In silico analysis of kaempferol as a competitor of estrogen on estrogen receptor alpha of endometrial cancer

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Abstract. In-silico study of kaempferol has been performed to determine its potential as a natural estrogen competitor in an effort to suppress the growth of endometrial cancer. This study used a method of molecular decelerating of kaempferol against estrogen receptor alpha, which is compared with natural estrogen in the body. The research process was conducted at the Laboratory of Biology Department of Science and Technology Faculty of UIN Sunan Gunung Djati Bandung, from December 2017 until February 2018. The software used in this study is PyRx-Virtual Screening Tools (for molecular docking process) and Discovery Studio (for pose visualization and data analysis). The results showed that kaempferol is a potential natural estrogen competitor. It is indicated by the lower binding affinity value of kaempferol (−7.0 kcal/mol) than the binding affinity of natural estrogen (−6.7 kcal/mol). The low-affinity binding value shows a stable interaction. This means that Kaempferol is a potential phytoestrogen for cancer therapy without side effects.

1. Introduction

Endometrial cancer is a gynaecologic malignant tumour that often occurs in women. Endometrial cancer begins when cells in the inner lining of the uterus (endometrium) grow uncontrollably. According to Cancer Research UK, 73% of these diseases are diagnosed in women of middle age (40 to 70 years of age) and about 25% are diagnosed at age of 75 or older. Age-related rates are increasing in countries where an economic transition from low to high happens [1]. One of the factors that cause endometrial cancer is estrogen uses in hormone therapies. Estrogen is used without progesterone combination in hormone therapy for contraception [2]. Estrogen initiates the growth of endometrial cancer cells through estrogen receptor Alpha (REα) [3]. Another estrogen use is hormone therapy with tamoxifen. Tamoxifen is used in treatments of breast and endometrial cancer. Tamoxifen is an artificial estrogen that acts as an anti-estrogen at the binding of REα and blocks its signalling pathway in cancer cells [4]. Unfortunately, in practice, the use of tamoxifen has a side effect. It triggers other endometrial cancer. Therefore, a natural estrogen (phytoestrogen) is required for hormone therapies.

Phytoestrogens are the right choice for hormone therapy. One of the phytoestrogen types assumed to have pharmacological activities of anticancer is kaempferol. Kaempferol is a type of flavonoid. Vega et al reported that they have isolated kaempferol and tested its activity as an inhibitor of cancer cell growth in Erlich carcinoma [5]. Likewise, Yoshida et al reported that the treatment of cancer cells with
kaempferol can suppress cancer cell populations with low effects on normal cells and can strengthen the toxic effects of chemotherapy to fight against cancer cells [6]. Inspired by the two previous reports, a study of kaempferol in suppressing endometrial cancer cells is necessary.

In-Silico study is a simulation method in an attempt to explain the pharmacological potential of a compound. One of the most commonly used methods is molecular docking [7]. This method is very effective to explain the pharmacological potential of a compound [8]. Therefore, in-silico study with molecular docking method is chosen to investigate the potential of kaempferol as an estrogen competitor that can bind to estrogen receptor alpha.

2. Material and methodology

2.1. Tools and materials

2.1.1. Tools. The required tools consist of hardware and software. The hardware involved Intel Celeron N3050 with HD graphics, 2 GB RAM, 64-bit operating system, and 500 GB Hard disk. The used software are PyRx-AutoDock Vina and Discovery studio.

2.1.2. Material. The material comprises a three-dimensional structure of estrogen receptor alpha (REα) with the code of 3DT3, which is downloaded from the website of protein data bank http://www.rcsb.org/pdb/explore/explore.do/structureId=3DT3 and a three-dimensional structure of kaempferol and 17β-estradiol (natural estrogen for control experiment), downloaded from the site of databank zinc.docking.org. The three-dimensional structure of kaempferol is in the form of .mol2 code ZINC03869768 and three-dimensional structure 17β-estradiol is in the form of .mol2 code ZINC03815415.

2.2. Procedure

2.2.1. Protein preparation. The REα protein is separated by water and ligand molecules with Discovery Studio version 4.5. Then, it is stored in a .pdb format. Next, with Autodock Vina 4.2, the hydrogen atom of the protein is displayed and stored in a .pdbqt format.

2.2.2. Gridbox determination. Determination of Gridbox of kaempferol is at the receptor site (REα) with the centre X: 39.3698, Y: -0.7673, Z: 14.1718 and the large dimension with the angstrom X: 25.00, Y: 25.00, Z: 25.00. Determination of 17β-estradiol ligand grid box is at the location of protein receptors with the centre 42.3941, Y: 1.5089, Z: 16.8038 and the large dimensions with the angstrom X: 25.00, Y: 25.00, Z: 25.00.

2.2.3. Molecular docking. Molecular docking in this study is docking- oriented because it determines the grid box as a target of molecular docking. Requirements for the docking with AutoDock Vina are that ligands and receptors must be in a .pdbqt format. There are grid box and configuration. The configuration must include receptor names, ligand names, file out with a .pdbqt format, a grid centre, and a grid size. The structural data needs to change from a .pdb extension format to a .pdbqt format. The .pdb format in the ligand and receptor shows no charge on the molecule, whereas the .pdbqt format indicates a partial charge on each atom.

2.2.4. Visualization and conformation analysis. Visualization of kaempferol poses and 17β-estradiol on receptors (REα) uses Discovery Studio software version 4.5.

3. Result and discussion

Analysis of molecular docking results was conducted on the resulted energy of binding affinity, the Root Mean Square Deviation (RMSD) value, the amount and type of interactions between the ligand with the amino acid in REα. The molecular docking process produces nine ligand-receptor conformations. The
best of the nine conformations is selected based on the lowest RMSD value. RMSD is the value of deviation between the moored ligand conformations of the x-ray ligand.

3.1. Visualization of conformation of docking result

In Figure 1 is the positions of kaempferol (blue) and 17β-estradiol (red) are on the same adhesive side of REα. This shows that both compounds have the same potential to fill the active side of REα. Based on figure kaempferol will be a strong competitor compound for estrogen.

The following is a clear visualization of the conformation of each ligand (kaempferol and 17β-Estradiol with REα).
Figure 2 shows a formed bond between the kaempferol and the amino acid residues of REα, namely Leusin 354, Aspartic Acid 351, Alanine 350, Triptofan 383, Leusin 536, Leusin 525, Tyrosin 526 and Aspartic Acid 532.

Figure 3 shows the 17β-estradiol pose in the REα bonding sac, showing the interaction of 17β-estradiol compounds with Leucine 536, Triptofan 383, Tyrosin 526 and Leusin 525 in REα.

The molecular docking result produces an affinity binding value to determine the conformation quality. The following table illustrates a comparison of the binding affinity values of both molecules resulted in the molecular docking.

| Ligand       | Binding Affinity (Kkal/mol) |
|--------------|----------------------------|
| Kaempferol   | -7.0                       |
| 17β-Estradiol| -6.7                       |

Table 1 shows that kaempferol has a low affinity binding value of -7.0 kcal / mol. This value is lower than the natural estrogen (17β-estradiol) with -6.7 kcal / mol. accordingly, the caemferol has a more stable bond to REα than the natural estrogen. This value is supported by the number of interactions formed between the ligand and REα. The interactions are illustrated in Table 2.

| Compound Name | Hydrogen Bond | Hydrophobic Interaction | Electrostatic Interaction |
|---------------|---------------|-------------------------|----------------------------|
| Kaempferol    | 2             | 6                       | 1                          |
| 17β-estradiol | 1             | 4                       | 0                          |

According to Table 2, the taempferol-REα conformation has a greater number of interaction than the 17β-estradiol-REα conformation. This supports the potential of kaemferol to be a natural estrogen-competitor.

4. Conclusion
The kaempferol can become an estrogen competitor to replace the role of 17βestradiol ligand (endogenous estrogen) to bind to REα. The lower affinity-binding value means the more stable interaction due to the stronger binding to REα, as a result it potentially plays as an estrogen competitor.
in Reα. Multiple molecular dynamics are required to know the stability of more detailed interactions in the body. In-vitro and in-vivo tests are necessary to determine the activity of kaempferol as anti-endometrial cancer.

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