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Molecular Docking Simulation Study to Identify Potential Phytocompound inhibitors for HER2 as Anti-breast Cancer Agents

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Abstract

The second most frequent kind of cancer among women worldwide is breast cancer. A large number of anticancer medications have been developed as a result of our growing understanding of the molecular mechanisms driving this cancer progression. However, throughout the past few decades, the usage of medications made chemically has not appreciably increased the general survival rate. As a result, innovative chemoprevention techniques and new strategies are required to enhance the effectiveness of current cancer therapies. In the process of finding new drugs, molecular docking is a crucial technique for looking for possible hits. In this study, we docked phytochemicals and looked at ligands' affinities for binding to HER2. Among the examined phytochemicals, Quercetin-3-O-rutinoside (Rutin), Tamarixetin, and Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside have demonstrated higher binding affinities and energies towards specific target protein HER2. These compounds may be preventing ATP from connecting to HER2's ATP-binding sites, which would decrease HER2's kinase activity and impede downstream signaling pathways that support cell growth and survival. These phytochemical compounds can be used in new ways to treat cancer. This *in-silico* work provides a strong ground for further investigation of their anticancer activity and further required to validate these results by *in vitro* and *in vivo*.

Keywords: HER2, Molecular Docking, Breast Cancer, Rutin, ATP, *Insilico*

Introduction

Cancer continues to be one of the world's most contagious illnesses. By 2020, India was expected to see 2.7 million new cases and almost 8.5 lakh deaths, according to the Indian Council of Medical Research (ICMR, 2020). Additionally, the Middle East and North Africa (MENA) region will see an increase in new cancer cases of over 20 million before 2025, according to the World Health Organization (Lewandowska*et al*., 2019; Canfell*et al*., 2020). This is a result of scarce resources in healthcare facilities, a dearth of cancer early detection programs, and subpar therapeutic strategies (Birnbaum *et al*., 2018; Horton *et al*., 2015). For instance, it is regarded as the second main cause of mortality in India, behind cardiovascular illnesses, surpassing breast cancer as the most common malignancy (Abdel *et al*., 2020; Al *et al*., 2010). Over ten years, the number of cancer cases grew by 44%, going from 3362 to 4849 (2000-2010) (Abdel *et al*., 2020). According to reports, effective cancer prevention and treatment methods include surgery, radiation, chemotherapy, hormone therapy, and immunotherapy (Canfell*et al*., 2020) have been reported.

Cancer is still regarded as a terrifying disease, despite substantial research into it and the development of related treatments (Boopathy*et al*., 2010). Its non-selectivity toward cancer cells over non-cancerous cells, however, limits its utility and makes therapy ineffectual. The primary issues with chemotherapeutic medications at the moment include nonspecific function, drug resistance, and significant adverse effects (Xu*et al*., 2001; Mansour *et al*., 2018). Therefore, there is a pressing need for in-depth research to discover complementary and alternative therapies. Researchers have discovered that a variety of natural materials, including herbs, can be employed as therapeutic agents in chemoprevention using high-

performance screening (Choudhari*et al*., 2020). These plants exhibit strong anti-cancer action, according to scientific studies on the effectiveness and safety of herbal treatments, and the majority of them are now undergoing clinical trials (Weli*et al*., 2014; Kang *et al*., 2005; Mahoammadi*et al*., 2016).

Humans receive medicinal plants as a gift from nature to aid in their quest for improved health. Since the beginning of time, mankind has recognized and utilized natural resources as the primary source of therapeutic medications. They continue to be a great source of powerful bioactive chemicals that can be utilized right away as medications (Wright, 2019; Siddiqui *et al*., 2020). Numerous plants have been reported to possess a variety of biological properties, including the ability to treat cancer, rheumatism, exhalation channel infections, menstrual disorders, wounds, skin illnesses, monthly irregularities, and menopausal symptoms (Talib*et al*., 2020; Ohue, 2019). Approximately 2,50,000 plant species make up the plant kingdom, but only about 10% of these have been examined or found as medicines for the treatment of various ailments (Iqbal *et al*., 2019; Ijaz*et al*., 2018). Therefore, various plant parts, such as the flower, seed, stem, bark, fruit, leaf, and embryo, contain phytochemical qualities of various plants and their derived bioactive analogues. Moreover, it is well known that medicinal plants produce a large number of bioactive metabolites with a variety of pharmacological properties, including anti-diabetic, anti-osteoporotic, antimicrobial, hepatoprotective, anti-inflammatory, antimalarial, anti-ageing, immunomodulator, antioxidant, antihypertension, anticancer, and others (Majalo*et al*., 2019; Patel *et al*., 2020).

There are several plant chemicals with various biological features that have not been well investigated for their anticancer action. Apples, onions, and tea are just a few examples of the many fruits and vegetables that contain quercetin-3-O-rutinoside, a flavonoid glycoside. According to studies (Apak*et al*., 2016), it contains anti-inflammatory, anti-diabetic, and antioxidant properties. Numerous cereal crops, such as wheat and maize, contain the hydroxamic acid derivative 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). Its antibacterial and insecticidal qualities have been demonstrated (Wang *et al*., 2022). A flavonoid called tamarixetin is present in many plants, including citrus fruits and onions. According to studies (Sarker*et al*., 2020), it possesses anti-inflammatory, anti-aging, and antioxidant properties. A flavonol glycoside called kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside is present in numerous plants, including strawberries and grapes. According to studies (Lee *et al*., 2010), it contains anti-inflammatory and antioxidant properties. A coumarin derivative called esculetin is present in many plants, including citrus fruits and horse chestnuts. It has been demonstrated to have anti-inflammatory, antioxidant, anti-diabetic, and cardiovascular disease prevention effects (Andrade *et al*., 2020). It is crucial to remember that even though these compounds anticancer activity hasn't been well investigated, they might still have other advantageous qualities and prospective use in medicine and health. Therefore, the purpose of this work is to use *in silico* docking simulation studies to forecast the anti-cancer efficacy of the chosen phytochemical compounds.

Materials and Methods

Selection and Preparation of Ligands

The three flavonoids (Quercetin-3-O-rutinoside, Tamarixetin and Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside) and two coumarin derivatives (Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and Esculetin) were selected for the docking simulation studies. These five compounds were retrieved from PubChem database in .SDF format and converted to PDB file format with using Open Babel software. These PDB files were converted to PDBQT in the AutoDock 4.2 software. The selected phytochemical compounds and their PubChem CID's were listed in Table 1 and structure are depicted in Fig. 1.

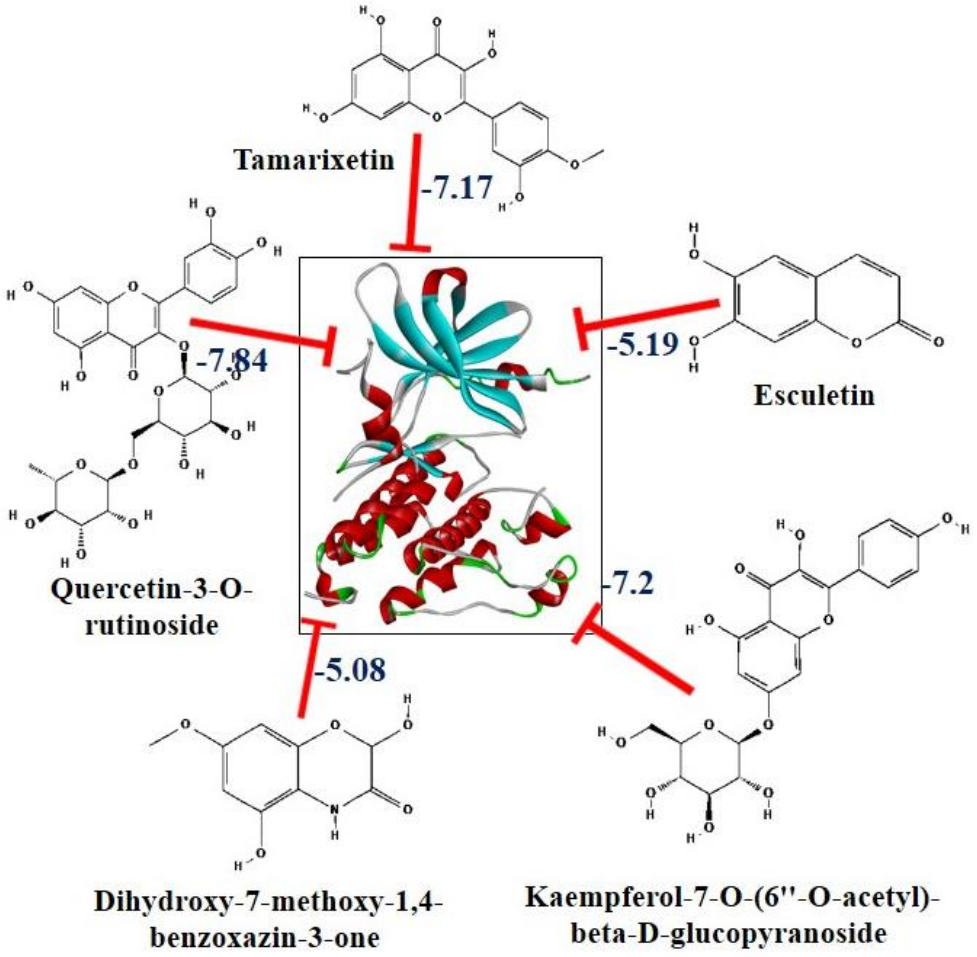

Fig. 1: Graphical abstract. Molecular docking of phytochemical compounds against HER2 with binding energies.

Table 1: List of phytochemical compounds with PubChem ID and structure selected for molecular docking against HER2.

Sl.No	Name of the Phytochemical Compound	PubChem CID	Molecular Formula
1	Quercetin-3-O-rutinoside (Rutin)	5280805	$C_{27}H_{30}O_{16}$
2	Tamarixetin	5281699	$C_{16}H_{12}O_7$
3	Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside	10095180	$C_{21}H_{20}O_{11}$
4	Dihydroxy-7-methoxy-1,4-benzoxazin-3-one	54061248	$C_9H_9NO_5$

5	Esculetin	5281416	$C_9H_6O_4$
6	Tak-285 (Original inhibitor)	11620908	$C_{26}H_{25}ClF_3N_5O_3$

Selection of Target Protein

According to the Indian Council of Medical Research (ICMR), the most common types of cancer in India are breast, lung, and cervical cancer, followed by oral, colorectal, and prostate cancer (ICMR, 2020). Target proteins with PDB IDs for anticancer activity vary depending on the specific type of cancer and the therapeutic approach. For breast cancer, lung cancer and cervical cancer, the target proteins or enzymes are Human epidermal growth factor receptor 2 (HER2) - PDB ID : 3RCD, Epidermal growth factor receptor (EGFR) - PDB ID : 4ZAU, and Poly(ADP-ribose) polymerase-1 (PARP-1) - PDB ID : 6BK4, respectively (Deonarain*et al*., 1994). In this study, HER2 target enzyme was selected to target the most prominent breast cancer (Fig. 2).

Protein Preparation for Docking Simulation

X-ray crystallographic structure of HER2 in complex with TAK-285 (PDB ID: 3RCD; resolution: 3.21 Å; R-value free: 0.294)) was retrieved from RCSB protein database (https://www.rcsb.org/structure/3RCD). The protein has A, B, C and D chains with 338amino acid sequence length. For the energy minimization of target protein, AutoDock version 4.2 andAutoDock Tools (ADT) version 1.5.6 was used (Davella*et al*., 2021). All the chains are similar, therefore except A, the B, C and D chains were removed from the complex. Water and co-crystal ligand molecules were eliminated from PDBs structure. Gasteiger charges and hydrogen atoms were added to each atom and the non-polar hydrogen atoms were merged.

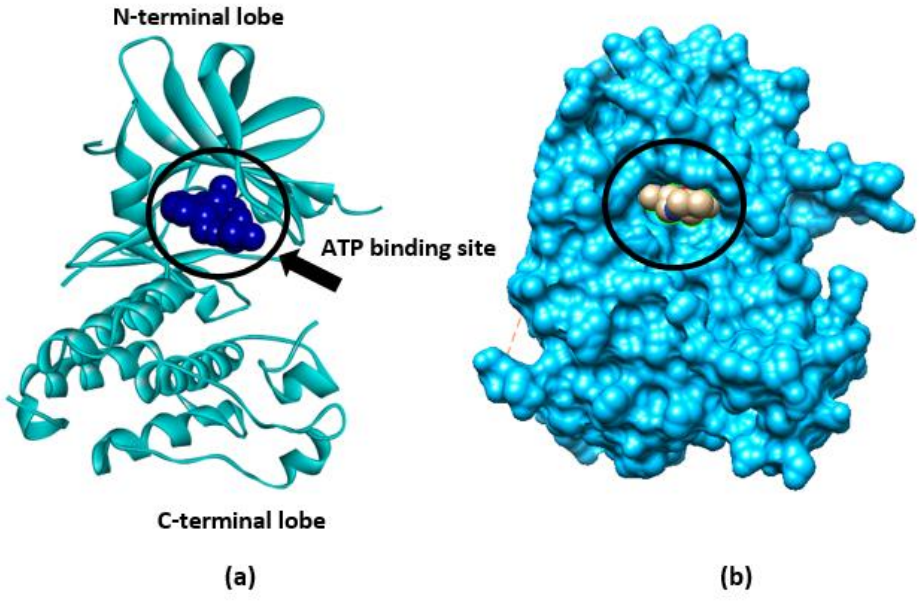

Fig. 2: Crystallographic structure of HER2 (PDB code: 3RCD) with its inhibitor.

(a) With ATP binding site highlighted in CPK space-filling sphere model with dark blue colour; (b) HER2 surface representation with ATP binding pocket in gray colour. The visualizations of 3D structure and surface representation are made using the crystal structure of HER2 by Chimera 1.6 software

Molecular Docking Simulation Studies

The target protein was converted to PDBQT (Protein Data Bank, Partial Charge (Q), & Atom type (T)) format utilizing ADT tools in order to run a docking simulation. In each experiment, the optimum position for each ligand in the receptor's active site was determined using the Lamarckian genetic algorithm (LGA) search approach.A grid box of 40x40x40 points in x, y, and z directions were built and centered on the center of the ligand in the complex with a spacing of 0.375 A^0 for 3RCD enzyme. The number of points for 3RCD in x, y, and z was 12.48. 2.964 and 28.015 consequently. The final step was applying a Lamarckian genetic algorithm to the following protocol: 150 run trails, 50,000,000 energy evaluations, 30,000 generations at most, 200 population members, a mutation rate of 0.02, a crossover rate of 0.8, and a docking simulation elitism value of 1. The docking results were assessed by classifying the docking energy anticipated by docking confirmations. The interaction of the protein-ligand was assessed using the softwares Chimera (Davella*et al*., 2020) and Biovia Discovery Studio. In order to determine the optimum binding mechanism for the protein inhibitors, the docked ligand-receptor complexes were ultimately evaluated in terms of energy, hydrophobic interaction, and hydrogen binding.

Results

Molecular Docking Simulation:

The current study is an effort to identify anti-breast cancer compounds that may be considered for drug development to treat breast cancer.The top hits represented high binding energies with HER2 ranging from −5.08 to −7.84 kcal/mol, as shown in Table 2. Post-docking analysis of HER2 revealed that all five compounds (Quercetin-3-O-rutinoside (Rutin); Tamarixetin; Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside; Dihydroxy-7-methoxy-1,4-benzoxazin-3-one and Esculetin) were also found to bind inside the kinase domain, surrounded by interacting residues with noticeably high binding energy value that ranged from −5.08 to −7.84 kcal/mol.

Table 2: Binding energy values and the important amino acids in the active sties of HER2 protein which responsible for interactions.

Sl.N.	Ligand	Binding energy (kcal/mol)	ATP binding sites	No. of H bonds	H bond interactive amino acids	Other binding interactive amino acids
1	Quercetin-3-O-rutinoside (Rutin)	-7.84	Lys-75 Thr-862	7	Gln-799, Met-801, Asp803,Asn850, Arg849,Lys753, Cys805,	Leu800,Val734, Thr862,Val851, Leu726,Asp808, Phe1004,Gly804, Leu796, Leu785, Ser783, Thr798
2	Tamarixetin	-7.17	Met-774 Phe864, Thr862	4	Asp863,Lys753, Met801, Gln799	Gly865, Leu796, Met774, Phe864, Leu785, Thr862, Ser783, Thr798,

						Ala751, Leu800, Leu852, Leu726, Val734,
3	Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside	-7.2	Thr862, Phe864, Met774,	6	Asp-863, Lys-753, Thr-862, Ser-783	Leu726, Gly727, Gly729, Asn850, Met774, Phe864, Leu785, Val782, Arg784, Leu796, Val797, Thr798, Ile752, Ala751, Leu852, Val734
4	Dihydroxy-7-methoxy-1,4-benzoxazin-3-one	-5.08	Leu755, Thr862, Met774, Phe864	2	Ser783, Asp863	Phe864, Met774, Leu785, Leu755, Gly865, Leu796, Lys753, Thr862, Thr798, Arg784
	Esculetin	-5.19	Thr862,	3	Met801, Asp863	Val734, Ala751, Leu852, Leu726, Lys753, Ser783, Thr862, Thr798, Gln799, Leu800
5	TAK-285	-9.7	Thr862,	4	Met801,Leu785, Gly727, Gly729	Met774, Arg784, Phe752, Gln799, Ser783, Thr798, Thr862, Ala751, Val797,Phe1004, Leu852, Leu800, Gly804, Leu726, Thr733, Ser728, Gly732, Val734, Lys753, Leu796, Asp863

HER2 Binding interactions with Quercetin-3-O-rutinoside:

Among the five phytochemical compounds, Quercetin-3-O-rutinoside showed lowest binding energy which shows highest affinity to inhibit HER2. Quercetin-3-O-rutinoside showed a binding energy value of −7.84 kcal/mol with the docked HER-2. Quercetin-3-O-rutinoside interacted with 7 active site amino acids of HER2 namely Gln799,Met801, Asp803, Asn850, Arg849, Lys753, and Cys805by forming 7 hydrogen bonds (Table 2 and Fig. 3), elevenVdW bonds with Leu-800, Val-734, Val-851, Asp-808, Phe-1004, Gly-804, Leu-796, Leu-785, Ser-783, Thr-798, three Pi-Sigma bond withAla-751, Leu-852 and Leu-726 amino acid residues of HER2. To validate the docking procedure, co-crystal ligand inside the PDB file (TAK-285) of 3RCD was extracted and re-docked with its target. The RMSD values of target was below 2 Å.

The crystal structure of HER2 complexed with TAK-285 (PDB ID: 3RCD) indicated Met801, Leu785, Gly727, Gly729, and Thr862 to be among the significant interacting residues which forms hydrogen bond. This inhibitor interactive with Met774, Arg784, Phe752, Gln799, Ser783, Thr798, Thr862, Ala751, Val797, Phe1004, Leu852, Leu800, Gly804, Leu726, Thr733, Ser728, Gly732, Val734, Lys753, Leu796

and Asp863 amino acid residues of HER2 with van der Waals, Pi-Sigma and other hydrophobic interactions. The X-ray co-crystallized structure of TAK-285 with HER2 demonstrated that it interacts with the expected residues in the respective ATP pocket. But only it binds only one amino acid (Thr-862) of ATP binding Binding-site interacting residues, as reported in the PDB, are shown in Table 2.

HER2 Binding interactions with Tamerixetin:

The Tamarixetin showed a binding energy value of −7.17 kcal/mol with the docked HER-2.The ligand Tamarixetin established 4 H-bonds with Asp863, Lys753, Met801 and Gln799, VdW bond interactions with Gly865, Leu-796, Met-774, Phe-864, Leu-785, Thr-862, Thr-798, Ser-783, Leu-800, Leu726 and Val-734, Pi-Sigma bond interaction with Leu-852 and Pi-Alkyl interactions with Ala-751 amino acid residues of HER-2 protein. This compound also binds to ATP binding sites (Met774, Phe-864, and Thr-862) of HER2 protein (Table 2, Fig. 4).

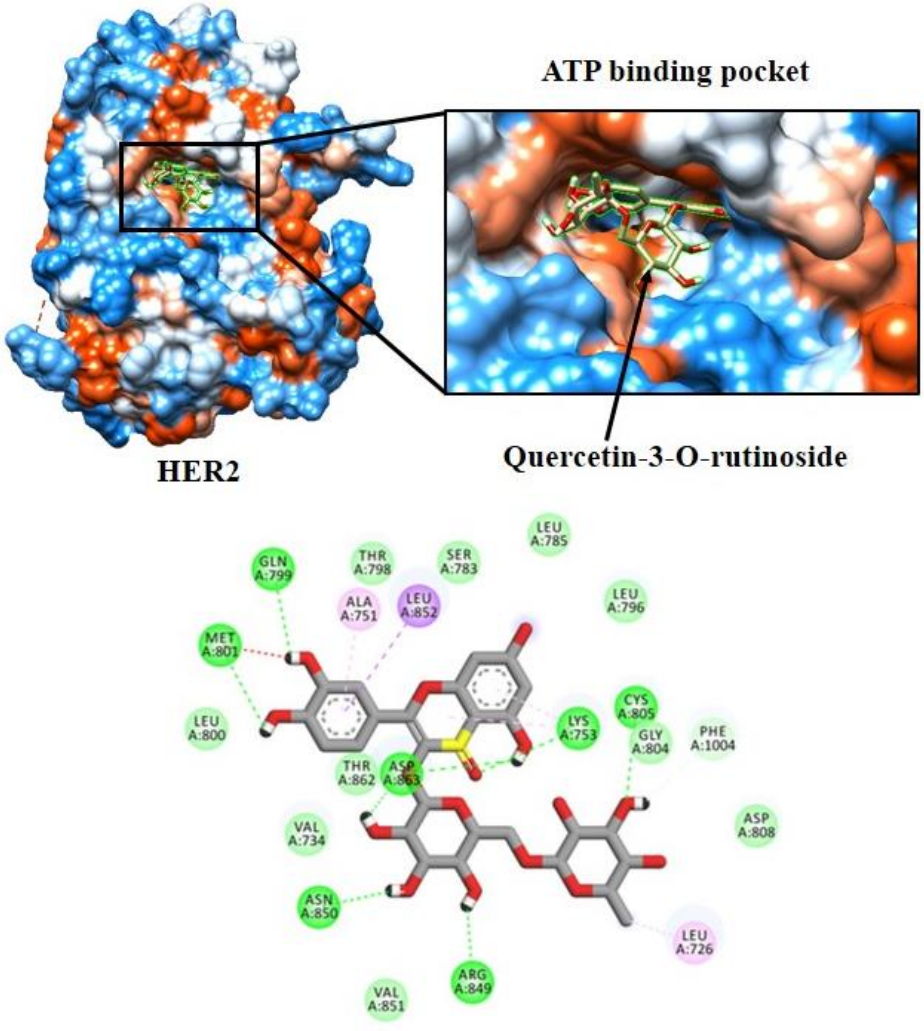

Fig. 3:Molecular surface representation of HER2 with Quercetin-3-O-rutinoside in stick format. Alongside 3D complex, 2D interaction plots indicate important binding site interactions between ligand and binding-site residues (green colour dash lines and balls indicates hydrogen bond interactions)

HER2 Binding interactions with Kaempferol-7-O-(6''-O-acetyl)-beta-D-glucopyranoside:
Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside showed -7.2 kcal/mol binding energy and it established six H-bonds with Asp-863, Lys-753, Thr-862 and Ser-783, twelve VdW bonds with Leu-726, Gly-727, Gly-729, Asn-850, Met-774, Phe-864, Val-782, Arg-784, Leu-796, Val-797,Ile=752, Leu-852, three Pi-sigma bond with Ala-751, Thr-798, Leu-785 and one Pi-Alkyl interaction with Val-734 amino acid residues of HER2 (Table 2, Fig. 5). Among the total eleven ATP binding sites, Thr-862,Phe-864 and Met-774 amino acids residues of HER2 are involved in interaction with Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside.

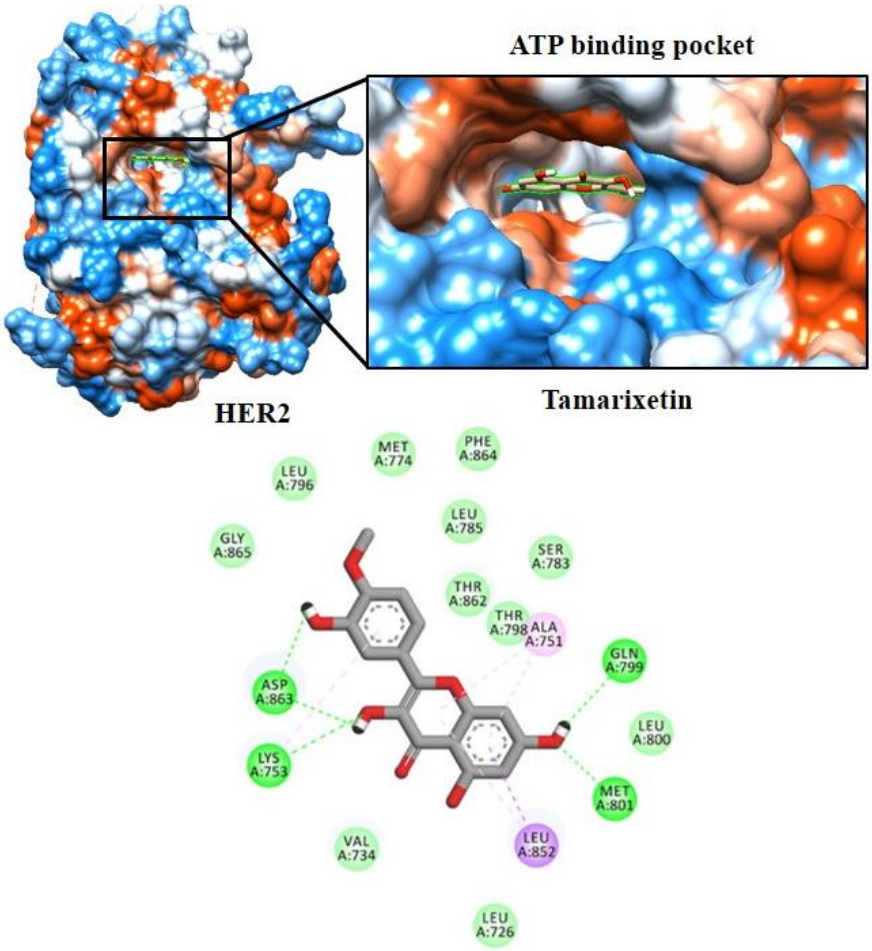

Fig. 4:Molecular surface representation of HER2 with Tamarixetin in stick format. Alongside 3D complex, 2D interaction plots indicate important binding site interactions between ligand and binding-site residues (green colour dash lines and balls indicates hydrogen bond interactions)

HER2 Binding interactions with Dihydroxy-7-methoxy-1,4-benzoxazin-3-one:
Dihydroxy-7-methoxy-1,4-benzoxazin-3-one showed -5.08 kcal/mol binding energy value with HER2. This compound established two H-bonds with Ser-783, Asp-863; eight VdW bonds with Phe-864, Met-774, Leu-785 Leu-755, Arg-784, Thr-798, Thr-862 and Lys-753; and one Pi-Alkyl bond interaction with Leu-796 amino acid residues of HER2 (Table 2, Fig. 6). This compound binds with Leu-755, Thr-862,

Met-774 and Phe-864 amino acid residues of HER2 which the ATP binding sites are showed strongest affinity and inhibition of HER2 activity.

HER2 Binding interactions with Esculetin:

Esculetin established three H-bonds with Met-801 and Asp-863 amino acid residues of HER2. Seven VdW bond interactions formed with Leu-800, Gln-799, Thr-798, Thr-862, Ser-783, Lys-753 and Leu-726; two Pi-Sigma bond interactions formed with Val-734, Ala-751 and one Pi-Sigma interaction with Leu-852 amino acids of HER2 protein. Only one ATP binding site (Thr-862) was involved in interaction with Esculetin among eleven(Table 2, Fig. 7).

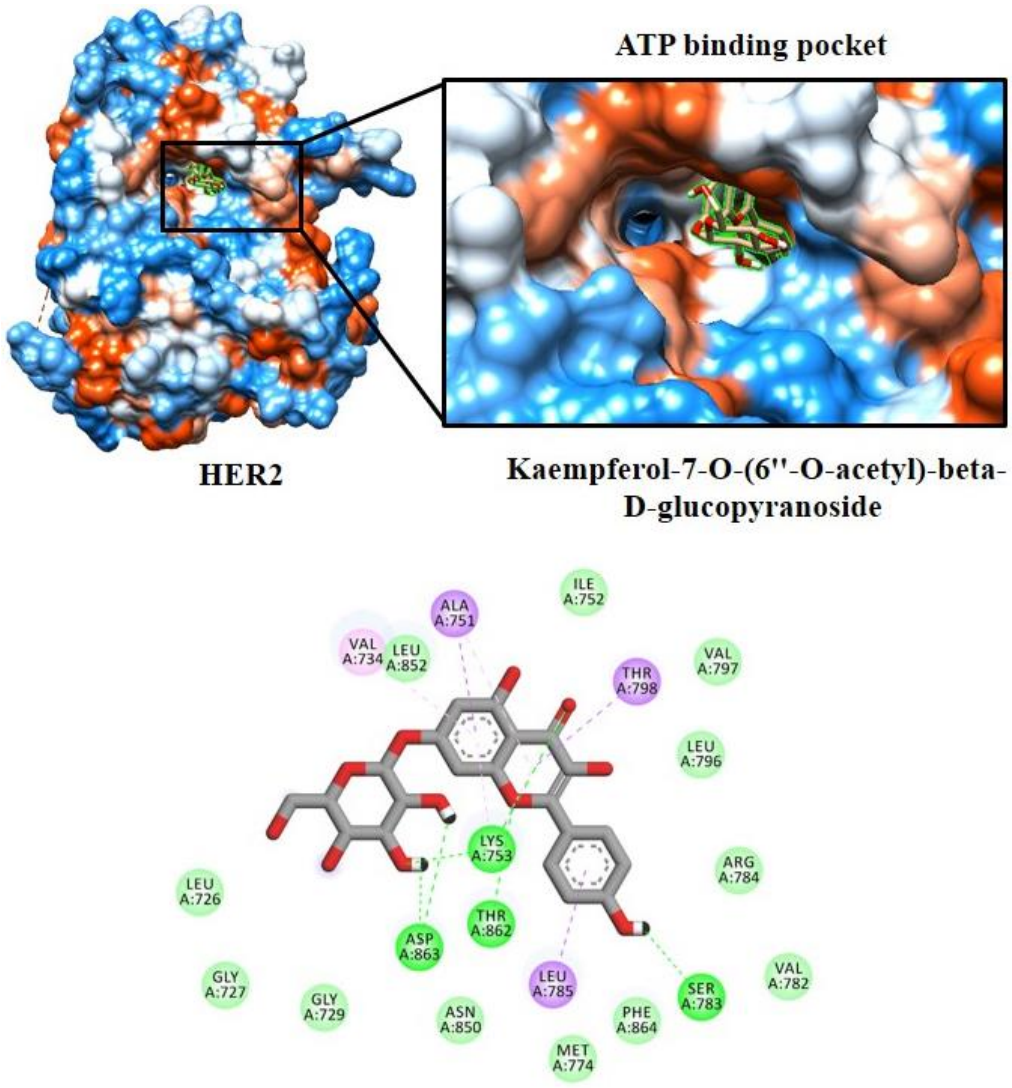

Fig. 5:Molecular surface representation of HER2 with Kaempferol-7-O-(6”-O-acetyl)-beta-D-glucopyranoside in stick format. Alongside 3D complex, 2D interaction plots indicate important binding site interactions between ligand and binding-site residues (green colour dash lines and balls indicates hydrogen bond interactions)

Based on the results of this study, the natural compound candidates have potential as biologically active compounds with improved stability in HER2. Designing HER2 inhibitors with carbonyl, carboxyl, and hydroxyl groups available for H-bond formation may improve protein-ligand stability.

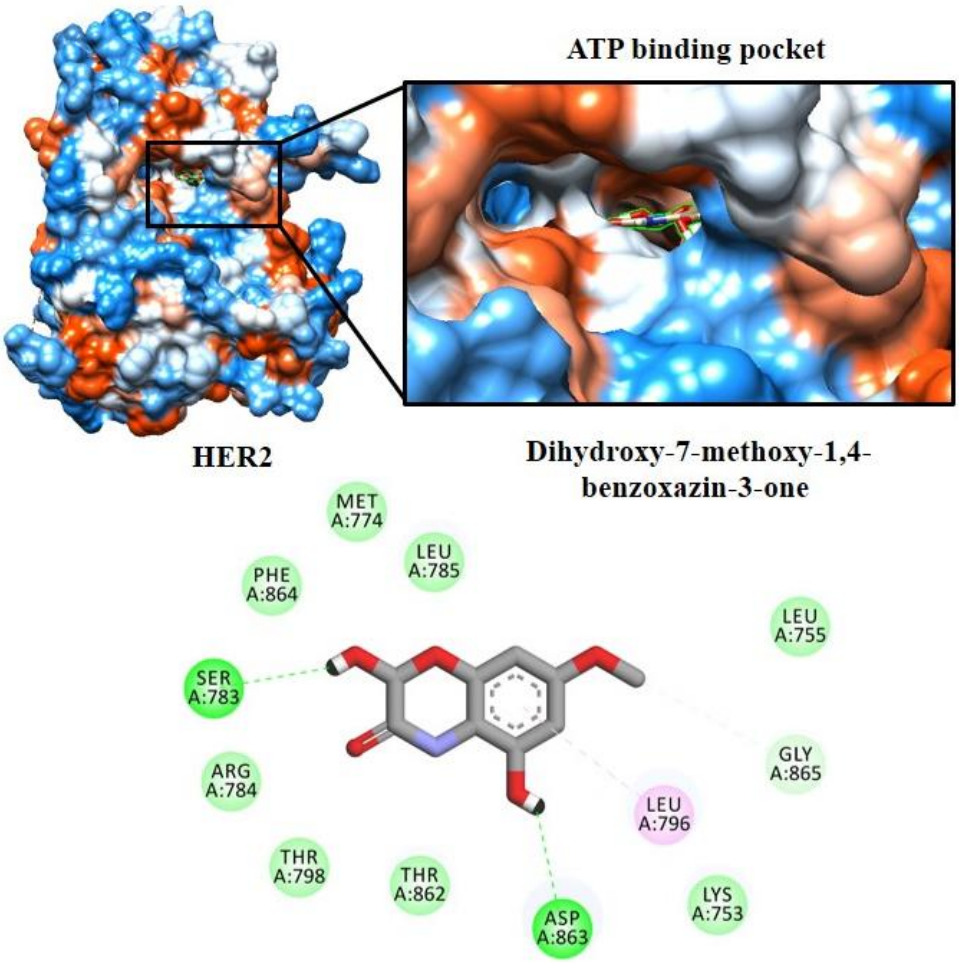

Fig. 6:Molecular surface representation of HER2 with Dihydroxy-7-methyoxy-1,4-benzoxazin-3-one in stick format. Alongside 3D complex, 2D interaction plots indicate important binding site interactions between ligand and binding-site residues (green colour dash lines and balls indicates hydrogen bond interactions)

Discussion

Human epidermal growth factor receptor 2 is another name for HER2 (Hu *et al*., 2018). It is a kind of protein that controls cell division and proliferation. HER2 is a member of the receptor tyrosine kinase family of receptors, which are found on the surface of cells and are in charge of relaying signals from the outside to the inside of the cell (Ayati*et al*., 2020). Growth factors are one type of signaling molecule that can activate HER2, which can then set off a series of biological events that ultimately result in cell growth and proliferation. Many cancer medicines target HER2, which plays a significant role in the initiation and advancement of some cancer types, including breast and gastric cancer (Lamichchane*et al*., 2023).

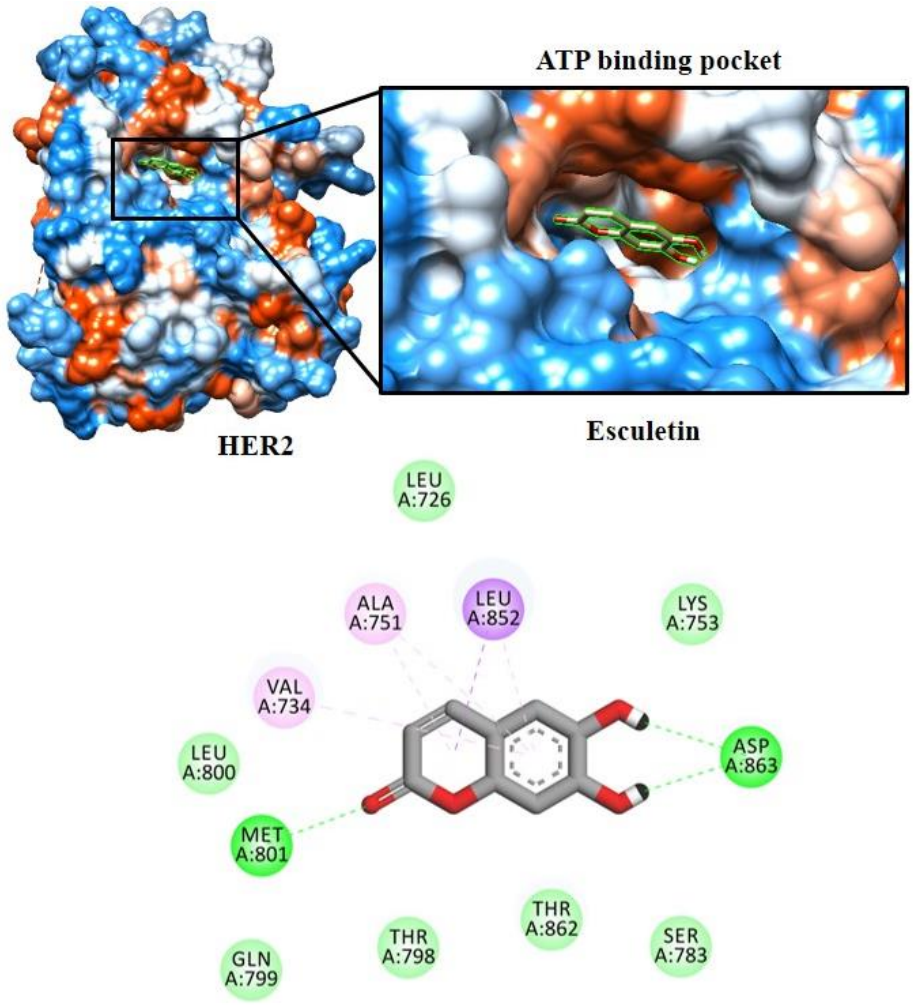

Fig. 7:Molecular surface representation of HER2 with Esculetin in stick format. Alongside 3D complex, 2D interaction plots indicate important binding site interactions between ligand and binding-site residues (green colour dash lines and balls indicates hydrogen bond interactions)

The current study's objective was to identify additional HER2 protein inhibiting agents. In order to find effective anti-breast cancer chemicals, the current *in silico* investigation was conducted. The intracellular kinase domain of HER2 (PDB ID: 3RCD) contains the protein's ATP-binding site. Several amino acid residues that help coordinate ATP and maintain the protein's active conformation make up the binding site. The amino acid residues that are implicated in the ATP binding site of HER2 are Lys-753, Asp-813, Glu-818, Lys-819, Ala-755, Gly-776, Leu-809, Leu-866, Met-774, Thr-862, and Phe-864 (Sait*et al*., 2020). Their enzymatic activity depends on these amino acid residues (Fujiki*et al*., 2015). As a result, targeted treatments that block their action have been developed for breast cancer patients who are HER2-positive. In this study, it was examined to see if the chosen phytochemical compounds bind to these ATP binding sites or not andalso compared with the original HER2 inhibitor drug TAK-285.

Molecular docking simulation confrmed the inhibitory action of selected phytochemical compounds in binding to HER2. The two compounds, Dihydroxy-7-methoxy-1,4-benzoxazin-3-one and Esculetin showed a free binding energy value ((ΔG bind) of −5.08 and −5.19 kcal/mol) in binding to HER-2, respectively. However, it showed weaker binding energy in binding to HER2 compared to Quercetin-3-O-rutinoside (Rutin) (-7.84 kcal/mol), Tamarixetin (-7.17 kcal/mol) and Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside (-7.2 kcal/mol), as selected compounds and original durg TAK-285 (-9.7 kcal/mol) as HER2 tyrosine kinase inhibitor. We confrmed that the main amino acids inside the active site of the HER2, which is responsible for essential interactions, are Lys-753, Asp-813, Glu-818, Lys-819, Ala-755, Gly-776, Leu-809, Leu-866, Met-774, Thr-862 and Phe-864.

Strong interactions between residues imply that binding with inhibitory compounds may be stable, leading to an inhibitory reaction (Iqbal *et al*., 2014). The top one (Quercetin-3-O-rutinoside) best virtual hits in complex with HER2 revealed strong binding affinities and highlighted several H-bonds and hydrophobic interactions between functional groups, and side chains of essential residues. The HER2 that complexed with TAK-285 showed the highest binding energy (−9.7 kcal/mol). Other three compounds showed binding energy in the range from −7.84 to −7.2 kcal/mol. Moreover, common interacting residues that displayed VdW and H-bond with different compounds were Leu-800, Val-734, Thr-862, Leu-726, Leu-796, Leu-785 and Ala-751, respectively (Table 2).

The kinase domain of HER2 contains the N-lobe and the C-lobe, which together make up the domain's ATP binding site (James *et al*., 2018; Solassol*et al*., 2019). The catalytic loop (amino acids 773–781) and the hinge region (amino acids 809–812), which joins the N-lobe and C-lobe, are two of the conserved amino acids that make up HER2's ATP binding site. The coordination of Mg2+ ions and the binding of ATP are both impacted by these residues (Bracht*et al*., 2010; Yusuf *et al*., 2018). In this investigation, three compounds-Rutin, Tamarixetin, and Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside were found to have greater affinity for HER2's ATP binding sites (Table 2). According to these findings, these three compounds may attach to the ATP binding site and prevent ATP from binding, hence disrupting downstream signaling pathways that support cell growth and survival and reducing the kinase activity of HER2.

This study finds that Rutin, Tamarixetin, and Kaempferol-7-O-(6"-O-acetyl)-beta-D-glucopyranoside have all demonstrated strong anti-cancer efficacy when tested against the HER2 protein using molecular docking simulations. To evaluate the practical value of these phytochemical compounds as an anti-cancer drug inducer, however, numerous *in vitro* and *in vivo* investigations are required.

Conclusion

One of the most common cancers in women worldwide is breast cancer. The development of drugs and the identification of specific inhibitors of several overexpressed proteins caused by breast cancer have both greatly benefited from the use of computational methods. The ATP binding site entry is blocked by binding to the important amino acids, which may decrease the function of HER2. This study identifies five phytochemical compounds with strong binding affinities to HER2, the most prevalent target protein linked to breast cancer. These virtual hits may be taken into consideration for early drug development against breast cancer after being tested through *in vitro* and *in vivo* investigations because of this prediction of binding to the ATP binding site of HER2.

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