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Molecular Docking for Evaluation of Piperine Affinity to the Colon Cancer Receptor

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Abstract.
Piperine is an alkaloid found in the plants of the *Piperaceae* family, such as *Piper nigrum* and *Piper retrofractum*. These two plants are commonly used as flavoring agents in daily meals. The piperine extract is reported to be effective in inhibiting the growth of colon cancer cells in laboratory experiments. Between 35-77% of colon cancer cases are caused by excessive expression of the epidermal growth factor receptor. In developing a new compound for commercial drugs, a study of the compound affinity to the receptor is necessary. Hence, the purpose of this study was to comprehensively evaluate the affinity of piperine to the colon cancer receptor using molecular docking software. The AutoDock software was used as a tool for the analysis. Three compounds were analyzed, i.e., the tested ligand (piperine), a native ligand (N-acetyl-D-glucosamine), and a positive control ligand (Gefitinib). Further, the binding score of piperine to the receptor was compared to the binding score of native ligands and positive control ligands. The binding score of the three compounds was found to be -3.82 kcal/mole (piperine), -4.16 kcal/mole (Gefitinib), and -3.75 kcal/mole (N-acetyl-D-glucosamine). It can therefore be concluded that there is a similar affinity between piperine and the control ligand. Therefore, piperine can be recommended and developed as a new drug for colon cancer treatment.

Keywords: piperine, in silico, colon cancer, molecular docking

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1. Introduction

The number of cancer incidence increased in recent years. National Cancer Institute predicts as many as 1,685,210 new cases of cancer will be diagnosed in the United States, and 595,690 people will die because of the disease. Breast, lung, colon, and prostate cancer are the most common cancers incident predicted [1]. Cancer may be a hereditary disease [2], but incorrect lifestyles such as smoking and consuming instant foods and drinks often increase the incidence of cancer significantly [3–5]. Development of the anticancer drug is urgently conducted to help the patient to fight cancer. Indonesia is rich with biodiversity that can be used for medical treatment. One of the biodiversity is pepper, which is included in the Piperaceae family plant, such as Piper nigrum L and

Piper retrofractum. Phytochemical compounds contained in these plants are piperine alkaloids. The biological activity of piperine has been reported by many researchers, such as increases in the absorption of nutrients in the body, anti-inflammatory, analgesic, anticarcinogenic, antimutagenic, and antitumor [6–8]. Piperine also reported inhibiting the growth of colon cancer cells in laboratory work [9, 10]. Ranged 35-77% of cases of colon cancer were found to be an excessive expression of epidermal growth factor receptor (EGFR) because of carcinogenic stimulation. EGFR is needed during nucleus cells [11]. Malfunctions of EGFR caused cancer. The malfunction can be caused by several conditions, such as mutation, amplification, and excessive expression of EGFR (overexpression) [12]. Based on the previous study showed that piperine potentially developed as a colon cancer therapeutic agent.

In this study, in silico analysis of piperine affinity toward EGFR will be conducted to study the possibility of piperin being a new drug for colon cancer [13–15]. The piperine affinity in the form of a binding score of piperine to EGFR will be compared to the affinity of native ligand (N-Acetyl-D-Glucosamine (NAG 1032)) and the commercial drug for colon cancer that is commonly used, i.e., Gefitinib. The lowest binding score is the best affinity to the receptor [16, 17].

2. Method

Evaluation study of piperine affinity to EGFR was started by developing the piperine compound using an applications Marvin Sketch-512.2 and then stored in the format ".cdx". The native and standard ligand (Gefitinib) were downloaded from the official website or http://www.drugbank.ca or http://pubchem.ncbi.nlm.gov/. File ligands downloaded as a 3D conformation with SDF format. Files that have been downloaded, are opened by using the DS Visualizer. On "show structure in the 3D window" right-click and then click the 3D image is then stored with the file name of the ligand in the form of GDP ((* .pdb). The next step was preparing the protein target. In this study, the protein targets were EGFR with PDB ID 1IVO (the responsible protein for colon cancer).

The receptor target was downloaded from the protein data bank (www.rcsb.org). Further, the elimination of the nuisance components was then conducted for files downloaded so that only the remaining protein targets using Discovery Studio Visualizer applications. The preparation result was then stored in a PDB format file (*.pdb). Using the Auto-DockTools application, the polar hydrogen atom was added to the protein targets to give partial load (partial charges) to the target protein. Also, the protein target was necessary to add the charge through Kollman Charges. After that, the protein target

file was saved in the format of *.pdbqt. File from previous preparation that includes Target.pdbqt, Ligan.pdbqt, grid parameter file (*.gpf), and a docking parameter file (*.dpf) were stored in a single folder on Cygwin Terminal. The docking process was done using Autogrid Autodock 4.2 and 4.2 through the Cygwin terminal. Autodock applications were conducted in the Linux setting. The docking was performed by pressing ”CTRL + ALT + T,” then typing on a terminal ADT command and pressing enter. The evaluation was based on the docking score of each compound. The binding score of piperine to the receptor compared to the binding score of native ligand and positive control ligand.

3. Result and Discussion

Commercially, Gefitinib is used for the treatment of colon cancer. With the rise of cancer cases in the latest decade, developing a cancer drug is necessary to conduct. One of the methods of developing a new drug was done by applying a natural product for treatment. One of the natural products that have been studied for colon cancer treatment is pepper which is commonly added to the food for daily meals. Inside the pepper containing piperine. As reported by Kim et al (2010) and Yaffe et al (2015), Piperine is effective to inhibit the growth of colon cancer cells in laboratory work [9, 10]. To know how potential piperine able to be an alternative compound for colon cancer treatment, besides a laboratory experiment that had been conducted by Kim et al. and Yaffe et al., the affinity of piperine toward responsible protein targeting for colon cancer is also necessary to be evaluated. The responsible protein target for colon cancer was reported to be the epidermal growth factor receptor (EGFR) [12]. Ranged 37%-77% of colon cancer cases were reported because of the excessive expression of the EGFR [18, 19]. The affinity of Piperine toward colon cancer receptors can be evaluated using a simple method called in silico molecular docking method. In this research, in silico evaluation was using AutoDock Tools. To determine the active site of EGFR, the visualization of the target protein using the DS Visualizer was conducted. The Visualization results of EGFR can be seen in Figure 3. The yellow color in Figure 3 shows the active binding site of the receptor target.

N-Acetyl-D-Glucosamine is the native ligand (compound) for EGFR. Whereas Gefitinib is an EGFR inhibitor that is commercially used for colon cancer. Gefitinib response is dependent on EGFR mutation. Gefitinib prevents phosphorylation, thereby inhibiting downstream signaling and blocking EGFR-dependent proliferation [20]. The visualization of the three tested ligands is shown in Table 1.

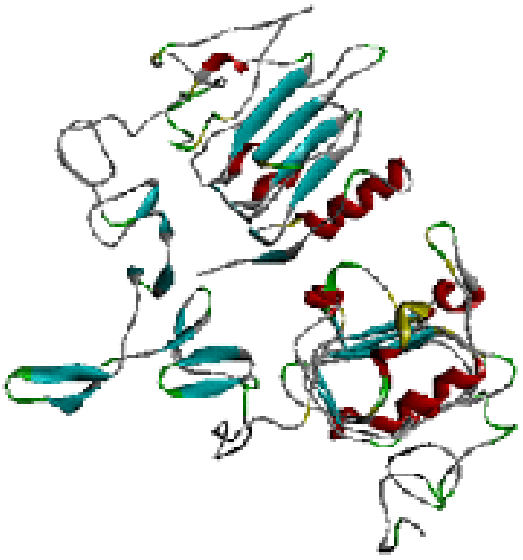


Figure 1: Visualization of the active site of EGFR (1IVO).

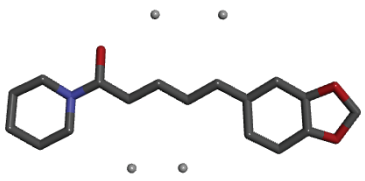
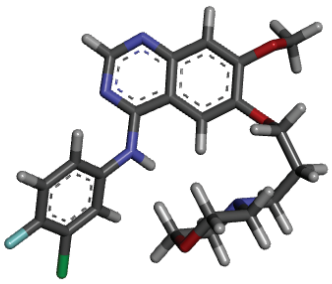
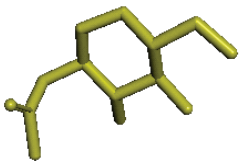
No	*Ligand*	*3D structure*
١	Piperine	
٢	Gefitinib	
٣	N-Acetyl-D-Glucosamine (NAG ١٠٣٢)	

Figure 2: The binding energy and interaction Ligand-receptor.

The docking process of the prepared receptor target (EGFR) and ligands produces ten (10) conformations with information on binding energy for each conformation. Based on the binding energy, the evaluation of the best conformation can be conducted. The best affinity is shown from the lowest binding energy for each conformation. The best

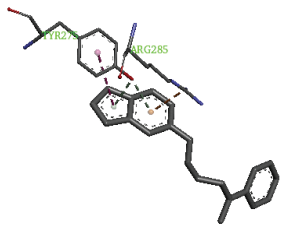
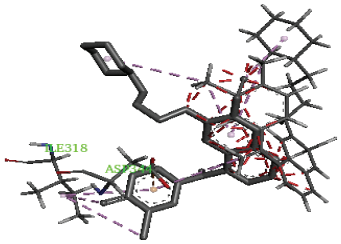
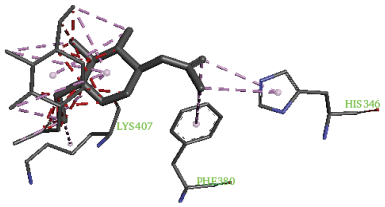
Ligand	*Bounding Energy (Kcal/mole)*	*Interaction*
Piperine	-٣,٨٢	
Gefitinib	-٤,١٦	
N-Acetyl-D-Glucosamine (NAG ١٠٣٢)	-٣,٧٥	

Figure 3: THE BINDING ENERGY AND INTERACTION LIGAND-RECEPTOR.

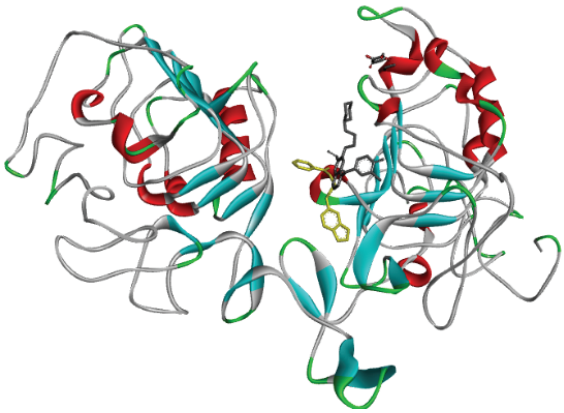


Figure 4: The overlap interaction of piperine, gefitinib, and NAG.

binding energy and the interaction between each ligand to the receptor target can be seen in Table 2.

Interaction of piperine and EGFR was done by binding residue Tyrosine and Arginine to the active site of piperine. The interaction of Gefitinib to EGFR was done by binding the active site of gefitinib to residues of EGFR, i.e. asparagine and isoleucine. The

interaction of the native ligand (NAG) gave the lowest binding energy. This may be due to the NAG interacting with the more active site of EGFR compared to the other two ligands tested. NAG bonded to lysine, phenylalanine, and histidine residues of EGFR. Overlap visualization of the interaction of the three ligands (compounds) tested toward the EGFR can be seen in Figure ??. It can be seen that the three ligands tested bind the EGFR at a similar area of the active site of EGFR with similar binding energy. Therefore, it may be concluded that piperine is a possible compound to be proposed as an alternative drug for colon cancer. Chemical structure modification is may possible to conduct to increase the affinity of piperine toward the EGFR.

4. Conclusion

Based on in silico analysis, the affinity of piperine toward EGFR is similar to the affinity of Gefitinib and NAG toward EGFR. The binding energy of piperine toward EGFR was found to be -3.82 kcal/mol, which is similar to the binding energy of commercially anticancer colon drugs i.e., gefitinib was found to be -4.16 kcal/mol. The structure modification may conduct to increase the affinity of piperine toward EGFR.

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