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 unspecific 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.

α-Humulene is a monomeric 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 IC50 value from the 11 plants of the Zingiberaceae group [8]. However, no study was found to elucidate α-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 α-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/. 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.

α-Humulene structure optimization

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

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**Table 1. Validation parameters of HER-2 target protein and 03Q native ligand**

| Protein target | Ligand         | Conformations | RMSD (Å) | Energy Vdw, Hb. desolv (kcal/mol) | Energy elec (kcal/mol) | Binding energy (kcal/mol) |
|----------------|----------------|---------------|----------|-----------------------------------|------------------------|--------------------------|
| HER-2          | 03Q native ligand | 1*            | 0.62     | -13.57                            | -0.01                  | -10.60                   |
|                |                | 2             | 0.71     | -12.74                            | -0.02                  | -10.50                   |
|                |                | 3             | 0.72     | -13.13                            | -0.09                  | -10.49                   |
|                |                | 4             | 1.18     | -13.22                            | -0.04                  | -10.28                   |
|                |                | 5             | 0.69     | -13.43                            | -0.04                  | -10.24                   |
|                |                | 6             | 2.59     | -11.92                            | -0.03                  | -9.78                    |
|                |                | 7             | 2.76     | -10.92                            | +0.03                  | -9.74                    |
|                |                | 8             | 3.53     | -13.49                            | +0.01                  | -8.97                    |
|                |                | 9             | 2.61     | -11.25                            | +0.02                  | -8.24                    |
|                |                | 10            | 2.51     | -12.71                            | -0.01                  | -7.90                    |
In silico molecular docking of alpha-humulene as HER-2 inhibitor

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 IC_{50} of 81.9 μg/mL against the MCF-7 breast cancer cell line [11].

| Protein target | Ligand | Conformations | Energy Vdw.Hb. desolv (kcal/mol) | Energy elec (kcal/mol) | Binding energy (kcal/mol) |
|----------------|--------|---------------|-------------------------------|------------------------|--------------------------|
| HER-2          | α-humulene | 1*            | -7.48                         | -0.02                  | -7.50                    |
|                |        | 2             | -6.39                         | -0.00                  | -6.39                    |
|                |        | 3             | -6.39                         | +0.01                  | -6.38                    |
|                |        | 4             | -7.31                         | -0.02                  | -7.33                    |
|                |        | 5             | -6.39                         | +0.01                  | -6.38                    |
|                |        | 6             | -6.39                         | +0.01                  | -6.38                    |
|                |        | 7             | -6.39                         | -0.00                  | -6.39                    |
|                |        | 8             | -6.39                         | +0.01                  | -6.38                    |
|                |        | 9             | -6.39                         | -0.00                  | -6.39                    |
|                |        | 10            | -6.39                         | -0.00                  | -6.39                    |
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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.

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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.

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