in protein macromolecules. Amino acid residues that interact with the ligand will determine the type of bond that occurs between the ligand and the protein. The following is an image of the visualization results of amino acids bound to the ligand and target receptor.

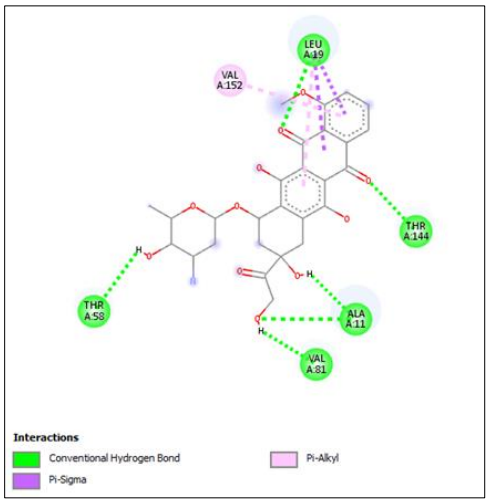

Figure 3 2D visualization results of the interaction between doxorubicin and KRAS protein

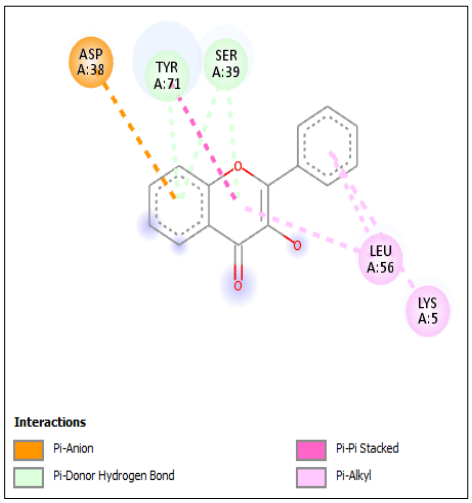

Figure 4 2D visualization results of the interaction between flavonol and KRAS protein

Table 3 Visualization of Amino Acid Ligands And Kras Proteins

No.	Ligand and Reseptor	Binding Site
1.	Doxorubicin and KRAS protein	Leu A19;Val A152; Thr A144; Ala A11 ; Val A81; Thr A58;
2.	Flavonol and KRAS protein	Asp A38; Tyr A71; SerA39; Leu A56; Lys A5

Based on the results of amino acid visualization that has been done through Biovia Discovery Studio software. Amino acids involved in the neuraminidase binding site have a role in binding the functional groups of the docked compounds.

The amino acids that bind to the active site have their own characters where these characters can determine the type of interaction that occurs with the docked compound. Based on the molecular docking of the doxorubicin compound to the KRAS protein, it can bind to the amino acid leucine (Leu A19) through hydrogen bonds, pi-alkyl, and pi-sigma. In addition, through hydrogen bonds it binds to the amino acids threonine (Thr A144; Thr A58), valine (Val A81), alanine (Ala A11), phenylalanine (Phe A82). Flavonol compounds against KRAS protein can bind to the amino acid asparagine (Asp A38) through the Pi-anion bond, tyrosine (Tyr A71) through the Pi-Donor hydrogen bond and Pi-pi Stacked bond, serine (Ser A39) through the Pi-Donor hydrogen bond and through the pi-alkyl bond with the amino acids leucine (Leu A56) and lysine (Lys A5).

In this interaction, the receptor and ligand bind through several types of bonds, each type of bond has its own properties. The following are the functions of each bond that occurs in the molecular docking of the test ligand against the KRAS protein:

- Hydrogen bonds are bonds that occur between hydrogen atoms in one molecule with one of the elements (N, O, F) in another molecule which is the strongest dipole-dipole force. In addition, hydrogen bonds are described as a form of electrostatic interaction between hydrogen atoms bound to electronegative atoms with other electronegative atoms. While in hydrogen bonds there are characteristics of the protons that make up the atoms, namely the dynamic movements of protons in the bond [24].
- Phi-alkyl bonds function to donate the dipole moment of the drug through the transfer of electron charge. The dipole moment is important in the orientation of the molecule when interacting with the binding site [25] [26].
- The π donor hydrogen bond interaction is a hydrogen bond that occurs between the hydrogen bond donor atom and the π ring which functions as a hydrogen bond acceptor [27]
- Pi-pi Stacked as a type of noncovalent interaction that is attractive and non-destructive. Pi-pi Stacked interaction can be used for wide applications such as immobilization, specific recognition, and material construction as long as the material maintains its aromatic groups [28]

Table 4 Validation Of Docking Results

Konformation	**RMSD result**			
	Doxorubicin		**Flavonol**	
	rmsd/ub	**rmsd/lb**	**rmsd/ub**	**rmsd/lb**
1	0.0	0.0	0.0	0.0
2	8.955	2.7	4.07	2.534
3	25.993	24.13	5.976	3.158
4	8.309	2.401	16.536	15.266
5	7.693	2.842	13.491	11.208
6	14.358	10.488	15.71	14.794
7	14.978	11.442	16.582	14.698
8	10.464	5.207	5.68	1.303
9	16.932	12.054	13.409	10.997

Based on the table above, it shows 9 conformations between the KRAS protein and the ligand doxorubicin, flavonol. The first conformation of each docking result is used in the docking process because it has the lowest RMSD scoring value. According to Bitencourt-Ferreira & de Azevedo (2019) an RMSD value <2 Å can be used as a benchmark for the success of a docking method. The lower the RMSD value (<2 Å) indicates the success of the docking process, because the smaller the RMSD value indicates that the predicted ligand binding is better because it is closer to the native ligand conformation [29].

4. Conclusion

Based on the research results, it can be concluded that flavonol compounds have the potential to inhibit liver cancer because they have values close to the positive control value, namely doxorubicin of -7.6 kcal / mol. Amino acids involved in flavonol compounds are asparagine (Asp A38), tyrosine (Tyr A71), serine (Ser A39), leucine (Leu A56), lysine (Lys A5). Based on the RMSD value which has a value of 0.0 for each compound, it indicates that the docking results that have been carried out have valid values, because they have a value of less than 2 Å.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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