Bioinformatics Study of Sea Cucumber Peptides as Anti-breast Cancer Through Inhibiting the Activity of Overexpressed Protein (EGFR, PI3K, AKT1, and CDK4)

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ABSTRACT: Breast cancer is the most common type of cancer in women globally. The overexpressed proteins, including EGFR, PI3K, AKT1, and CDK4, have a role in the growth of breast cancer cells. The 3D peptide structure of sea cucumber Cucumaria frondosa was modeled and then docked with EGFR, PI3K, AKT1, and CDK4 proteins using AutoDock Vina software. The docking result, which has the best binding affinity value, is continued with molecular dynamics simulation. The docking results showed that all peptides bind to the active sites of the four proteins. WPPNYQW and YDWRF peptides bind to proteins with lower binding affinity values than positive controls. The four proteins were in a stable state when complexed with the WPPNYQW peptide, which was seen from the RMSD and RMSF value. PI3K-YDWRF and AKT1-YDWRF complexes are stable, characterized by high RMSD values and increased volatility in several amino acids. WPPNYQW peptide has high potential as an anti-breast cancer agent because it binds to the active sites of the four proteins with low binding affinity values and stable interactions. Meanwhile, the YDWRF peptide interacts with the four proteins with low binding affinity values, but the interaction is only stable on PI3K and AKT1 proteins.

KEYWORDS: Sea cucumbers, peptide, EGFR, PI3K, AKT1, CDK4

Introduction

Breast cancer is the most common type of cancer in women worldwide, followed by cervical cancer and ovarian cancer.\textsuperscript{1} In 2019, there were approximately 268,600 cases of breast cancer, and 82% of patients were found to have breast cancers aged less than 50 years, with a mortality rate as high as 90%.\textsuperscript{2} The highest mortality is found in developing countries due to the lack of resources for proper diagnosis and treatment.\textsuperscript{3} In addition, cancer treatment methods, such as chemotherapy, also have many adverse side effects for the skin, hair, bone marrow, blood, gastrointestinal tract, and kidneys. They can even cause chronic side effects such as drug resistance, carcinogenicity, and infertility.\textsuperscript{4} Therefore, an alternative breast cancer therapy that is cheaper and has minimal side effects is needed.

Marine life comprises thousands of bioactive metabolites to be explored for anticancer.\textsuperscript{5} Sea cucumbers are marine invertebrates that have a high potential to be used as an antinbreast cancer agent. Previous studies reported that sea cucumber (Holothuria tubulosa) coelom fluid extract could inhibit the growth of triple-negative breast cancer cells (MDA-MB231) by disrupting the cell cycle and inducing autophagy.\textsuperscript{6} Sea cucumber extract TBL-12 can inhibit PCa cell (prostate cancer cell line) proliferation and metastasis and can induce apoptosis through the intrinsic apoptosis pathway.\textsuperscript{7} Holothuria parva methanol extract induces apoptosis by releasing cytochrome c from mitochondria and by inducing caspase-3 activation in mouse hepatocellular carcinoma models.\textsuperscript{8} The anticancer activity of sea cucumbers is thought to be caused by the presence of anticancer compounds such as holothurin A (HA), frondoside A, 24-dehydroechinoside A (DHEA), frondanol A5, okhtosides B,\textsuperscript{9} colochiosides A, cucumariosides A2–2, ds-echinoside A, echinoside A, glycosides 1 & 2, intercedensides A, B, and C, philinopside A, philinopside E, stichoposide C, and stichopo- side D.\textsuperscript{10} In previous studies, these compounds were shown to have anticancer activity by various mechanisms. For example, frondoside A from Cucumaria frondosa can induce apoptosis by activating p53 and significantly increasing caspase-3/7 and caspase-9 activity in ER-MDA-MB-231 breast cancer cells.\textsuperscript{11} Holothurin A and 24-glycosides isolated from Pearsonothuria graeffei inhibited HepG2s cell metastasis by suppressing Matrix Metalloproteinase-9 (MMP-9) and Nuclear Factor-kappaB (NF-kB) expression and by inducing Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) expression.\textsuperscript{12} However, there has been no research on peptides from sea cucumber as an anticancer agent. Peptides have several advantages as an alternative to anticancer drugs compared to compounds, eg, they have low toxicity, have multiple targets, and do not accumulate tissues because they are quickly metabolized.\textsuperscript{13} Therefore, peptides can be used as an excellent alternative to anticancer drugs. In this study, peptides from sea cucumbers (C. frondosa) are tested to inhibit the proteins causing breast cancer growth and development, namely, Epidermal Growth...
EGFR, PI3K, AKT1, and CDK4 are proteins having a role in the growth of breast cancer cells. EGFR is a receptor family of tyrosine kinase receptors with a role in regulating cell growth and survival. In an in vivo study, overexpression of EGFR led to the transformation of mouse cells into malignant cells and increased proliferation and resistance to apoptosis. EGFR is overexpressed in about 14% of breast cancer cases due to the amplification of the EGFR gene. One of the strategies to inhibit the activity of this protein is to inhibit its phosphorylation activity. Lapatinib and gefitinib are compounds that have been shown to inhibit EGFR phosphorylation activity. PI3K is a central protein in the PI3K/AKT/mTOR pathway regulating cell growth and proliferation. In most breast cancer cases, the PI3K gene undergoes mutations or amplifications, resulting in the PI3K/AKT/mTOR pathway. One strategy to inhibit the activity of this protein is to inhibit its phosphorylation activity using the wortmannin compound that binds to the ATP binding pocket of PI3K. Apart from PI3K, AKT1 also has a central role in the PI3K/AKT/mTOR pathway. AKT1 plays a role in cell proliferation, metabolism, and growth and is overexpressed and overactivated in various types of breast cancer cells. Therefore, different inhibitor compounds were developed to stop the activity of this protein. CDK4 plays a crucial role in breast cancer cell growth and is often overexpressed. Cyclin D1 protein is also overexpressed in most breast cancer cells and requires CDK4 to induce breast cancer growth. Therefore, inhibiting CDK4 activity is one of the best strategies for inhibiting breast cancer growth.

A number of researches have been done on the compounds from sea cucumbers that can potentially be a breast cancer drug. However, there is still no research on the peptide’s potential of sea cucumbers as antitumor agents. This study presents the potential of sea cucumber peptides in inhibiting the growth of breast cancer cells by inhibiting the activity of the EGFR, PI3K, AKT1, and CDK4 proteins using a bioinformatics approach. This study aims to find and explain peptides’ potential in sea cucumber (C. frondosa) in inhibiting the activity of breast cancer-related proteins.

### Methods

#### Breast cancer-related protein preparations

Breast cancer-related proteins were determined with the help of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.genome.jp/kegg/pathway.html). The 3D structure of the protein kinase domain EGFR (1XKK), PI3K (1E90), AKT1 (6HHF), and CDK4 (2W9Z) was obtained from the RCSB PDB database (https://www.rcsb.org/). Proteins were prepared by removing water molecules and ligand contaminants using BIOVIA Discovery Studio 2019 software (Dassault Systèmes BIOVIA, San Diego, California, USA).

#### Peptide preparation

The peptides contained in sea cucumbers (C. frondosa) are WPPNYQW, YDWFRE, EMFWR, EEEALALVDNGSGMCK, KMLWK, MMSLHL, RMCCCSPLK, TEFHLL, VELWR, VMLGMLWTLLLRT, WNWKVL, and WNWKV. Peptides structure were predicted using PEP-FOLD (web-server modeled://263bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/). The peptides were then prepared using PyRx 0.8 software by minimizing the conformational energy.

#### Inhibitor preparation as positive controls

The 3D structure of the inhibitor compounds of each protein in the form of gefitinib (CID: 123631), wortmannin (CID: 312145), AZD5363 (CID: 2522436), and abemaciclib (CID: 46220502) was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The inhibitors were prepared using PyRx 0.8 software by minimizing the conformational energy.

#### Molecular docking

Protein-ligand docking was performed using AutoDock Vina software integrated with PyRx 0.8. The docking result with a lower binding affinity value than the inhibitor was taken to be analyzed and continued with molecular dynamics simulations. Visualization of docking results was carried out using BIOVIA Discovery Studio 2019 by looking at the protein’s ligand-binding position.

#### Molecular dynamics simulation

The peptides with the lowest binding affinity value and inhibitor as a positive control continued with molecular dynamics simulation using Yet Another Scientific Artificial Reality Application (YASARA) software. The parameters used were according to cell physiological conditions (37°C, 1 atm, pH 7.4, 0.9% salt content) for 20 ns, which are autosaved every 25 ps. 20 ns is the duration commonly used to determine the stability of the protein-ligand complex. The simulation was run using the md_run macro program, and the results were displayed using the md_analyze, md_analyzeres, and md_analyzebindenergy macro program.

### Results

#### Molecular docking results

The molecular docking simulation aims to obtain peptides that bind to the protein’s active site and have a low binding affinity. The peptide has a high potential to inhibit the activity of the target protein. The results of protein-peptide docking
show that all peptides (WPPNYQW, YDWRF, EMEWR, EEELAALVLDNGSMCK, KMLWK, MMSLHL, RMCCCSPLK, TEFHLL, VELWR, VMLGMLWTLRL, WNWKL, and WNWKV) interact on the active site of the protein (Figure 1). Besides, WPPNYQW and YDWRF peptides always have lower binding affinity than inhibitors (Table 1). This result shows that the two peptides have high potential as inhibitors of EGFR, PI3K, AKT1, and CDK4 proteins because they bind strongly to these proteins’ active sites, more potent than the inhibitors.

Based on docking results, the WPPNYQW and YDWRF peptides are shown to have high potential as EGFR, PI3K, AKT1, and CDK4 inhibitors because they bind to the ATP binding pocket and have lower binding affinity values than control inhibitors (Figure 2 and Table 2).

The docking results showed that all peptides bind to EGFR in the ATP binding pocket and gefitinib as an inhibitor. Besides, two peptides have a lower binding affinity than gefitinib, namely, WPPNYQW of -9.5 kcal/mol and YDWRF of -9.3 kcal/mol. These two peptides bind together with the amino acid residue Lys745, which plays an important role in binding ATP. Therefore, these two peptides block ATP from binding to EGFR in the ATP binding pocket. Hydrogen bonds dominate protein-peptide interactions. WPPNYQW peptides form chemical interactions consisting of eleven hydrogen bonds (Ala722, Lys745, Met793, Asp837, Met793, Asp855, Ala743, Leu788, Gly721, Thr854, and Leu718) and seven hydrophobic interactions (Met766, Phe856, Leu718, Trp880, Lys879, Leu858, and Leu777). Meanwhile, YDWRF peptide forms eight hydrogen bonds (Cys797, Tyr998, Arg841,....

Table 1. Peptide docking results.

| LIGAND                        | BINDING AFFINITY (KCAL/MOL) |
|-------------------------------|-----------------------------|
|                               | EGFR | PI3K | AKT1 | CDK4 |
| Inhibitor (positive control)  | -8.2  | -9   | -9.8 | -9   |
| WPPNYQW                      | -9.5  | -10.6| -11.3| -9.4 |
| YDWRF                        | -9.3  | -9.4 | -10.7| -9.3 |
| EMEWR                        | -8.3  | -8.2 | -9.4 | -7.4 |
| EEEAALVLDNGSMCK              | -6.1  | -6.8 | -6.1 | -5.7 |
| KMLWK                        | -7.8  | -7.5 | -8.9 | -6.7 |
| MMSLHL                       | -6.8  | -7.9 | -8.5 | -7.1 |
| RMCCCSPLK                    | -7.2  | -6.9 | -7.6 | -5.3 |
| TEFHLL                       | -8.3  | -8.4 | -9.5 | -7.6 |
| VELWR                        | -8.5  | -8.3 | -8.9 | -8   |
| VMLGMLWTLRL                 | -4.7  | -5.3 | -8.1 | -8   |
| WNWKL                        | -8.7  | -8.9 | -8   | -9.2 |
| WNWKV                        | -9.5  | -9   | -8.5 | -8.9 |

Abbreviations: Akt1, Protein Kinase B1; CDK4, Cyclin-Dependent Kinase 4; EGFR, Epidermal Growth Factor Receptor; PI3K, Phosphatidylinositol-3 kinase.
Figure 2. WPPNYQW (orange) and YDWRF (green) peptides bind to EGFR (A), PI3K (B), AKT1 (C), and CDK4 (D) on the same side as the inhibitor. WPPNYQW and YDWRF peptides have a similar binding mode to the inhibitor indicating the peptides have the potential to act as protein inhibitors. AKT1 indicates Protein Kinase B1; CDK4, Cyclin-Dependent Kinase 4; EGFR, Epidermal Growth Factor Receptor; PI3K, Phosphatidylinositol-3 kinase.

Table 2. The interaction of WPPNYQW and YDWRF peptides with EGFR, PI3K, AKT1, and CDK4 proteins.

| PROTEIN          | LIGAND         | BINDING AFFINITY (KCAL/MOL) | POSITION OF CHEMICAL BONDS                      | HYDROGEN BOND          | HYDROPHOBIC INTERACTION |
|------------------|----------------|-----------------------------|------------------------------------------------|------------------------|-------------------------|
| EGFR Inhibitor   | WPPNYQW        | -9.5                        | Ala722, Lys745, Met793, Asp837, Met793, Ala743, Leu788, Gly721, Thr854, Leu718 | Trp880, Lys779, Leu858, Leu777 |                         |
| EGFR Inhibitor   | YDWRF          | -9.3                        | Cys797, Tyr998, Arg841, Asp800, Leu1001, Met1002, Val717, Phe795 | Lys745, Glu804, Cys797, Leu1001, Met1002, Arg841, Val726, Ala743 |                         |
| PI3K Inhibitor   | WPPNYQW        | -10.6                       | Lys807, Asn951, Lys833, Asp841, Asp964, Arg841, Glu880, Tyr867, Val882 | Trp812, Ile811, Ile885, Ile963, Ile879, Met953 |                         |
| PI3K Inhibitor   | YDWRF          | -9.4                        | Asp950, Lys833, Thr886, Ala885, Val882, Arg947, Trp812, Ile885, Met953 |                         |                         |
| Akt1 Inhibitor   | WPPNYQW        | -11.3                       | Gln79, Tyr272, Thr279, Glu79, Leu78, Asp274, Asn279, Thr272, Ile290, Thr272 | Arg947, Trp80, Ile84, Ile186, Leu210, Ala12, Leu201, Gly85, Ile290, Thr211, Trp80 |                         |
| Akt1 Inhibitor   | YDWRF          | -10.7                       | Gln79, Thr72, Thr272, Val147, His95, Ala10, Ile12 |                         |                         |
| CDK4 Inhibitor   | WPPNYQW        | -9.4                        | Gly15, Ile12, Asp99, Asp97, Thr102, Ile12, Val14, Ala16, Tyr17, Thr177, Val147, Val176, Cys215, Val16, Ala33, Ala157 |                         |                         |
| CDK4 Inhibitor   | YDWRF          | -9.3                        | Asp105, Val14, Thr102, Val96, Thr177, Thr272, Val20, Ala33 |                         |                         |

Abbreviations: Akt1, Protein Kinase B1; CDK4, Cyclin-Dependent Kinase 4; EGFR, Epidermal Growth Factor Receptor; PI3K, Phosphatidylinositol-3 kinase.
Asp800, Leu1001, Met1002, Val717, Phe795) and eight hydrophobic interactions (Lys745, Glu804, Cys797, Leu1001, Met1002, Arg841, Val726, and Ala743). The number of hydrogen bonds formed is predicted to have an essential role in the stability of protein-peptide interactions.41 These results reveal that the WPPNYQW and YDWRF peptides have potential as EGFR inhibitors through the competitive ATP inhibitor mechanism (Figure 2A). The interaction of WPPNYQW and YDWRF peptides with EGFR have lower binding affinity values than interaction with inhibitors (Table 2).

The docking results showed that all peptides bind to the ATP binding pocket in the catalytic domain of the PI3K p110 subunit, and two peptides, WPPNYQW and YDWRF, have lower binding affinity values than wortmannin as inhibitors. The binding affinity value for the interaction between PI3K and WPPNYQW and YDWRF peptides is -10.6 kcal/mol and -9.4 kcal/mol, respectively. Hydrogen bonds dominate the interactions that form. The WPPNYQW peptide forms ten hydrogen bonds (Lys807, Asn951, Lys833, Asp841, Asp964, Asp836, Asp841, Glu880, Tyr867, and Val882) and six hydrophobic interactions (Arg947, Trp812, Met953, Ile881, Ala885, and Leu1090). Meanwhile, the YDWRF peptide forms seven hydrogen bonds (Asp950, Lys833, Thr886, Ala885, Val882, Asp950, and Ser806) and eight hydrophobic interactions (Lys883, Trp812, Tyr867, Val822, Ile881, Met953, Ile963, and Ile879). These two peptides have more hydrogen bonds than wortmannin as a positive control. Therefore, the WPPNYQW and YDWRF peptides have high potential as PI3K protein inhibitors through the competitive ATP inhibitor mechanism (Figure 2B and Table 2).

The docking results showed all the peptides were bound in the ATP binding pocket of AKT1 the same as AZD5363. However, two peptides, namely, WPPNYQW and YDWRF, have lower binding affinity than AZD5363, ie, -11.3 kcal/mol and -10.7 kcal/mol. The WPPNYQW peptide forms seven hydrogen bonds (Gln79, Leu78, Asp274, Asn279, Tyr272, Ile290, and Thr211) and seven hydrophobic interactions (Ala58, Trp80, Ile84, Ile186, Leu210, Lys268, and Leu264). Meanwhile, the YDWRF peptide forms seven hydrogen bonds (Thr82, Phe293, Gly294, Leu295, Glu85, Ile290, Thr211, and Thr211) and five hydrophobic interactions (Trp80, Tyr272, Cys296, Lys268, and Val270). The results reveal that WPPNYQW and YDWRF peptides have high potential as PI3K protein inhibitors through the competitive ATP inhibitor mechanism (Figure 2B and Table 2).

The docking results between CDK4 and peptides showed that the peptides bind to CDK4 in the ATP binding pocket with varying binding affinity values. Two peptides, namely, WPPNYQW and YDWRF, had lower binding affinities (WPPNYQW, -9.4 kcal/mol; YDWRF, -9.3 kcal/mol) than abemaciclib as positive controls. The WPPNYQW peptide forms seven hydrogen bonds (Val14, Ala16, Tyr17, Thr177, Glu144, Asp99, and Ile12) and seven hydrophobic interactions (Ile12, Val14, Ala16, Tyr17, Thr177, Glu144, and Asp99). Meanwhile, the YDWRF peptide forms six hydrogen bonds (Asp105, Val14, Thr102, Val96, Thr177, and Asp99) and four hydrophobic interactions (Ile12, Leu147, Val20, and Ala33). The number of chemical interactions that are formed will increase the stability of the protein–peptide interaction. Based on these docking results, the WPPNYQW and YDWRF peptides are shown to have high potential as CDK4 inhibitors because they bind to the ATP binding pocket and have lower binding affinity values than abemaciclib (Figure 2D and Table 2).

**Molecular dynamics simulation results**

Molecular dynamics simulations were carried out to analyze the structural stability and conformational fluctuations of the EGFR, PI3K, AKT1, and CDK4 proteins that interact with the WPPNYQW and YDWRF peptides. The molecular dynamics simulation result can be seen in Figure 3.

The EGFR, PI3K, and AKT1 proteins complexed with the WPPNYQW peptide had a stable RMSD value of less than 3 Å from the start to the end of the simulation, which is not much different from inhibitor compounds. Meanwhile, the RMSD CDK4 complexed with inhibitor ligand and WPPNYQW peptide showed a different trend. CDK4 complexed with inhibitor (abemaciclib) had an unstable RMSD value during the simulation, which was more than 3 Å from the 4 ns time to the end of the simulation. The CDK4 complex with the WPPNYQW peptide was unstable at 6–8 ns time but returned to stability from 10 ns until the end of simulation. This result shows that CDK4 is more stable when interacting with the WPPNYQW peptide than the inhibitor (abemaciclib). Different results were shown when the four proteins interacted with the YDWRF peptide. PI3K and AKT1 proteins were stable from the beginning to the end of the simulation when complexed with the YDWRF peptide. Meanwhile, EGFR and CDK4 proteins were unstable when complexed with the YDWRF peptide characterized by high RMSD values (Figure 3).

EGFR in the form of a complex with peptides has a lower RMSD value than EGFR itself, which indicates that the EGFR complex with peptides is more stable (Figure 3A and E). The RMSD values of the PI3K and the PI3K-peptides complex tended to be the same during the simulation, indicating the complex’s stability (Figure 3B and E). The RMSD value of AKT1 with the AKT1-peptides complex was not much different during simulation, and even the AKT1-YDWRF was more stable because it had a lower RMSD value (Figure 3C and E). The CDK4-WPPNYQW RMSD value is not much different from CDK4. When interacted with YDWRF, CDK4 had a significant increase in the RMSD value, indicating a change in the structure of the CDK4 protein (Figure 3D and E).

In EGFR complexed with peptides and inhibitors, several unstable amino acids are involved, including Lys754, Lys867,
However, amino acids are very unstable when EGFR is complexed with YDWRF, namely, Val1010, Val1011, Asp1012, and Ala1013. These residues cause the RMSD value of the EGFR-YDWRF complex to tend to be higher. Meanwhile, PI3K and AKT1 complexed with peptides showed RMSF values which were not significantly different than when the two proteins were complexed with an inhibitor. CDK4 complexed with YDWRF has very unstable residues, namely, Pro245, Arg246, Gly247, and Ala248 (Figure 4). These residues play a role in the structural instability of the CDK4-YDWRF protein, which can be seen from the high RMSD value of the CDK4-YDWRF complex. From the RMSD and RMSF values of the protein-ligand complex, all proteins are found to be stable when are complexed with the WPPNYQW peptide. In contrast, the only proteins that were stable when interacted with YDWRF were PI3K and AKT1. EGFR and CDK4 are unstable because some residues have high flexibility when the protein is complexed with the YDWRF peptide. PI3K and AKT1 proteins were stable from the beginning to the end of the simulation when complexed with the YDWRF peptide. Meanwhile, EGFR and CDK4 proteins were unstable when complexed with the YDWRF peptide characterized by high RMSD values (Figure 3).

Hydrogen bonds have an important role in the stability of protein conformation and the stability of protein-ligand interactions. The numbers of hydrogen bonds of the complexes during

![Figure 3](image-url)
the simulation is shown in Figure 5. The numbers of hydrogen bonds complexed four proteins with the WPPNYQW and YDWRF peptides were not significantly different compared to the four proteins complexed with the inhibitors as positive controls. This indicates that the complex structures are stable.

The molecular dynamic binding energy represents the stability of protein-ligand interactions during the simulation. The simulation results show that all protein-peptide complexes have lower molecular dynamic binding energy values than protein-inhibitor complexes. Based on the molecular dynamic
binding energy, the stability level of the protein-peptide interactions are still below the protein-inhibitor (Figure 6).

Discussion
EGFR is a ligand epidermal growth factor (EGF) receptor, with an essential role in the growth and development of cancer cells. These receptors are overexpressed in various types of cancer cells, including breast cancer cells. Activation of EGFR signaling is induced by the EGF ligand, causing EGFR to form dimers, and then tyrosine residues appear in the kinase domain, causing effector proteins to be recruited. Phosphotyrosine motive in the kinase domain with ATP activates several pathways such as the KRAS/BRAF/MEK/ERK pathway, PI3K pathway, AKT pathway, and Signal Transducer and Activator of Transcription (STAT) pathway that stimulate proliferation, angiogenesis, migration, survival, and cell adhesion. Therefore, one strategy to inhibit EGFR phosphorylation activity is to block the interaction between the tyrosine kinase EGFR domain and ATP. The ATP binding pocket is the region where the EGFR binds to ATP. This region is located near the C-helix and A-loop structures, to be precise around the amino acids Leu718, Val726, Gly745, Leu788, Gly796, Cys797, Leu844, and Asp855.

This study reveals that the WPPNYQW and YDWRF peptides have potential as EGFR inhibitors through the competitive ATP inhibitor mechanism. Inhibition of EGFR activity can inhibit breast cancer cell growth through a variety of mechanisms. Inhibition of EGFR activity can reduce collagenase expression (MMP1) and Meiosis-specific Serine/Threonine Protein Kinase 1 (MEK1), which play a role in cancer cell invasion and metastasis. EGFR inactivation also induces apoptosis by decreasing the expression of antiapoptotic proteins such as Secreted Frizzled Related Protein 1 (SRFP1), Baculoviral IAP Repeat Containing 5 (BIRC5), and BAG Co-chaperone 1 (BAG1). EGFR inhibition can also lead to cell cycle arrest in cancer cells. However, the use of EGFR inhibitors for cancer therapy as a single agent has a low success rate because other pathways play a role in cancer growth, such as the PI3K pathway.

PI3K is the central protein in the PI3K/AKT/mTOR pathway. PI3K is activated by receptor tyrosine kinases, such as EGFR. Once activated, the catalytic domain in the p110 subunit catalyzes the transfer of phosphate from ATP to Phosphatidylinositol Biphosphate (PIP2) to become Phosphatidylinositol triphosphate (PIP3). PIP3 is what then activates AKT1 through the phosphoinositide-dependent kinase 1 (PDK1) protein recruit. This pathway, called the master regulator for cancer, is responsible for regulating cell growth and proliferation. One strategy to inhibit this pathway is to inhibit the activity of the PI3K protein by blocking the binding between PI3K and ATP so that the activation mechanism of AKT1 cannot occur.

The docking results revealed that all peptides bind to the ATP binding pocket in the catalytic domain of the PI3K p110 subunit, and two peptides, WPPNYQW and YDWRF, have lower binding affinity values than wortmannin as inhibitors.
That result shows that the WPPNYQW and YDWRF peptides have potential as PI3K inhibitors. The inhibition of PI3K activity can prevent the uncontrolled proliferation of cancer cells.\(^65\) Inhibition of PI3K activity, can also suppress the expression of ZAK, TACC1, ZFR, and ZNF565 genes, resulting in cell death.\(^68\) In addition, PI3K inactivation also results in inhibition of Cyclin D1 activity, which plays an important role in proliferation and cycle regulation. PI3K inactivation can also induce apoptosis by increasing the amount of cleaved caspase-3 protein.\(^69\)

Akt is one of the central proteins in the PI3K/AKT/mTOR pathway, activated by PI3K. This protein is overexpressed in ER+ or HER+ breast cancer cells and plays an important role in proliferation, metabolism, antiapoptosis, and cell survival.\(^24\) The activation stage of Akt1 is initiated by the PH domain that binds to PIP3 on the cell membrane previously phosphorylated by PI3K. The interaction between the Akt1 PH domain and PIP3 changes the Akt1 conformation so that it allows PDK1 to phosphorylate Akt1. After being phosphorylated, the ATP binding pocket in the kinase domain is exposed, and Akt1 will return to the cytoplasm to phosphorylate its target proteins.\(^40\) Akt1 then binds to ATP and transfers phosphate from ATP to its target proteins to become the active protein product. Therefore, many Akt1 inhibitor compounds have been developed, which target ATP binding pockets that compete with ATP to bind to the ATP binding pocket so that Akt1 cannot phosphorylate the substrate.\(^61\) One of them is AZD5363, which binds to Akt1 in the ATP binding pocket, thus preventing ATP from binding to Akt1. Consequently, there was no phosphorylation of the target protein from Akt1.\(^33\)

Inhibition of the activity of the Akt1 protein will induce apoptosis and cell cycle arrest in cancer cells.\(^62\) The results reveal that WPPNYQW and YDWRF peptides have high potential as an Akt1 inhibitor, even better than AZD5363. Akt1, which is inhibited, will increase the activity of Checkpoint kinase 2 (Chk2) protein. This protein will degrade Cell Division Control Protein 2 (Cdc2), resulting in cell arrest in the G2-M phase.\(^63\) Akt1 inhibition can also inhibit the activity of proteins that play an important role in cell proliferation such as Mammalian Target of Rapamycin (mTOR), IKB Kinase (IKK), and Cyclin D1, and increase the activity of antiproliferative proteins such as Glycogen Synthase Kinase 3 Beta (GSK3β) and Forkhead box protein O1 (FOXO1).\(^64\)

When overexpressed, CDK4 causes uncontrolled cell growth and proliferation.\(^65\) In the G0/G1 phase, CDK4 binds to Cyclin D1 and then phosphorylates the retinoblastoma protein (Rb), thus inducing cells to enter the G1/S phase.\(^66\) Previous studies reported that the CDK4/Cyclin D/Rb pathway causes the growth and development of various cancer types. One strategy to inhibit this pathway is by inhibiting the phosphorylation of Rb through the CDK4/Cyclin D complex.\(^34\) Therefore, a CDK4 inhibitor was developed that targets the ATP binding pocket of CDK4 so that CDK4 cannot bind to ATP and phosphorylate Rb. This inhibitor binds to CDK4 in the ATP binding pocket located between the residues of His95, Val96, Asp97, Arg101, Thr102, and Ghu144.\(^67\) Some compounds with an activity as competitive ATP inhibitors include abemaciclib, palbociclib, and ribociclib have entered the clinical research stage.\(^34\)

The docking results between CDK4 and peptides showed that WPPNYQW and YDWRF peptides had lower binding affinity than abemaciclib as inhibitor. It indicates that both peptides have high potential as CDK4 inhibitors. The inhibition of CDK4 activity will stimulate cancer cells to undergo cell cycle arrest. When the CDK4/Cyclin D complex is active, Cyclin D-CDK4 will phosphorylate RB so that RB is released from E2F, and then E2F can bind to DNA as a transcription factor for genes that play an important role in the running of the cell cycle.\(^68\) CDK4 inhibition will prevent RB phosphorylation so that RB will continue to bind to E2F (E2 Factor) protein, and E2F protein cannot bind to DNA as a transcription factor.\(^69\) This interaction causes cell arrest in phase G1.\(^70\)

Molecular dynamics simulations were carried out to analyze the structural stability and conformational fluctuations of the EGFR, PI3K, Akt1, and CDK4 proteins that interact with the WPPNYQW and YDWRF peptides. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), number of hydrogen bonds, and molecular dynamic binding energy analyses are also used to obtain important information regarding the stability and flexibility of protein-ligand complexes. High deviation and variability during the simulation indicate low stability.\(^71\) Proteins stable during the simulation are marked with an RMSD value of not more than 3 Å. A value more than that indicates that the protein has undergone some structural changes.\(^72\) RMSF represents a shift in each amino acid residue on a protein. Amino acids with a high RMSF value indicate that these amino acids are unstable or have high fluctuations during the simulation.\(^73\) The stability of the RMSD and RMSF values is important to conclude a good binding affinity for protein-ligand interactions.\(^74\) The conformational stability of the complex also can be determined from the number of hydrogen bonds during the simulation. Hydrogen bonds are required to form the secondary structures of proteins such as the α-helix and the β-sheet. Hydrogen bonds make these structures very stable.\(^42\) Hydrogen bonding also facilitates the stability of protein-ligand interactions. The more hydrogen bonds, the more stable the interaction.\(^41\) The stability of the protein-ligand interaction during the simulation can be determined from the molecular dynamic binding energy value. The molecular dynamic binding energy is influenced by the value of the potential energy and the solvation energy of complexes, ligands, proteins, and ligands. The more positive the molecular dynamic binding energy value, the better the protein-ligand binding.\(^43\)

As the result of this study, the EGFR, PI3K, Akt1, and CDK4 protein were in a stable state when complexed with the
WPPNYQW peptide, which was seen from the RMSD and RMSF value. PI3K-YDWRF and AKT1-YDWRF complexes are stable, characterized by high RMSD values and increased volatility in several amino acids.

Our result suggested that sea cucumber peptides namely WPPNYQW and YDWRF maybe are promising inhibitor candidates of key target proteins that highly expressed in breast cancer. Further research is needed to validate the in vitro and in vivo activity of the sea cucumber peptides in inhibit cancer progression.

Conclusion
In this study, two sea cucumber peptides had high potential as ant-breast cancer agents, namely, WPPNYQW and YDWRF peptides. The two peptides bind to the active sites of EGFR, PI3K, AKT1, and CDK4 proteins with lower binding affinity values than inhibitors. The interactions between WPPNYQW peptide and the four proteins are stable. While the YDWRF peptide interacts stably with PI3K and AKT1 proteins, it is unstable when interacting with EGFR and CDK4 proteins. Therefore, the WPPNYQW peptide has more potential as an ant-breast cancer agent than the YDWRF peptide.

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Availability of Data and Materials
The data underlying this article are available in the article.

Ethical Approval
This article does not contain any studies with human participants or animals performed by any of the authors.

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