Molecular Docking and Molecular Dynamic Simulation on the Interaction of Saffron's Active Compunds with HER-2 Protein

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Abstract

Human epidermal growth factor receptor-2 (HER-2) is an essential oncogene in breast cancer. HER-2 causes 25% of breast cancer, and this type of cancer tends to grow and spread faster than others but had a good response to HER-2 targeted therapy. This study aims to analyze chemical compounds in saffron plants (*Crocus sativus*) that potential to breast anticancer activity by inhibiting HER-2 receptor (PDB ID: 3RCD). The study employed *in silico* research such as molecular docking using AutoDock Tools software, and visualization with Biovia Discovery Studio. In addition, molecular dynamic simulation was conducted using GROMACS software, with visualization performed using Grace. The molecular docking results showed that Crocetin has a lower binding energy value of -8.37 kcal/mol compared to Herceptin, which is -7.11 kcal/mol and the lowest energy among Saffron bioactive compounds. These results indicated that the affinity of Crocetin in binding to HER-2 receptor is better than Herceptin. The molecular interactions were hydrogen, hydrophobic, electrostatic, and unfavorable bonds. The MD results showed that the RMSD value meets the 0.2-0.5 nm stability requirements. According to the data analysis, Herceptin appears to have a more stable RMSF value when compares to Crocetin. The Rg graph of both complexes showed stability until the end of the simulation. The H-bond results show that the Herceptin complex has more hydrogen bonds than the Crocetin complex. These results showed that the chemical components of saffron plants have the potential as breast anticancers by inhibiting the HER-2 receptor.

Keywords: *anticancer, Crocus sativus, HER-2 receptor, molecular docking, molecular dynamic*.

INTRODUCTION

Breast cancer is the most common cancer in women worldwide. The morbidity and mortality rates of breast cancer have significantly increased over the past decades, it is an urgent need to provide the most effective prevention taking into account that modifiable risk factors might be crucial in providing the reduction of breast cancer incidents (*e.g.*, lack physical activity, high body mass index, alcohol intake, smoking, insufficient vitamin supplementation, and exposure to artificial light) (Łukasiewicz, *et al.,* 2021). Breast cancer

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usually starts from ductal hyperproliferation and then develops into benign tumors or even metastatic carcinoma after being continuously stimulated by various carcinogenic factors (Sun, *et al*., 2017). Breast cancer can be classified based on its anatomical origin, whether lobular or ductal, as well as hormone reception and expression of Human Epidermal Growth Factor Receptor 2 (HER-2). Hormone receptor-positive breast cancer, especially if nonmetastatic, may be amenable to hormone-blocking therapy. HER-2-positive tumors are generally responsive to HER-2 monoclonal antibodies. Hormone receptor-positive, HER-2 negative is breast cancer's most common expression status (Watkins, 2019).

The HER-2 is an epidermal growth factor receptor (EGFR) from the tyrosine kinase group. HER-2 is an essential oncogene in breast cancer on human chromosome 17 (17q12) (Sun, *et al*., 2017). The group with high HER-2 can contribute 10-15% of breast cancer cases and its status is highly relevant in choices regarding the appropriate management of breast cancer patients. The HER-2 enriched cancers grow faster than luminal cancers and usually have a poor prognosis. The HER-2 overexpression is one of the earliest processes during breast carcinogenesis. Additionally, HER-2 increases the detection rate of metastatic or recurrent breast cancer from 50% to more than 80% (Łukasiewicz, *et al.,* 2021). Also, *in silico* approach with HER-2 receptor as targeted therapy can be an approach to find potential drugs candidate for breast cancer (Mutiah, *et al*., 2021).

Crocus sativus L, commonly known as Saffron, comes from the crocus genus in the Iridaceae family. Saffron has been featured in traditional Chinese, Ayurvedic, and Greek recipes. *Crocus sativus* is a sedative, expectorant, anti-asthma, anticancer, and antihyperlipidemia (Zakiyah, *et al*., 2021). Fewer than 50 constituents, however, have been identified so far. The three main biologically active compounds are crocin, picrococin, and safranal (Mzabri, *et al*., 2019). Based on chemical analyses of dry stigma of saffron extracts, carotenoids, namely crocin and crocetin and the monoterpene aldehydes picrocrocin and safranal are the most important active carotenoid secondary metabolites of saffron (Samarghandian, *et al.,* 2014). Crocin, a monoglycosyl or di-glycosyl polyene ester, give deep red color of of saffron's stigma. Picrocrocin (C16H26O7) is the main factor influencing the bitter taste of saffron. Safranal was responsible for the aroma of fresh Saffron. Crocetin contains anthocyanin pigments that gives Saffron its color because it is located in the central core of crocin (Mzabri, *et al.,* 2019). There is also the compound dimethylcrocetin, which can inhibit the interaction between DNA and proteins, which are essential for forming cellular DNA (Afifah, *et al.,* 2020). Saffron extract in combination with sodium selenite or sodium arsenite may have synergistic effects and have an important role in cancer chemoprevention. Saffron has inhibitory effect against malignant cells with dose dependent as well. Saffron pretreatment for five consecutive days prior to the administration of antitumor drugs including cisplatin significantly inhibited by inducing cellular DNA damage (Mzabri, *et al.,* 2019). Saffron aquaeous extract could decrease tumor volume in mice breast tumor tissue induced by the 4T1 cells by increasing expression of p53. Previous research report have never screened Saffron's active compound on the HER-2 receptor. This is important to find any compounds from Saffron that has potential as anticancer. Therefore, in this study, the affinity of five Saffron's active compound was screened for HER-2 receptors through *in silico* approach.

MATERIALS AND METHODS

Materials

The material used is HER-2 protein (GDP ID: 3RCD) which was downloaded from https://www.rcsb.org/. Herceptin as a positive control and Saffron's active compounds in stigma, there are safranal, crocetin, crocin, dimethylcrocetin,

and picrocrocin whose molecular structures were downloaded from https://pubchem.ncbi.nlm.nih.gov/.

The tools used include hardware in the form of a set of ASUS laptops with specifications for Processor type 11th Gen Intel(R) Core(TM) i3-1115G4 @ 3.00GHz 3.00 GHz, Random Access Memory (RAM) specifications of 4 GB (Gigabyte), CPU Intel I3-1115G4/BGA, SSD 512 G and Windows 11 (64 bit). The hardware for molecular dynamics is a Personal Computer (PC) with system specifications Ubuntu 18.04.1 LTS, AMD Ryzen 7 2700x Eight-Core Processor x 16, GNOME 3.28.2, 64-bit, 1 TB HDD connected to the internet for Molecular Dynamics.

The software used is AutoDock Tools (http://autodock.scripps.edu/), PyMOL 2.5 produced by Schrödinger (https://pymol.org), MarvinSketch produced by ChemAxon (https://chemaxon.com/products/marvin), GROMACS (https://www.gromacs.org/), Biovia Discovery Studio (https://www.3ds.com/products-services/biovia/), Protein Data Bank (https://www.rcsb .org/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), CHARMM-GUI (https://www.charmm-gui.org/), and Grace (https://plasmagate.weizmann.ac.il/Grace/).

Ligand-Receptor Preparation

Receptor preparation was carried out by eliminating water molecules and reference ligands then adding hydrogen atoms and optimizing by adding hydrogen and adding a computed gasteiger charge using AutodockTools 4.0. Ligand preparation was done by downloading the Saffron's ligand from the site (https://pubchem.ncbi.nlm.nih.gov/), optimizing by setting torsion tree with choose torsion and set the number of active torsion.

Molecular dynamic simulation using the HER-2 receptor complex with positive control and the best test ligand with docking results. Then, the complex was input on the CHARMM-GUI website (https://www.charmm-gui.org/) to create the topology, waterbox, add ions, and select the force field.

Validation Method of Docking

The validation method of docking is done by redocking a native ligand (TAK-285) in grid box that taken from the center of the ligand with Autodock 4.0. The grid box measurement for this research was X: 12.48, Y: 2.964, and Z: 28.015. The results of the receptor validation were interpreted with the value of Root Mean Square Deviation (RMSD). Receptors can be said to be valid if they meet the criteria for the RMSD value 2Å (Rena, *et al.,* 2022).

Docking Ligand-Protein

Ligand-protein docking was done by detecting cavities where the drug will bind or

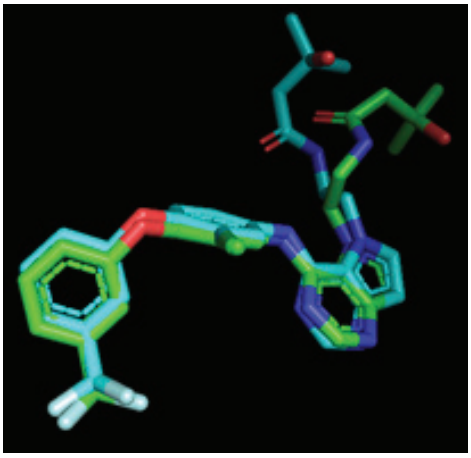

Figure 1. RMSD analysis results of TAK-285.

Table 1. The minimum energy of positive control and Saffron's active compounds.

Ligands	Binding Energy	Inhibition Constant
Herceptin (positive control)	**-7.11**	**6.15**
Safranal	-4.93	242.05
Crocetin	**-8.37**	**732.09**
Crocin	21.79	11.65
Dimethyl Crocetin	-6.49	3 8.7
Picrocrocin	-6.02	17.45

Remark : bold font indicates ligand with the best docking results.

interact with receptors. Place the 3-Dimensional structure of the compound into cavities selected. The docking of compounds on the receptor is done automatically by Autodock 4.0. The parameter measured is the energy value and interaction bond.

Molecular Dynamic

Molecular dynamics consists of minimization, equilibration, and production processes. System minimization is the process of reducing potential energy in the system, equilibration is the process of making the system at a temperature of 300K and pressure of 1 atm, and production produces a trajectory. Next, the results were analyzed using the parameters RMSD, RMSF, radius of gyration, and hydrogen bond. The molecular dynamic results were visualized using Grace software.

RESULTS

The result of RMSD<2 Å indicates that the docking procedure is valid. The smaller the RMSD value, the closer the docked ligand pose will be to the pose of the natural ligand (Rena, *et al*. 2022). Based on the results obtained, native ligand has RMSD value of 1.802 Å. RMSD analysis was carried out by comparing the 3D complex structure of the PDB with the redocking results, the results of this comparison can be seen in Figure 1.

The docking of ligands with receptors can be seen through the results of the binding energy or Rerank score (Mutiah, *et al*., 2021). A low binding energy value indicates the best affinity. The smaller the inhibition constant value, the better the inhibitory activity. The more negative the binding energy value and the lower the inhibition constant value indicates that the bond between the ligand and protein has good stability, the stronger the bond formed (Sohrab, *et al.,* 2022). Based on the docking results, Crocetin has the lowest energy than other compounds. The data was shown at Table 1.

In this study, there was an interaction of the ligand with the active amino acid present at the HER-2 receptor. Active amino acids with conventional hydrogen bonds in herceptin are Ser 783, Met 801, Gly 804, Asp 863, Met 801, and Gln 799. Active amino acid with carbon hydrogen bonds in herceptin is Gly 804. Active amino acids with hydrophobic (π-sigma and π-alkyl) are Thr 798, Leu 852, Ala 751, Met 801, and Leu 726.Z In other side, herceptin has steric bond that shown in unfavorable bond, the active amino acid is Ser 783. The data was shown at Table 2. The unique binding site in Herceptin interaction with HER-2 protein involve Ser 783, Asp 863, Gln 799, Leu 852, Ala 751, Met 801, and Leu 726. This binding site also found in Saffron's active compounds interaction with HER-2 protein. The comparation about interaction between atoms of Herceptin and Crocetin as the best ligand was shown at Table 3.

Saffron's active compounds that has same hydrogen bond with Ser 783 are crocin and picrocrocin, with Asp 863 are crocin and dimethyl crocetin, with Gln 799 are crocetin and picrocrocin. Saffron's active compounds that

Table 2. Results of docking and chemical bonds of positive control and Saffron's active compounds to HER-2 receptor.

No	Ligands	Category	Chemical Bond	Amino Acid Residue and Bond Distance (Å)
1	Herceptin	Hydrogen bond	*Conventional Hydrogen Bond*	**SER 783** (2.69Å); MET 801 (2.02Å); GLY 804 (3.06Å); **ASP 863** (2.00Å); MET 801 (2.73Å); **GLN 799** (2.67Å)
			Carbon Hydrogen Bond	GLY 804 (3.00Å)
		Hydrophobic	*π-sigma*	THR 798 (3.21Å); **LEU 852** (3.34Å; 3.88Å; 3.86Å)
			π-alkyl	**ALA 751** (3.62Å, 4.34Å, 4.44Å); **MET 801** (5.05 Å); **LEU 726** (5.48Å)
		Unfavorable	*Unfavorable Donor-Donor*	**SER 783** (3.14Å)
2	Safranal	Hydrogen bond	*Conventional Hydrogen Bond*	THR 862 (1.82Å)
		Hydrophobic	*Alkyl*	LEU 785 (5.05, 4.19); LYS 753 (4.66)
			π-alkyl	PHE 864 (4.96, 5.49)
3	Crocetin	Hydrogen bond	*Conventional Hydrogen Bond*	ALA 730 (2.72); PHE 731 (2.08); GLY 732 (2.87); LYS 753 (1.78); **GLN 799** (2.34)
		Hydrophobic	*Alkyl*	VAL 734 (4.44, 4.00); **ALA 751** (3.43); **LEU 726** (4.45, 4.11); CYS 805 (4.73); VAL 734 (5.13); **LEU 852** (4.88)
			π-alkyl	PHE 1004 (4.10, 407)
		Electrostatic	*Attractive charge*	LYS 753 (4.31)
4	Crocin	Hydrogen bond	*Conventional Hydrogen Bond*	THR 862 (2.50); **ASP 863** (2.83); **SER 783** (2.20); GLN 799 (2.81)
			Carbon Hydrogen Bond	GLY 865 (3.59)
		Hydrophobic	*Alkyl*	VAL 734 (4.30, 5.47); LYS 753 (4.01)
		Unfavorable	*Unfavorable Bump*	**ALA 751** (2.18); **ASP 863** (2.21)
			Unfavorable Negative-Negative	GLU 770 (5.18); ASP 808 (5.24)

No	Ligands	Category	Chemical Bond	Amino Acid Residue and Bond Distance (Å)
5	Dimethylcrocetin	Hydrogen bond	*Conventional Hydrogen Bond*	CYS 805 (2.05)
			Carbon Hydrogen Bond	ASP 808 (3.50); **ASP 863** (3.44, 3.41)
		Hydrophobic	*Alkyl*	VAL 734 (5.21); **ALA 751** (4.83, 3.45); LYS 753 (4.74); CYS 805 (3.86); **LEU 726** (4.95); LEU 800 (4.80); **MET 801** (4.96, 5.23) ; **LEU 852** (5.01, 5.13, 4.20, 4.21); LYS 53 (4.23, 4.95)
			π-alkyl	PHE 864 (4.57); PHE 1004 (4.31, 5.48)
6	Picrocrocin	Hydrogen bond	*Conventional Hydrogen Bond*	LYS 753 (2.23); **SER 783** (2.84); THR 862 (2.42Å, 2.15); **GLN 799** (2.10, 2.19)
		Hydrophobic	*Alkyl*	VAL 734 (4.42, 5.23); **ALA 751** (3.23); LYS 753 (3.85, 4.01, 4.43)

Remark: bold font indicates the same type of amino acid residue between the test ligand and the positive control.

has hydrophobic bond with Leu 852 are crocetin and dimethylcrocetin, with Ala 751 are crocetin; dimethylcrocetin; and picrococin, with Met 801 is dimethylcrocetin, with Leu 726 are crocetin and dimethylcrocetin. The amino acid that has unfavorable bond in herceptin is Ser 783 but in crocin and picrococin this amino acid has hydrogen bonds. Beside that, there is electrostatic bond in crocetin that no one else had. The chemical bond was shown at Table 2 and Figure 2.

The RMSD value of the protein-ligand complex is represented on a graph of the RMSD value during a simulation time of 20 ns, as shown in Figure 3. The RMSF showed protein stability indicated by the absence of sharp fluctuation spikes in the residues making up the target protein. In Figure 3, the residual RMSF values in the target proteins. Hydrogen bond analysis was conducted by observing the donor-acceptor pair between the target protein and the selected ligand and the hydrogen bond occupancy. Hydrogen bonding data on selected protein-ligand complexes are presented in Figure 3.

DISCUSSION

Crocetin provides proapoptotic effects on MCF-7 breast cancer cells, showing caspase-dependent pathways through increased Bax protein expression (International BMR, 2020). In interaction there are many bonds that happen between ligands and target protein. In this research there is hydrogen bond interaction that occur between a hydrogen bond donor atom and an acceptor atom like N, O, P, and S. These interactions are considered classical hydrogen bond donors, and hydrogen atoms can also be donors if connected to these types of atoms. Carbon hydrogen bond interactions are weaker than conventional hydrogen bonds, and a carbon atom can be a donor if it is in an acetylene group or next to an oxygen or nitrogen atom. There are also π-donor hydrogen bond interactions, which occur

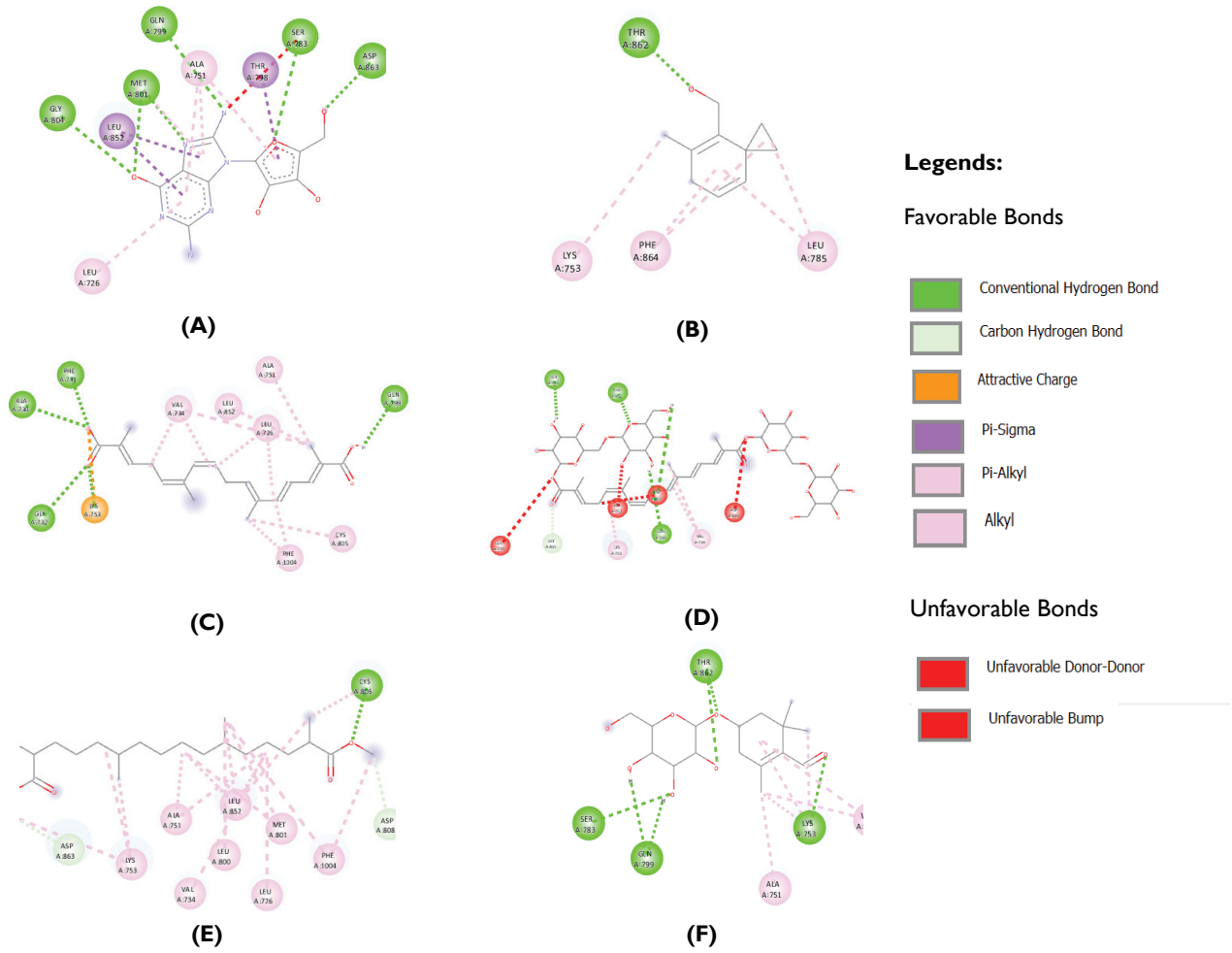

Figure 2. Amino acid- ligand binding of (A) Herceptin; (B) Safranal; (C) Crocetin; (D) Crocin; (E) Dimethyl crocetin; (F) Picrocrocin, where green line represent hydrogen bonds, orange line represent ionic bonds, purple line represent hydrophobic bonds, red line represent steric bond.

between a π ring that functions as a hydrogen bond acceptor and a hydrogen bond donor atom (Gómez, *et al.*, 2020).

Another type interaction in this research was hydrophobic Interaction that divide into 2 types, alkyl interactions and π-alkyl interactions. Alkyl interactions have alkyl groups that non-polarized and non-π systems that can be found in aliphatic amino acid side chains such as alanine, valine, leucine, isoleucine, methionine, selenomethionine, cysteine, proline. π-alkyl interactions or CH-π interactions occur between a hydrogen and a π ring system, provided that the hydrogen acting as the donor is connected to a non-aromatic carbon atom and meets the appropriate distance and relative position requirements (Gómez, *et al.*, 2020).

There is an interaction that can reduce stability and result in an increase in the binding energy of the ligand-receptor bond because this type of bond shows a repulsive force that occurs between two molecules. Different types of unfavorable interactions can happen between atoms, such as steric bumps, which happen when the distance between atoms is less than a certain threshold, repulsive charge interactions between atoms with the same charge, acceptor-acceptor clashes when

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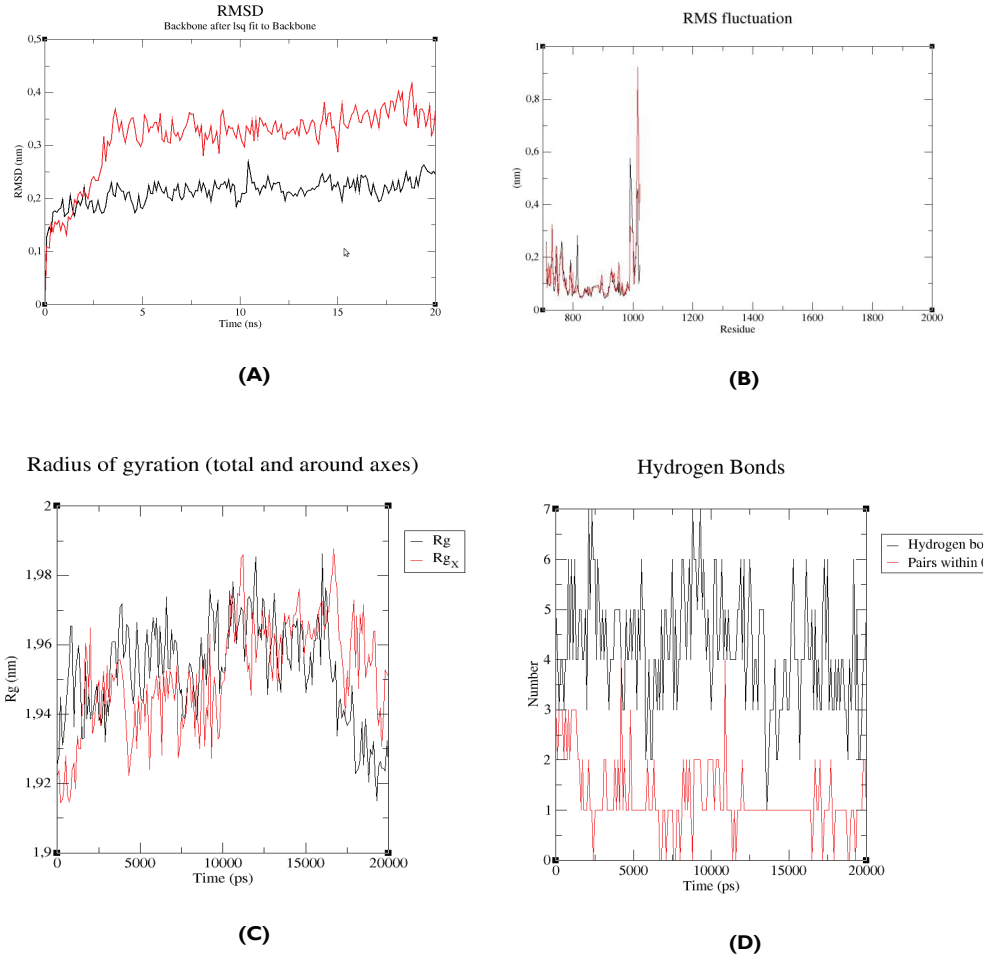

Figure 3. The RMSD (A); RMSF (B); Radius of Gyration (C); and Hydrogen Bond (D) values of the selected protein-ligand complex for 20 ns (black=Herceptin; red=Crocetin).

two acceptor atoms are too close, donor-donor clashes between two donor atoms, and metal repulsion between a metal ion and a donor atom. Factors like distance, charge, and the presence of certain atoms can cause these interactions (Gómez, *et al.*, 2020).

Molecular Dynamic

In the backbone RMSD simulation, there was an increase at the beginning, especially for the Crocetin test compound. Then, both began to stabilize at a time close to 5 ns until the end of the simulation, which is 20 ns. The RMSD value of 0.2-0.5 nm is acceptable for the system and can be said to be stable (Elfita, *et al.,* 2023). The RMSD value requirement is said to be stable if it is less than 0.3 nm (Supandi, *et al.,* 2021). The RMSF is a parameter that describes the fluctuation of ligand interaction with each amino acid residue (Zubair, *et al.,* 2021). The lower the RMSF value, the more stable the interaction between the ligand and the amino acid. Based on the RMSF results on both complexes shows that the herceptin complex is more stable than the Crocetin complex.

The radius of Gyration is a parameter that describes the compactness of protein structure and

Table 3. Description interaction of results of docking and chemical bonds of positive control and Saffron's active compounds to HER-2 receptor.

Herceptin				
No	**Category**	**Chemical Bond**	**From**	**To**
1.	Hydrogen Bond	Conventional Hydrogen Bond	Serine 783 (H-Donor)	Herceptin O atom (H-Acceptor)
			Methionine 801 (H-Donor)	Herceptin N atom (H-Acceptor)
			Glycine 804 (H-Donor)	Herceptin O atom (H-Acceptor)
			Aspartic Acid 863 (H-Donor)	Herceptin O atom (H-Acceptor)
			Herceptin O atom (H-Donor)	Methionine 801 (H-Acceptor)
			Herceptin N atom (H-Donor)	Glycine 799 (H-Acceptor)
		Carbon Hydrogen Bond	Glycine 804 (H-Donor)	Herceptin O atom (H-Acceptor)
		π-Sigma	Threonine 798 (C-H)	Herceptin (Pi-Orbitals)
			Leucine 852 (C-H)	Herceptin (Pi-Orbitals)
2.	Hydrophobic	π-Alkyl	Herceptin (Pi-Orbitals)	Alanine 751 (Alkyl)
			Herceptin (Pi-Orbitals)	Methionine 801 (Alkyl)
			Herceptin (Pi-Orbitals)	Leucine 726 (Alkyl)
			Herceptin (Pi-Orbitals)	Alanine 751 (Alkyl)
3.	Unfavorable	Unfavorable Donor-Donor	Serine 783 (H-Donor)	Herceptin N atom (H-Acceptor)

Crocetin				
No	**Category**	**Chemical Bond**	**From**	**To**
1.	Hydrogen Bond	Conventional Hydrogen Bonds	Alanine 730 (H-Donor)	Crocetin O atom (H-Acceptor)
			Phenylalanine 731 (H-Donor)	Crocetin O atom (H-Acceptor)
			Glycine 732 (H-Donor)	Crocetin O atom (H-Acceptor)
			Lysine 753 (H-Donor)	Crocetin O atom (H-Acceptor)
			Crocetin H atom (H-Donor)	Glutamin 799 (H-Acceptor)
2.	Hydrophobic	Alkyl	Valine 734 (Alkyl)	Crocetin (Alkyl)
			Valine 734 (Alkyl)	Crocetin (Alkyl)
			Alanine 751 (Alkyl)	Atom C crocetin (Alkyl)
			Crocetin (Alkyl)	Leucine 726 (Alkyl)
			Crocetin (Alkyl)	Cysteine 805 (Alkyl)
			Crocetin (Alkyl)	Valine 734 (Alkyl)
			Crocetin (Alkyl)	Leucine 852 (Alkyl)
		π-Alkyl	Phenilalanin 1004 (Pi Orbitals)	Crocetin (Alkyl)
3.	Electrostatic	Attractive Charge	Lysine 753 (Positive)	Crocetin O atom (Negative)

the binding pattern of the drug with protein (Priya, *et al.,* 2017). Based on the graph, the radius of gyration value ranges from 1.92-1.98 nm during the simulation time (20,000 ps). The graph shows that the Rg values of both ligand-protein complexes fluctuate but are still in the range of 1.92-1.98 nm until the end of the simulation, and the movement of the ligand-protein complexes resemble each other (Elfita, *et al.*, 2023).

The graph shows the results of hydrogen bonding interactions over a simulation time of 20,000 ps. Based on the graph, the hydrogen bonding interaction in both complexes is relatively stable per unit time. Hydrogen bonds were detected to be stable from the beginning of the simulation until the time of 20,000 ps in both complexes. The Herceptin complex has more hydrogen bonds. The lower the hydrogen bonds, the more deviations can be observed, which correlates with the RMSD results (Priya, *et al.,* 2017).

CONCLUSION

These results showed that the chemical components of saffron plants have the potential as breast anticancers by inhibiting the HER-2 receptor. Crocetin and Herceptin showed stability in interaction to HER-2 Protein.

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