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DECIPHERING HUMAN ESTROGEN RECEPTOR-2 INHIBITOR FROM *Momordica charantia*: COMPUTATIONAL MODELS AGAINST BREAST CANCER

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Abstract
HER-2, or Human Epidermal Growth Factor Receptor-2, is a constituent of the epidermal growth factor receptor family, possessing tyrosine kinase properties. Its over-expression has been correlated with breast cancer. On the other hand, the pharmacological potential of *Momordica charantia* has been attributed to the phytocompounds. Herein, the inhibiting potential of phytocompounds from *M. charantia* against HER-2 was investigated using computational approaches. Maestro Schrodinger software (2021 v 12.1) was used to perform molecular docking, molecular mechanics generalized Born surface area (MM/GBSA), and pharmacokinetics prediction of a hundred phytocompounds from *M. charantia* against HER-2. The result revealed that among the phytocompounds, five (5) showed promising inhibitory potential comparable to the standard drug, Gefitinib. The MM/GBSA result showed that Rutin, Quercetin, Isoquerrcitrin, Folic Acid, and riboflavin formed a more stable complex with HER-2 than Gefitinib. The pharmacokinetics profile of the hit compounds showed that the hit compounds except riboflavin violated two or more of Lipinski's rule of five. In conclusion, the bioactive compounds found in *M. charantia* could potentially act as primary candidates for the creation of effective inhibitors targeting HER-2 in the treatment of breast cancer.
Keywords: Breast cancer; HER-2; *Momordica charantia*; Molecular Docking; ADMET

Introduction

Breast cancer stands as a pervasive and critical health concern, ranking as the most commonly diagnosed cancer among women globally and the second most frequently occurring cancer overall (Kolak *et al.*, 2017). This disease entails the uncontrolled growth of breast cells, resulting in the formation of tumors that, if left undetected and untreated, can spread throughout the body, ultimately leading to potentially fatal consequences (Dervan, 2001). Breast cancer typically originates within the milk ducts or the milk-producing lobules of the breast. Initially, it may manifest in a non-invasive state referred to as "in situ," which poses a relatively lower immediate threat to life. However, as cancer cells progress, they can infiltrate the surrounding breast tissue, a process termed "invasion." This invasive

growth gives rise to the development of tumors, subsequently leading to the emergence of palpable lumps or areas of thickened tissue (Kolak *et al.*, 2017).

The global impact of breast cancer transcends geographical boundaries, affecting women across all age groups. While breast cancer can strike individuals of any age post-puberty, its incidence notably rises in the later stages of life. Notably, the death rate associated with breast cancer remained relatively static from the 1930s to the 1970s, a period marked by the predominance of surgical interventions, particularly radical mastectomy (Sofowora *et al.,* 2013). However, significant advancements occurred from the 1990s onward. During this era, countries initiated breast cancer early detection programs, integrated with comprehensive treatment strategies encompassing effective medical therapies, medications, radiation therapies, and surgical procedures. These combined efforts contributed to a notable improvement in survival rates for breast cancer patients (Dervan, 2001).

Mounting evidence underscores the profound influence of lifestyle and environmental factors on the onset of breast cancer. Factors including a high-fat diet, regular alcohol consumption, and inadequate physical activity have been extensively linked to an increased risk of developing this disease. Proactive measures aimed at addressing and mitigating these factors through primary prevention hold tremendous potential for substantially reducing both the incidence and mortality rates associated with breast cancer. Additionally, secondary prevention strategies encompass a range of diagnostic procedures, including mammography, ultrasonography, magnetic resonance imaging, and breast self-examinations, along with advanced and highly accurate imaging techniques. These combined approaches play a pivotal role in early tumor detection or the identification of potentially precancerous lesions, thereby significantly enhancing the prospects for timely and effective interventions (Kolak *et al.*, 2017).

Approximately 15-20% of breast cancer cases exhibit an oncogenic amplification of the ErbB2 gene, which is responsible for encoding the transmembrane protein known as HER2. This distinct subset of tumors has long been classified as "HER2-positive" breast cancers, a categorization that has remained consistent for nearly two decades (Awuchi, 2019). Identification of HER2-positive breast cancers is typically based on specific criteria, such as a 3+ score determined through immunohistochemistry (IHC) or a 2+ score via IHC, coupled with the detection of ErbB2 amplification through fluorescent in situ hybridization (FISH). While these tumors are characterized by clinical and biological aggressiveness, significant progress has been made in prognosis and treatment thanks to targeted therapeutic interventions (Mohamed *et al.*, 2013).

Momordica Charantia, commonly known as bitter melon or bitter gourd, is a tropical and subtropical vine that has gained attention for its notable pharmacological properties. This plant has been used traditionally in various cultures for its medicinal benefits (Khalid *et al.*, 2021). One of its most well-known properties is its potential to help manage diabetes (Oyelere *et al.*, 2022). Bitter melon contains compounds that may mimic the action of insulin, helping to regulate blood sugar levels. Studies have shown that bitter melon extracts can improve glucose tolerance and reduce blood sugar levels, making it a promising natural remedy for diabetes management. Additionally, it possesses antioxidant properties, which can help combat oxidative stress and inflammation, contributing to its potential role in preventing

chronic diseases (Li *et al.*, 2020).

Beyond its role in diabetes management, *Momordica charantia* has exhibited anti-inflammatory, antimicrobial, and anticancer properties. The plant's extracts have been studied for their potential to inhibit the growth of cancer cells and induce apoptosis, suggesting a possible role in cancer prevention and treatment. Moreover, bitter melon has been investigated for its effects on weight management and obesity-related conditions. While more research is needed to fully understand and harness its pharmacological potential, *Momordica charantia* continues to be a subject of interest in the field of natural medicine and holds promise as a source of beneficial compounds for various health applications (Khalid *et al.*, 2021).

Methods

Utilizing the Schrödinger suite's Maestro computational simulations were conducted using a HP desktop system featuring an Intel CORE i7 processor and 16GB RAM.

Protein preparation

The crystal structure of HER2 in complex with TAK-285 was downloaded from the Protein Data Bank (PDB) with the identification number, 3RCD. Initially, the protein was imported into the workspace, and missing loops and chains were filled using Prime. Following this, refinement of the chain involved the removal of water molecules. The protein was optimized using PROPKA at pH 7.5, and water molecules beyond a 5 Å radius were eliminated before protein minimization. Energy minimization was carried out using the OPLS3e force field to attain a lower-energy conformation for the protein (Bodun et al., 2023).

Ligand preparation

More than 100 phytochemicals reportedly found in Momordica charantia were downloaded from the PubChem database in SDF format. These phytochemicals underwent preparation using the LigPrep module with the OPLS3 force field (Omoboyowa *et al.,* 2022). Tautomer generation was omitted, and stereoisomer calculation was limited to a single isomer per ligand. The output was formatted in maestro format. Additionally, Gefitinib was chosen as a standard ligand. Similar preparation steps were applied to this reference compound, serving as a benchmark for comparative analysis.

Receptor grid generation

To prepare for ligand-receptor docking, a receptor grid was generated utilizing the Glide module, outlining the active site (Omoboyowa *et al.,* 2022). The grid was configured with specific parameters: a Van der Waals radius scaling factor of 1.0, a partial charge cutoff of 0.25, and default settings for site constraints, rotatable groups, and excluded volumes. The resulting receptor grid was positioned at coordinates X: 13.42, Y: 2.75, and Z: 27.53.

Molecular Docking

The molecular docking methodology provides a valuable approach for investigating the interactions between small molecules and the binding sites of target proteins (Omoboyowa *et al.,* 2023). This procedure was conducted utilizing the Glide module within the Maestro interface of Schrödinger. Flexible docking involved employing ligands previously filtered and prepared via the LigPrep module, along with the receptor grid generated from the receptor grid module. Initially, the program was run in HTVS (High Throughput Virtual Screening) docking mode, and the top fifty compounds were chosen based on their docking scores for further evaluation. Subsequently, another round of ligand docking was carried out using the SP precision mode. The top compounds were once again filtered based on their docking scores. Finally, the top ten molecules selected

from SP underwent docking using the XP precision mode to conclude the analysis.

ADMET and Pharmacokinetics Analysis

The top five ligands from XP docking, as well as the standard ligand Gefitinib, were analyzed for their ADMET and Pharmacokinetics parameters using the Qikprop module (Olugbogi *et al.*, 2023).

Results and Discussion

Breast cancer is a major public health concern around the world. While the fatality rate may fluctuate, the impact remains significant, necessitating immediate attention (Sun *et al.*, 2017). The economic consequences of the breast cancer epidemic are significant. A continuing increase in cases could lead to economic pressures and recession in several countries, emphasizing the importance of scientific intervention. Proteins, particularly HER2, play critical roles in cancer cell reproduction (Tarantino *et al.*, 2020). As a result, targeting certain proteins, such as HER2, has emerged as a critical strategy in the development of breast cancer therapies. The study of antagonistic actions against proteins such as HER2 is critical because it may hinder the generation of key factors involved in the progression of cancer (Miglietta *et al.*, 2021).

Table 1: Docking and MMGBSA scores of top compounds and Standard.

Compound Name	Docking Score	MM/GBSA Score	MM/GBSA Coloumb	MM/GBSA H-bond	MM/GBSA Lipo	MM/GBSA Solv Gb
Rutin	-13.779	-41.21	-37.40	-6.18	-19.49	52.71
Quercetin	-11.895	-58.94	-38.11	-4.51	-9.30	31.83
Isoquercitrin	-11.351	-46.48	-30.77	-4.13	-15.31	32.22
Folic Acid	-9.772	-59.26	-31.95	-0.53	-7.21	16.22
Riboflavin	-9.600	-56.44	-30.78	-2.20	-8.62	19.65
Gefitinib	-8.662	-52.94	-6.21	-0.83	-19.60	22.82

Molecular docking is of great importance in the design of new drugs. It correctly predicts the experimental binding mode and affinity of a ligand/molecule within the binding site of the protein target (Fan *et al.*, 2019).

Table 2: Interacting amino acids at the active site with the lead compounds.

Compound name	Docking score	H-bond	Interacting residue	Hydrophobic interaction	Other interaction
Rutin	-13.779	7	ASP 808, CYS 805, GLN799, THR798, ASP863, LYS753, ARG849	LEU726, CYS805, LEU785,MET774, MET801,LEU800, LEU796, VAL734, ALA751,PHE864, LEU852,PHE1004	None

Quercetin	-11.895	9	ASP863, LYS753, ASN850, ARG849, GLN799, MET801, CYS805, ASP808,	LEU852, ALA751, VAL734, LEU800, MET801,CYS805, PHE1004,LEU726	None
Isoquercitrin	-11.351	9	ASN850, ASP863, LYS753, THR862, ASP808, CYS805, MET801, GLN799	LEU850, VAL734, PHE731, ALA751, LEU726,PHE1004, CYS805, LEU800, MET801	None
Folic Acid	-9.772	4	ASP863, SER783, CYS805, ASP808	LEU796, ALA751, VAL734, LEU726, LEU784, PHE 864, MET774, LEU852, CYS805, PHE1004	None
Riboflavin	-9.600	6	LYS753, ASN850, ASP863, THR862, MET801	ALA751, PHE731, VAL734, LEU852, LEU800, MET801, CYS805, LEU726, PHE1004	None
Gefitinib	-8.662	1	MET801	ILE750, ALA751, VAL734, PHE731, ALA730, LEU726, LEU785, LEU796,VAL797, LEU800, LEU852, MET801, CYS805, PHE1004	None

The docking scores ranged from -13.779 to -9.600 kcal/mol, where lower scores indicated stronger binding affinity. Rutin, having the highest docking score of -13.779 kcal/mol, demonstrated seven hydrogen bond interactions with ASP808, CYS 805, GLN 799, THR798, ASP 863, LYS 753, and ARG 849. Additionally, hydrophobic amino acids such as LEU 726, CYS 805, LEU 785, MET 774, MET 801, LEU 800, LEU 796, VAL 734, ALA 751, PHE 864, LEU 852, and PHE 1004 interacted with Rutin at the active site.

Quercetin, ranking second with a docking score of -11.895 kcal/mol, formed nine hydrogen bonds with ASP 863, LYS 753, ASN 850, ARG 849, GLN 799, MET 801, CYS 805, and ASP 808. It also interacted with various hydrophobic amino acids, including LEU 852, ALA 751, VAL 734, LEU 800, MET 801, CYS 805, PHE 1004, and LEU 726. Isoquercitrin, with a docking score of -11.315 kcal/mol, established hydrogen bond contacts with ASP 145, LEU 83, and HIE 84. Similar hydrophobic interactions occurred with several amino acids such as LEU850, VAL 734, PHE 731, ALA751, LEU 726, PHE 1004, CYS 805, LEU 800, and MET 80.

Folic acid exhibited a favorable docking score of -9.772 kcal/mol, forming hydrogen bonds with ASP 863, SER 783, CYS 805, and ASP 808. Additionally, it interacted with hydrophobic amino acids such as LEU 796, ALA 751, VAL 734, LEU 726, LEU 784, PHE 864, MET 774, LEU 852, CYS 805, and PHE 1004 (Supplementary Figure 4). Riboflavin, with a docking score of -9.600 kcal/mol, engaged in six hydrogen bond interactions with LYS 753, ASN 850, ASP 863, THR 862, and MET 801. Hydrophobic contacts involved ALA 751, PHE 731, VAL 734, LEU 852, LEU 800, MET 801, CYS 805, LEU 726, and PHE 1004 (Supplementary Figure 5). Geftinib, the reference drug, demonstrated a docking score of -8.662 kcal/mol, forming one hydrogen bond interaction with MET 801 and interacting with hydrophobic amino acids such as ILE 750, ALA 751, VAL 734, PHE 731, ALA 730, LEU 726, LEU 785, LEU 796, VAL 797, LEU 800, LEU 852, MET 801, CYS 805, and PHE 1004 (Table 1; Supplementary Figure 6). This study's findings align with another study indicating the significant interacting amino acid residues at the HER2 catalytic site (Sait et al., 2020). Table 1 also presents the free binding energy for the top five compounds and the reference drug. The MMGBSA results consistently aligned with the superior docking scores of the compounds in comparison to the standard drug, with all compounds exhibiting better predicted MMGBSA scores than the standard drug's -52.94, and folic acid achieving a remarkably high MMGBSA score of -59.26 (Table 1).

Table 3: Pharmacokinetic properties and drug-likeness of top compounds and Standard.

Compound Name	QplogHERG	QPPCaco	QPPMDCK	QPlogKp	RuleofFive
Rutin	-5.552	0.522	0.149	-7.718	3
Quercetin	-5.311	5.408	1.754	-6.289	2
Isoquercitrin	-5.052	4.426	1.412	-6.303	2
Folic Acid	-2.589	0.028	0.010	-8.315	2
Riboflavin	-4.305	24.050	8.801	-5.489	0
Gefitinib	-6.093	1065.526	2289.157	-2.702	0

No of violations of Lipinski's rule of five (MW <= 500; LogP <= 5.00; HBD <= 5; HBA <= 10) (Range: maximum is 4)
QPlogHERG: Predicted IC50 value for the blockage of HERG K + channels. Concern below –5
QPPMDCK: Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier. QikProp predictions are for non-active transport. < 25 poor, > 500 great
QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier. <25 poor, > 500 great

Various criteria have been utilized to assess the drug-likeness of compounds, among which the Lipinski Rule of Five (RO5) is widely recognized (Karami et al., 2022). According to this rule, a molecule is deemed drug-like if it does not breach more than two of the following conditions: molecular weight less than 500 Da, octanol-water partition coefficient below 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors.

Table 4: Drug-likeness of top compounds and Standard.

COMPOUND NAME	MW	#H-bond acceptors	#H-bond donors	TPSA	iLOGP	log Kp (cm/s)
Rutin	610.52	16	10	269.43	0.46	-10.26
Quercetin	302.24	7	5	131.36	1.63	-7.05
Isoquercitrin	464.38	12	8	210.51	0.94	-8.88
Folic Acid	441.4	9	6	213.28	0.04	-9.76
Riboflavin	376.36	8	5	161.56	1.63	-9.63
Gefitinib	446.9	7	1	68.74	4.04	-6.11

The study's findings indicated that all compounds, except for Rutin, complied with the Lipinski rules. With an increasing number of compounds breaking these rules and entering the market, efforts have been made to adjust these parameters. The physicochemical properties, as calculated by Qikprop, are presented in Table 3. Pharmacokinetic properties of the compounds were assessed by predicting the IC50 value for HERG K+ channel blockade (QPlogHERG), MDCK cell permeability in nm/sec (QPPMDCK), and Caco-2 cell permeability in nm/sec (QPPCaco). Among the top compounds, except for Folic acid and Riboflavin, and the standard drug, fell within the acceptable range for HERG K+ channel blockade. Only the standard drug exhibited a favorable Caco-2 and MDCK cell permeability score, as shown in Table 4.

Conclusion

Medicinal plants are recognized as valuable sources of various bioactive compounds utilized in treating diverse ailments. In this investigation, I conducted in silico molecular docking analysis of phytoconstituents reportedly found in *M. charantia* against HER-2. Docking the HER-2 receptor with these phytochemicals revealed that the top five compounds, identified in this study, exhibited promising potential by displaying superior binding affinity to HER-2 compared to Gefitinib. However, further exploration through in vivo and in vitro approaches is recommended to elucidate the molecular mechanisms underlying the potential of these compounds as potent drugs against breast cancer.

References

Awuchi, C. G. (2019). Medicinal plants: the medical, food, and nutritional biochemistry and uses. *Inter J Adv Academic Res*, *5*(11), 220-241.

Bodun, D. S., Omoboyowa, D. A., Omotuyi, O.

I., Olugbogi, E. A., Balogun, T. A., Ezeh, C. J., & Omirin, E. S. (2023). QSAR-based virtual screening of traditional Chinese medicine for the identification of mitotic kinesin Eg5 inhibitors. *Comp Biol Chem*, *104*, 107865.

Dervan, P. A. (2001). *Understanding breast cancer*. McFarland.

Fan, M., Lee, J. I., Ryu, Y. B., Choi, Y. J., Tang, Y., Oh, M., and Kim, E. K. (2021). Comparative analysis of metabolite profiling of Momordica charantia leaf and the anti-obesity effect through regulating lipid metabolism. *Inter J Environ Res Public Health*, *18*(11), 5584.

Khalid, Z., Hassan, S., Shahzad, S., & Khurram, H. (2021). A review on biological attributes of *Momordica charantia*. *Adv Biosci Bioeng*, *9*(1), 8-12.

Kolak, A., Kamińska, M., Sygit, K., Budny, A., Surdyka, D., Kukiełka-Budny, B., & Burdan, F. (2017). Primary and secondary prevention of breast cancer. *Annals Agric environ Med*, *24*(4).

Li, Z., Xia, A., Li, S., Yang, G., Jin, W., Zhang, M., & Wang, S. (2020). The pharmacological properties and therapeutic use of bitter melon (*Momordica charantia L.*). *Current Pharma Rep*, *6*, 103-109.

Miglietta, F., Griguolo, G., Bottosso, M., Giarratano, T., Lo Mele, M., Fassan, M., and Dieci, M. V. (2021). Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer*, *7*(1), 137.

Mohamed, A., Krajewski, K., Cakar, B., & Ma, C. X. (2013). Targeted therapy for breast cancer. *The American J pathol*, *183*(4), 1096-1112.

Olugbogi, E. A., Arobadade, O. A., Bodun, D. S., Omoseeye, S. D., Omirin, E. S., Fapohunda, O., ... & Omotuyi, O. I. (2023). Identification of apposite antagonist for androgen receptor in prostate cancer: an in silico study of fenugreek compounds. *J Biomol Str Dyna*, 1-17.

Omoboyowa, D. A., Iqbal, M. N., Balogun, T. A., Bodun, D. S., Fatoki, J. O., & Oyeneyin, O. E. (2022). Inhibitory potential of phytochemicals from Chromolaena odorata L. against apoptosis signal-regulatory kinase 1: A computational model against colorectal cancer. *Computational Toxicol*, *23*, 100235.

Omoboyowa, D. A., Kareem, J. A., Saibu, O. A., Bodun, D. S., Ajayi, T. M., & Oyeneyin, O. E. (2023). Identification of Phyto-Compounds from Ilex kudingcha as Inhibitors of Sterol-14α-Demethylase Protease: A Computational Approach Against Chagas Disease. *Chem Africa*, *6*(3), 1335-1347.

Oyelere, S. F., Ajayi, O. H., Ayoade, T. E., Pereira, G. B. S., Owoyemi, B. C. D., Ilesanmi, A. O., & Akinyemi, O. A. (2022). A detailed review on the phytochemical profiles and anti-diabetic mechanisms of *Momordica charantia*. *Heliyon*, *8*(4).

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African J traditional, compl altern med*, *10*(5), 210-229.

Tarantino, P., Gandini, S., Nicolò, E., Trillo, P., Giugliano, F., Zagami, P., ... & Curigliano, G. (2022). Evolution of low HER2 expression between early and advanced-stage breast cancer. *European J Cancer*, *163*, 35-43.