**The potency of alpha-humulene as HER-2 inhibitor by molecular docking**

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**Abstract:** HER-2 overexpression is present in approximately 20% of breast cancer. This research aims to study the interactions of alpha-humulene to HER-2 protein by using *in silico* molecular docking. The experiment was carried out by HER-2 protein preparation (PDB ID 3PP0), docking validation, alpha-humulene optimization, and alpha-humulene docking. The results showed that alpha-humulene had binding energy of -7.50 kcal/mol, Van der Waals binding energy of -7.48 kcal/mol, and electrostatic energy of -0.02 kcal/mol. Alpha-humulene is potential as anti-breast cancer towards HER-2 *in silico*.

**Keywords:** alpha-humulene, anti-breast cancer, HER-2, in silico, molecular docking

**Introduction**

Breast cancer is a malignant tumor in the mammary gland tissue originating from the ductal epithelium or its lobules [1]. About 20% of breast cancer cases are caused by overexpression of HER-2 [2]. The human epidermal growth factor (HER-2) plays a role in proliferation, migration, cell survival and growth [3]. HER-2 overexpression is associated with more aggressive disease, a higher recurrence rate, and shortened survival. In addition, HER-2+ breast cancer has a higher preference for metastasis to the brain [4].

Breast cancer is generally treated by chemotherapy, surgery, radiotherapy, or combination. However, this therapy has several side effects, such as hair loss and drug resistance [5]. Hair loss after chemotherapy is caused by an unspecified drug target [6]. Conventional chemotherapy kills both cancer cells and normal cells. Therefore, it is urgent to develop anticancer drugs that kill cancer cells specifically. One of the drug sources is natural ingredients.

alpha-Humulene is a monocyclic sesquiterpene that is commonly found in the Zingiberaceae family (Figure 1) [7]. Extracts from Zingiberaceae were tested on MCF-7 breast cancer and HT-29 colon cancer cell lines and produced the small $IC_{50}$ value from the 11 plants of the Zingiberaceae group [8]. However, no study was found to elucidate alpha-humulene as anti-breast cancer *in silico*. Molecular docking is a computational simulation to predict the binding between a ligand and a protein, in which ligand is docked to the active site of the receptor [9]. This research aims to study alpha-humulene activity as anti-breast cancer by inhibiting HER-2 *in silico*.

**Methods**

**Protein preparation**

The HER-2 protein (PDB ID: 3PP0) was downloaded from [http://www.rcsb.org/](http://www.rcsb.org/). This target protein was prepared using Chimera 1.11.1 by separating the protein sequence from 03Q native ligand.

**Validation of docking**

The docking method was validated using the Autodock Tools application (Autodock 4.2 and Autogrid) by redocking 2-{2-[4-((5-chloro-6-[3-(trifluoromethyl) phenoxy]pyridine-3-yl)amino)-5H-pyrrolo[3,2-d] pyrimidin-5-yl]ethoxy}ethanol (03Q) native ligand to the prepared HER-2 protein. The grid box was arranged by adjusting the coordinate size of the grid center (X = 16.387 Å, Y = 17.394 Å, Z = 26.218 Å) and grid size (X = 40 Å, Y = 40 Å, Z = 40 Å). The validation parameter of docking protocol was the value of root mean square deviation (RMSD), which is valid if the value of RMSD is less than ≤ 2.0 Å.

**alpha-Humulene structure optimization**

The downloaded 3D structure of alpha-humulene was optimized using HyperChem 8. The optimization

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**Pharmacy Reports**  
https://doi.org/10.51511/pr.19
step was carried out using the AM1 (Austin Model 1) semi-empirical computational method and single-point calculations and geometry optimization.

**Docking α-humulene to the HER-2 protein target**

The optimized α-humulene was then docked to the prepared HER-2 target protein using the Autodock 4.2 program. The results gave α-humulene conformation with the lowest binding energy to the target protein. The interaction analysis showed the types of bindings such as hydrogen bonds, Van der Waals, hydrophobic, and electrostatics.

**Results**

**Preparation of HER-2 protein target**

The preparation of HER-2 target protein provided and the protein and 03Q native ligand (Figure 2).

**Docking validation**

The validation parameter of the docking protocol was the RMSD value. The obtained RMSD value was 0.62Å which is valid (< 2.0 Å). This validation also produced the binding energy between HER-2 target protein and 03Q native ligand. The binding energy of the selected conformation with the lowest RMSD value was -13.57 kcal/mol for Van der Waals, -0.01 kcal/mol for electrostatic, and -10.60 kcal/mol for free binding energy (Table 1). The visualization indicated the hydrogen bonding occurs between 03Q native ligand with MET 801 residue (Figure 3).

**Optimization of α-humulene**

The 3-dimensional structure (3D) of α-humulene was optimized using single-point calculations and geometry optimizations. The single point energy calculation of
α-humulene was -3800.11 kcal/mol, and the energy of geometric optimization was -4497.19 kcal/mol. The 3D structures of single-point and geometry optimization of α-humulene are displayed in Figure 4.

Docking α-humulene to HER-2 target protein

The docking process yielded ten bond conformations between α-humulene and HER-2 protein. The most stable conformation of α-humulene with the lowest binding energy had the Van der Waals and hydrophobic energy of -7.48 kcal/mol, electrostatic energy of -0.02 kcal/mol, and binding energy of -7.50 kcal/mol (Table 2). Visualization analysis showed that α-humulene did not form a hydrogen bonding with HER-2 protein target through MET 801 residue as 03Q native ligand.

Discussion

The docking protocol was valid, as shown by the RMSD of 0.62 Å (≤ 2.0 Å). The docking results showed that the binding energy of α-humulene to HER-2 protein was -7.50 kcal/mol, higher than 03Q native ligand with -10.60 kcal/mol. This finding implies that α-humulene is potential for HER-2 protein as indicated by the negative value of binding energy.

α-Humulene is one of the sesquiterpenes known for its anti-cancer activity. In silico study of eugenol sesquiterpenes against HER-2 protein obtained the binding energy value of -4.16 kcal/mol, higher than gefitinib with -7.05 kcal/mol as a control [10]. In vitro study indicated that α-humulene had a cytotoxic activity with an IC₅₀ of 81.9 μg/mL against the MCF-7 breast cancer cell line [11].
Conclusion

Based on this study, α-humulene has the potential as an anti-breast cancer agent by in silico through the inhibition of the HER-2 protein.

Acknowledgment

None.

Funding

The authors thank to Direktorat Jenderal Pembelajaran dan Kemahasiswaan Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia funded for this research in the Program Kreativitas Mahasiswa 2021 (grant number 015/E2/PPK/SPPK/PKM/2021).

Declaration of interest

The authors declare no competing interests.

Author contributions

IMHP conceptualized the study design; IMHP and IPAACP investigated the data; KDAP and GADP reviewed and edited the final version; IMHP acquitted the funding; NPLL supervised all experiments. All authors read and approved the final manuscript.

Received: 25 September 2021
Accepted: 10 December 2021
Published online: 12 January 2022

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