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Unlocking the Synergistic Power of Gallic Acid and Resveratrol: A Multi-Target Strategy to Combat Breast Cancer

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Abstract

Background:

Triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2, is among the most aggressive breast cancer subtypes. Standard treatments like chemotherapy, radiotherapy, and surgery often result in high rates of recurrence and metastasis due to issues like drug resistance, particularly to agents like doxorubicin. This underscores the need for alternative, less toxic therapies. Recently, plant-derived compounds such as gallic acid (GA) and resveratrol have shown potential in targeting multiple cancer pathways.

Objectives:

This study aims to explore the synergistic effects of GA and resveratrol by investigating their molecular docking with receptors commonly implicated in TNBC, including HER2, ER, PR, and BRCA1.

Methods:

Ligand and receptor preparations were conducted using Autodock PyRx software. Molecular docking analyses were performed to assess binding affinities and interactions with active site residues on these receptors, evaluating the therapeutic potential of the combined GA and resveratrol approach.

Results:

Docking results indicated strong binding affinities of GA and resveratrol with HER2, ER, PR, and BRCA1. Key interactions included multiple shared binding residues across these receptors. The compounds exhibited robust binding energies, particularly at HER2's active site, suggesting potential for pathway inhibition.

Conclusions:

This study underscores the promising synergistic potential of GA and resveratrol as a multi-target approach for TNBC. These findings offer an alternative to conventional treatments, potentially reducing reliance on chemotherapy and its associated toxicities, and suggest further research into plant-based integrative treatments.

Key Words: Gallic acid, Resveratrol, Multi-target therapy,Molecular docking,

Triple-negative breast cancer (TNBC),

1. INTRODUCTION

Breast cancer is a prevalent malignancy that primarily affects women and leads to a high number of cancer-related fatalities worldwide. Over 500,000 women worldwide lose their lives to breast cancer each year, accounting for over 1.7 million new cases of the illness that are reported[1]. One of the three main types of breast cancer is triple-negative breast cancer (TNBC), which is characterized by the absence of ER, PR, and HER2 receptors. Sixty percent of instances of breast cancer are hormone receptor-positive (ER and PR), whereas fifteen to twenty percent of cases are HER2-positive (HER-1 to HER-4)[2]. Twenty percent of instances of breast cancer are triple-negative (TNBC), which lacks the expression of HER-2, progesterone receptors (PR), or estrogen receptors (ER). Because of this absence,

TNBC is more aggressive and challenging to treat, which has a negative prognosis and low survival rates[3]. Triple-negative breast cancer (TNBC) is resistant to hormone and targeted therapies due to its unique traits, making it difficult to treat with surgery, chemotherapy, and standard radiation therapy, which often results in higher metastasis and recurrence[4]. Doxorubicin, or Adriamycin, is a highly effective chemotherapy drug for TNBC, but its use is limited due to frequent drug resistance[5]. There is an urgent need for new, effective anti-tumor drugs with fewer side effects and lower toxicity for TNBC.

Studies show that plant extracts, especially from traditional Chinese medicine (TCM), can induce apoptosis in cancer cells. For instance, Brucea javanica and Cordyceps sinensis extracts have been found to reduce TNBC growth and enhance cancer cell death[6]. Extracts of Cordyceps sinensis can reduce breast cancer growth by activating the NF-κB pathway and enhancing M1 macrophage polarization, sparking interest in plant-derived anti-tumor drugs[7]. Recent research confirms gallic acid (GA) as a promising cancer-fighting agent due to its anti-tumor effects[8]. Several studies have found that problems with the PI3K/AKT signaling pathway are strongly linked to the development of breast cancer[9].Activated AKT signaling promotes breast cancer cell growth, survival, and metastasis[10].

Resveratrol is a promising anti-cancer drug that has been shown in multiple trials to affect the three separate stages of carcinogenesis, namely initiation, promotion, and progression. It affects a number of signaling pathways that control angiogenesis, inflammation, apoptosis, cell division, growth, and metastasis[11]. Many studies highlight resveratrol's anti-proliferative and apoptotic effects. It induces cell cycle arrest by lowering cyclin D1 levels and increasing the tumor suppressor p53 and the cdk inhibitor p21[12]. Resveratrol can either promote or inhibit breast cancer cell growth, depending on cell type and conditions, so its exact mechanism is still unclear[13]. Efforts are underway to find cancer treatments beyond radiotherapy and chemotherapy, which can stress patients and worsen their health. Multi-target therapeutics overcome the limitations of single-target treatments, offering greater effectiveness and reduced resistance. This study evaluates the synergistic effects of resveratrol and gallic acid in treating breast cancer, highlighting how their combined action may enhance therapeutic

outcomes compared to individual treatments. It also highlights the promise of multi-target therapies, which could offer more effective and durable treatment solutions.

2. MATERIALS AND METHODS

2.1. Ligand Preparation (Molecular Docking)

For docking investigations, the 3D structures of the pure chemicals were retrieved from the PubChem(https://pubchem.ncbi.nlm.nih.gov/). The conjugate gradient optimization approach with 200 steps was used to optimize the ligands using the Universal Force Field (UFF) energy minimization parameter. In order to get the lowest free energy, energy reduction was done using open babel in PyRx [14]. The results were then transformed into PDBQT forms for molecular docking research.

2.2 Protein Structure preparation

The three-dimensional crystal structures of HER2, accession number 3RCD, ER, accession number 5WAC, and PR, accession number 1zuc [15] BRCA1 with accession number 1JNX[16]target proteins that were obtained from PDB. They are improved by utilizing Biovia Discovery Studio Visualize [21] to eliminate unnecessary water molecules, add hydrogen atoms, and prepare the protein for docking studies. The protein is then saved in PDB format. and saved as PDB format. Using the PyRx tool, these processed protein structures are transformed into a PDBQT file by choosing the create macromolecule option[14]. The default values for the extra configuration options were also applied.

2.3 Setting grid parameters

The areas of macromolecules known as active binding sites are where ligand molecules bind to prevent illness. The most preferred binding sites for docking investigations are protein-ligand binding sites. Protein interfaces therefore function as universal binding sites for all ligands. The grid box in this study is configured to examine the protein-ligand interactions of the ER, PR, HER-2, BRCA1, and VEGF proteins[2].

2.4 Molecular docking study

The investigation on molecular docking was conducted using pure chemicals and the three-dimensional structure of the ER(5WAC), PR(1ZUC), HER-2(3RCD), BRCA1 (1JNX) and VEGF (1VFP) utilizing the Autodock PyRx docking tool on receptors [2].

2.5 ADMET analysis

With the development of in silico methods, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) features were predicted at the conclusion of the drug discovery pipeline. Early on, ADMET characteristics are predictable. In the course of drug development, the ADMET investigations exclude failed drug compounds and concentrate primarily on promising therapeutic candidates. Thus, conventional in vitro and in vivo tests can be replaced by these ADMET investigations. Drug research would experience a major cost savings if these qualities could be predicted early[22].

3. RESULTS AND DISCUSSION

3.1 Receptor Profiling

The Progesterone Receptor (PDB code: 1ZUC), BRCA1 (PDB code: 1JNX), Estrogen Receptor (PDB code: 5W9C), VEGF Receptor (PDB code: 1VFP), and HER-2 Receptor (PDB code: 7PCD) are among the important receptors linked to breast cancer that have undergone receptor study. Every receptor's structure was downloaded in the.pdb file format from the Protein Data Bank (PDB). (Figure 1).

PROCHECK and ERRAT were used for receptor analysis. Ramachandran plots, which highlight particular geometric aspects and allow for evaluation of the overall structural quality, were used in the PROCHECK assessment. A Ramachandran plot's quality for protein structure analysis is determined by how much non-glycine residue is present in prohibited regions—less than 0.8% is the threshold.

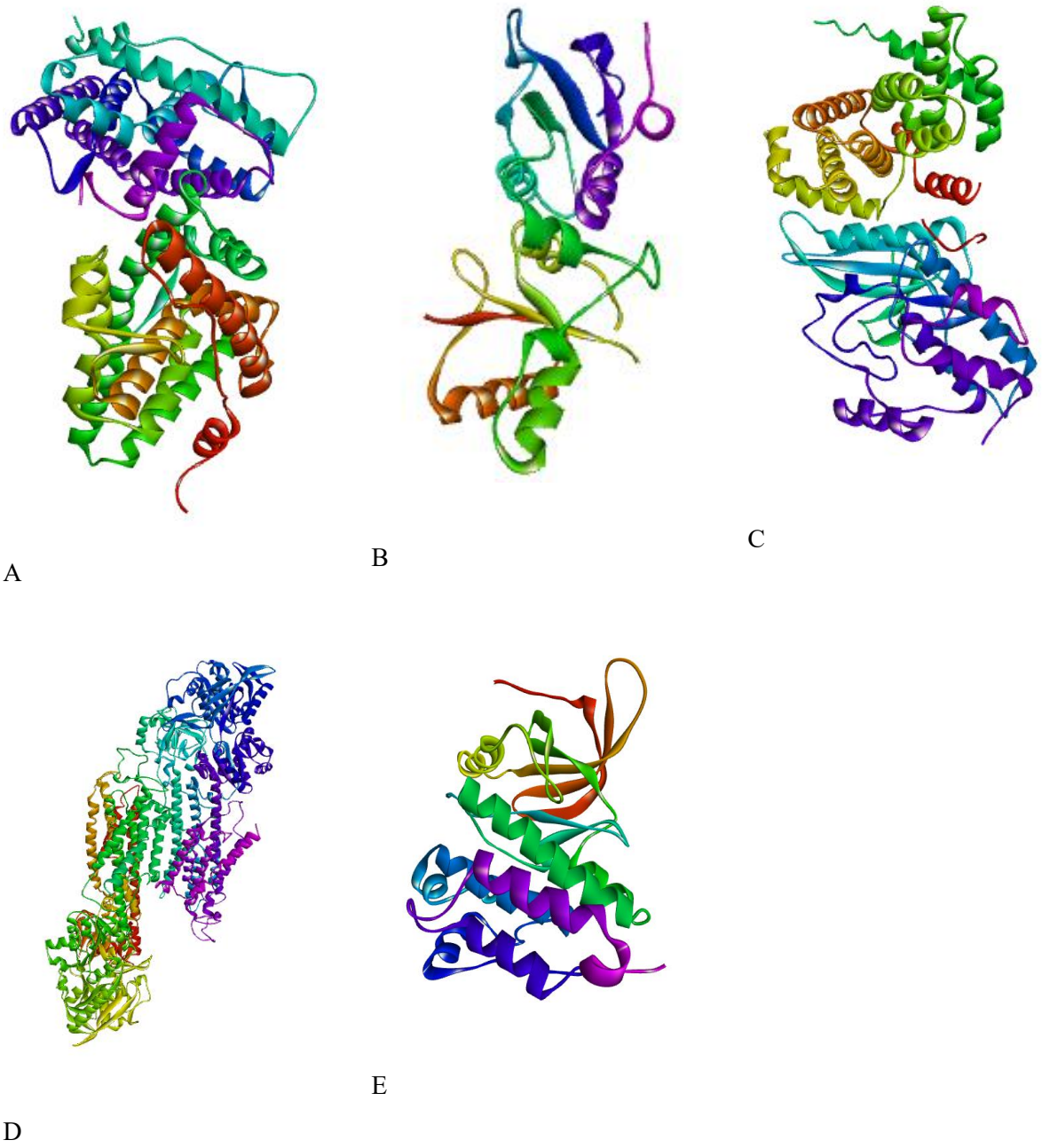


Fig 1: Progesterone receptor (1ZUC), BRCA1 (1jnx), estrogen receptor (5W9C), VEGF (1vfp), and HER-2 receptors (7PCD) are the receptors' respective structures.

The progesterone receptor (1ZUC) Ramachandran plot analysis shows that 93% of the amino acid residues are located in the most preferred regions and just 0.0% are found in the banned regions. Only 0.0% of the amino acid residues in the BRCA1(1vfp) receptor are located in the regions that are prohibited, compared to 82.8% in the most preferred regions. Only 0.0% of the amino acid residues in the prohibited regions of the estrogen receptor (5W9C) are found, compared to 95.6% in the most

favored regions. Just 0.0% of the VEGF (1vfp)'s amino acid residues are found in the prohibited regions, compared to 84.6% in the most recommended regions. The quantity of residues of amino acids in the most favored regions.

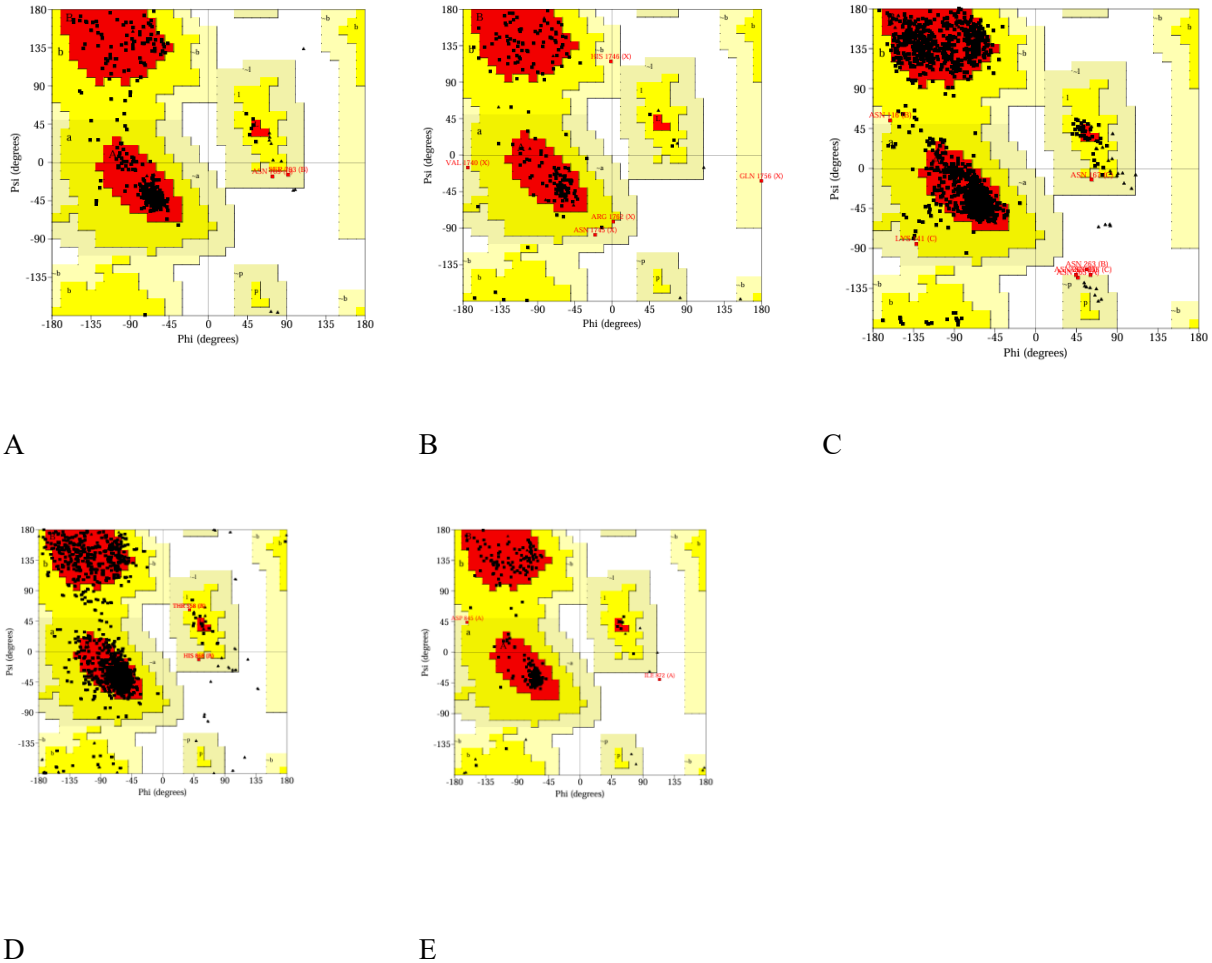
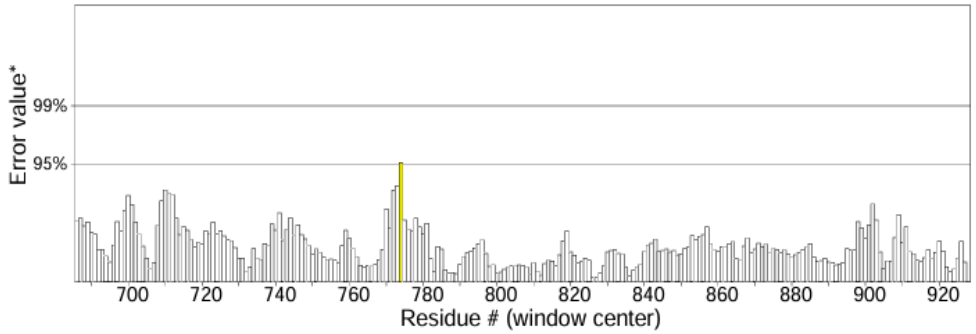
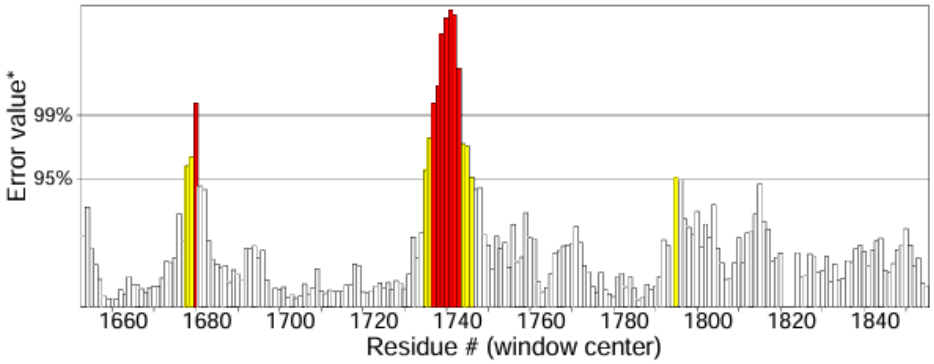


Fig 2: Progesterone receptor (1ZUC), BRCA1 (1jnx), estrogen receptor (5W9C), VEGF (1vfp), and HER-2 receptors (7PCD) are the receptors on the Ramachandran plot.

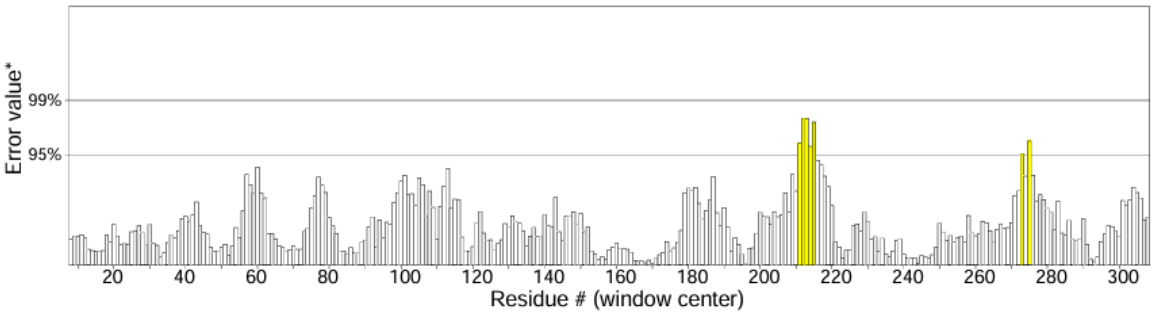
Overall quality factor**: 99.381



Overall quality factor**: 91.919



Overall quality factor**: 97.297



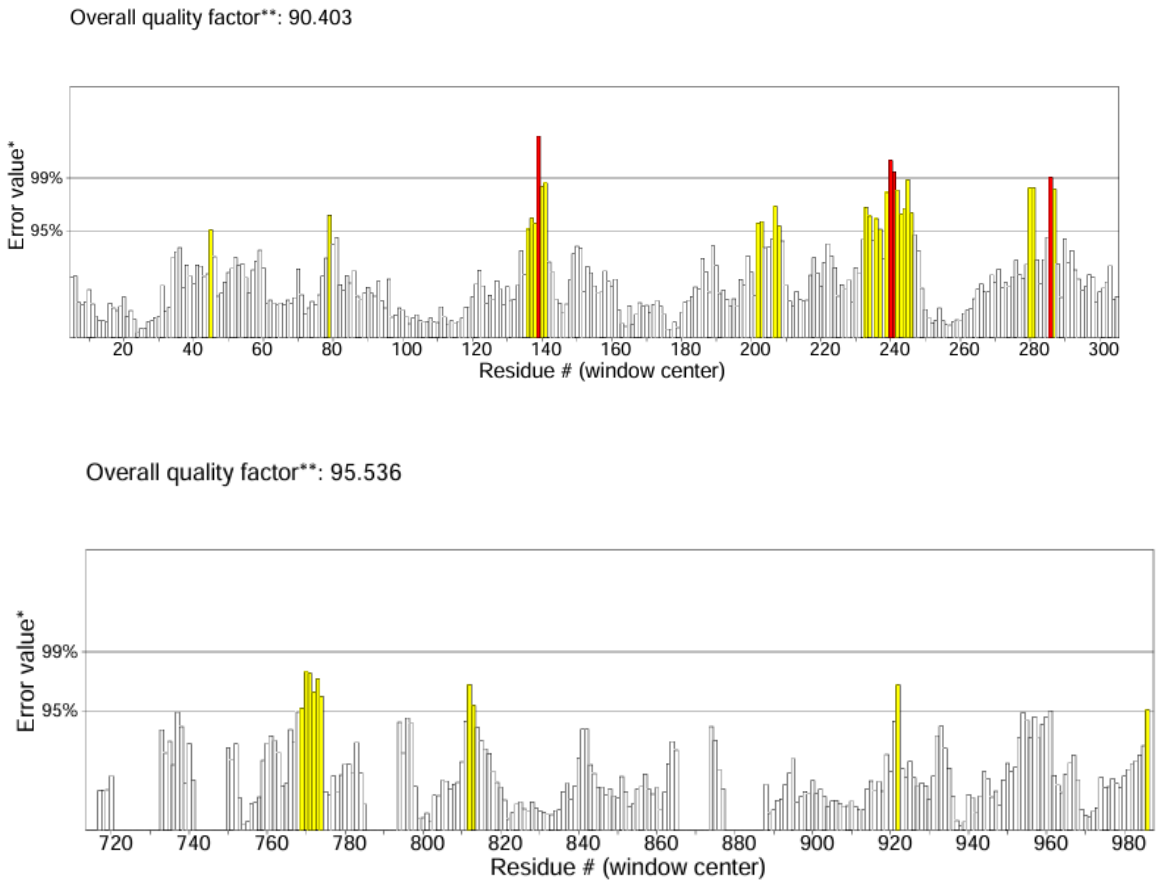


Fig 3: Results of the ERRAT analysis.

Based on the nature of atomic interactions, ERRAT assesses models by examining the statistical connections of unbound interactions between various atom types. Better model quality is indicated by a higher ERRAT score, with a score of more than 50% usually being suggestive of a stable protein structure.The quality factor scores, as indicated by the ERRAT analysis (Figure 3), were as follows: Progesterone receptor (1ZUC): 99.381%; BRCA1 (1JNX): 91.919%; estrogen receptor (5W9C): 99.247%; VEGF receptor (1VFX): 90.403%; and HER-2 receptor (7PCD): 95.536%. These findings imply that the HER-2 receptor (7PCD), BRCA1 (1JNX), estrogen receptor (5W9C), VEGF (1VFX), progesterone receptor (1ZUC), and estrogen receptor are all highly characterized by their protein structures. The investigation also shows that modeling flaws exist despite the excellent quality.

3.2 Results Analysis and Display of Molecular Docking Data

Discovery Studio software was used to show the docking results in both 2D and 3D formats (Figures X and Y). The interactions between the ligands with the lowest binding energies and the amino acid residues of the progesterone receptor (1ZUC), estrogen receptor (5W9C), VEGF receptor (1VFX), and HER-2 receptor (7PCD) were observed with the aid of these visualizations. The ligands created a variety of connections with the receptors, including hydrophobic bonds, Van der Waals contacts, conventional hydrogen bonds, Pi-Sigma bonds, and other kinds of molecular bonds, according to the docking study (Figure 5). The durability and strength of the ligand-receptor interactions are influenced by these connections.

Table 3.1 Chemical compounds' binding energies against receptors.

Receptor	Compounds	Binding Energy
1ZUC	Gallic Acid	-6.4
	Resveratrol	-8
1JNX	Gallic Acid	-4.8
	Resveratrol	-5.8
5W9C	Gallic Acid	-6.5
	Resveratrol	-7.5
1VFX	Gallic Acid	-6.4
	Resveratrol	-9
7PCD	Gallic Acid	-5.7
	Resveratrol	-7.6

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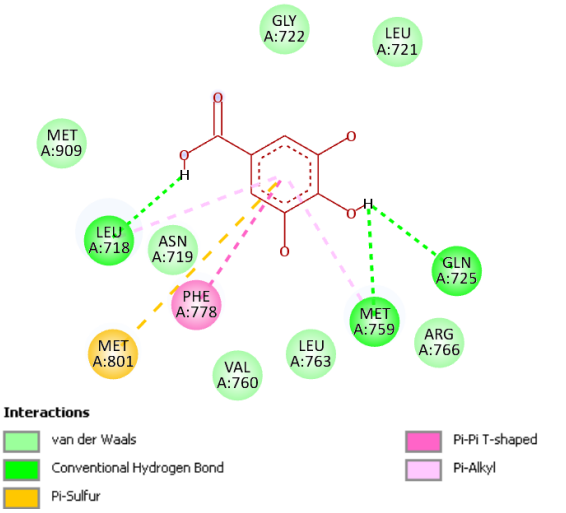

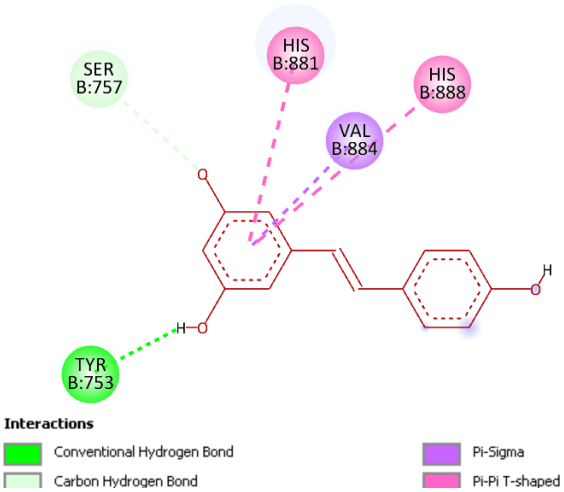

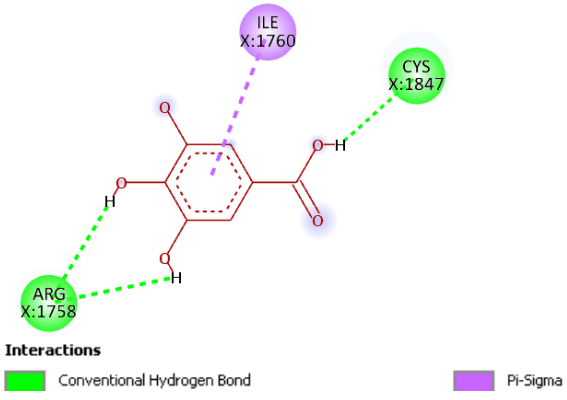

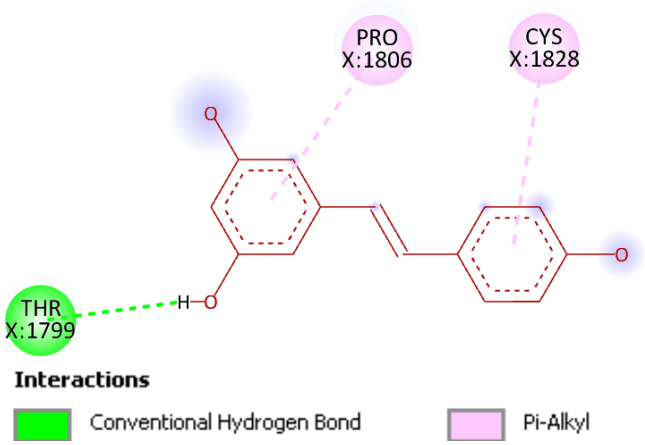

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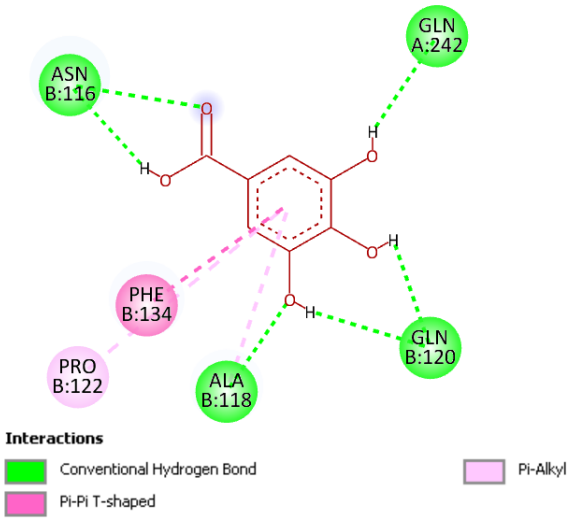
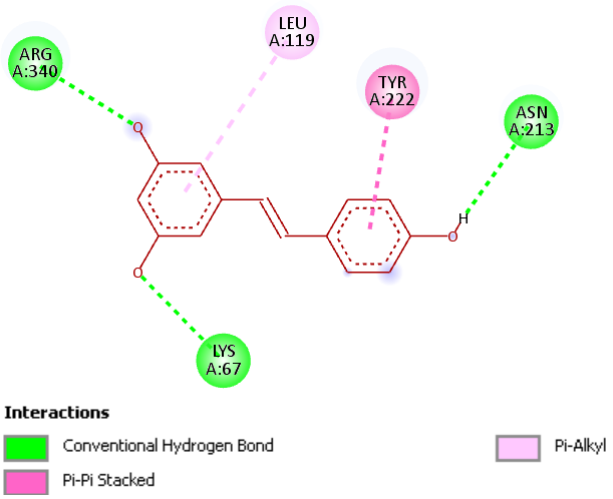
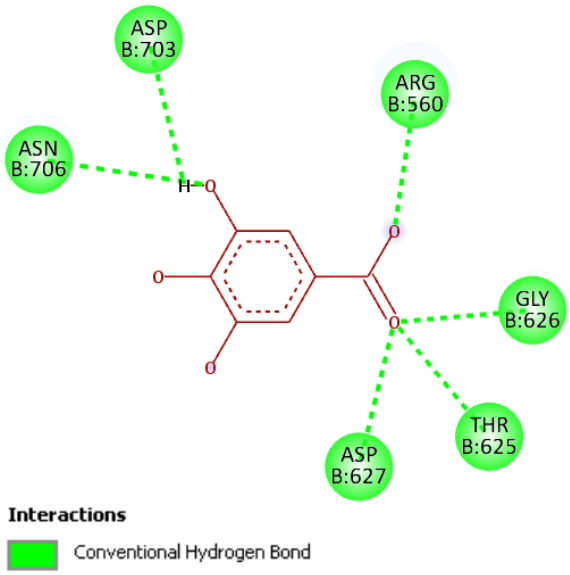
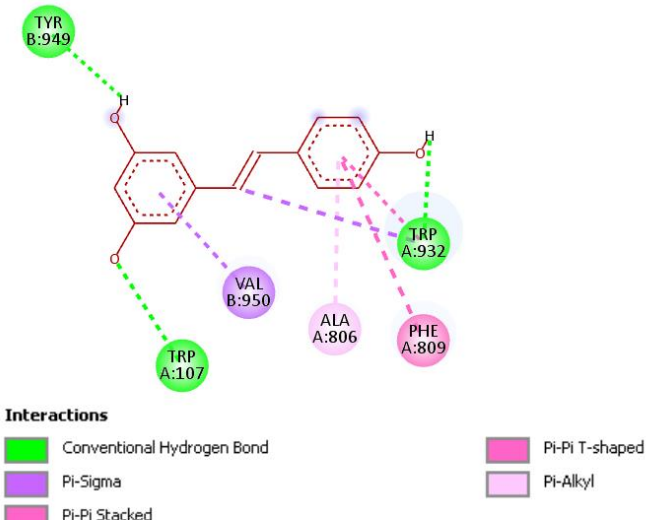








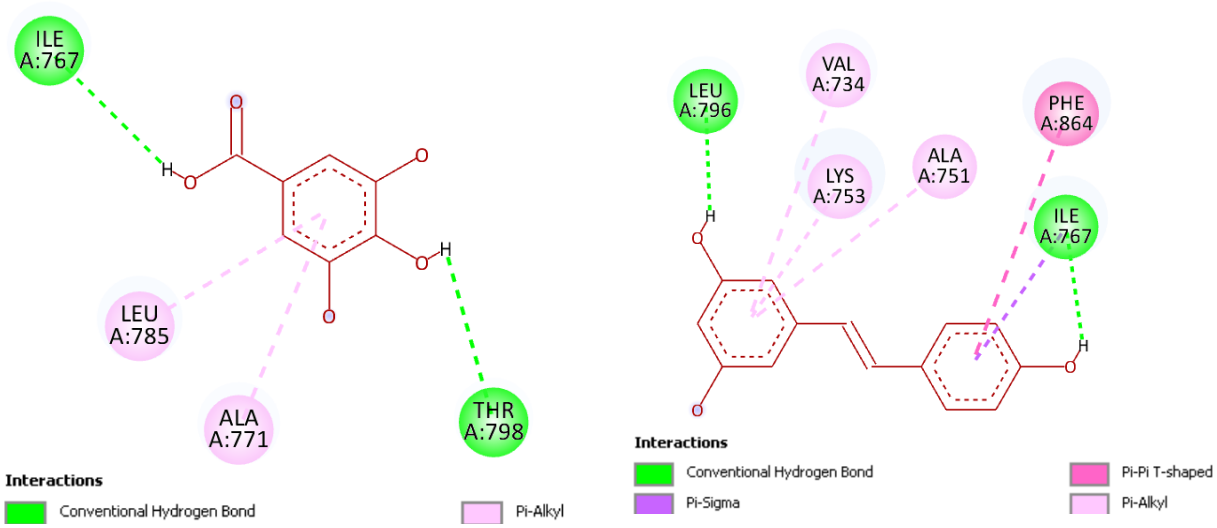
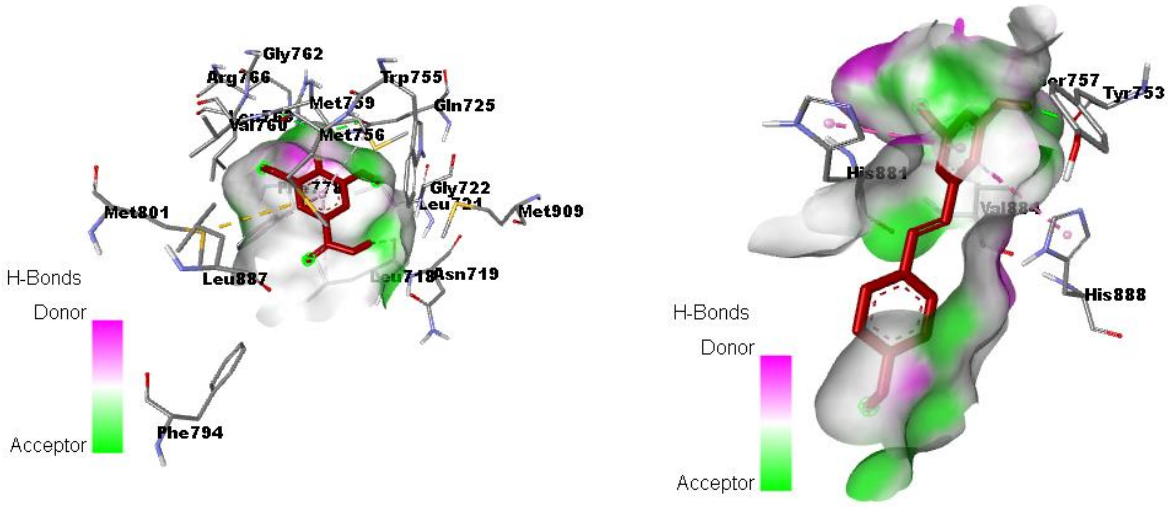
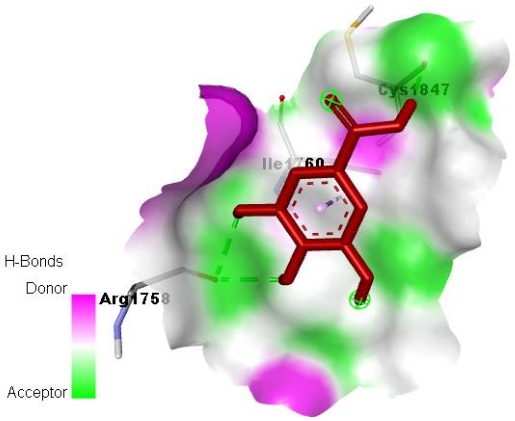
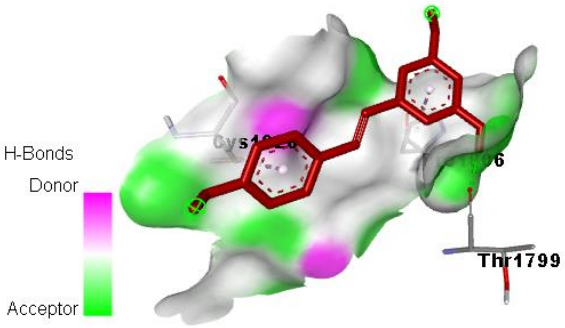
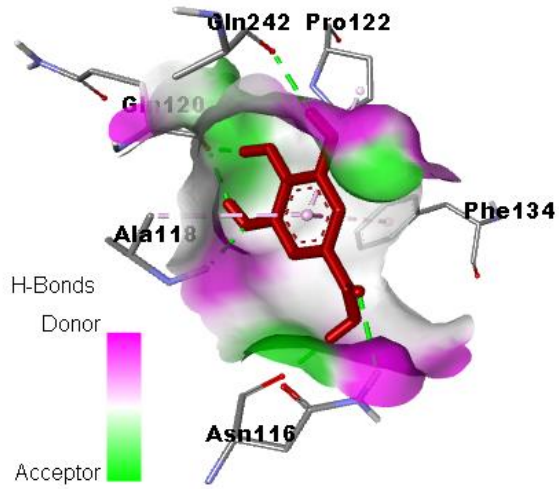


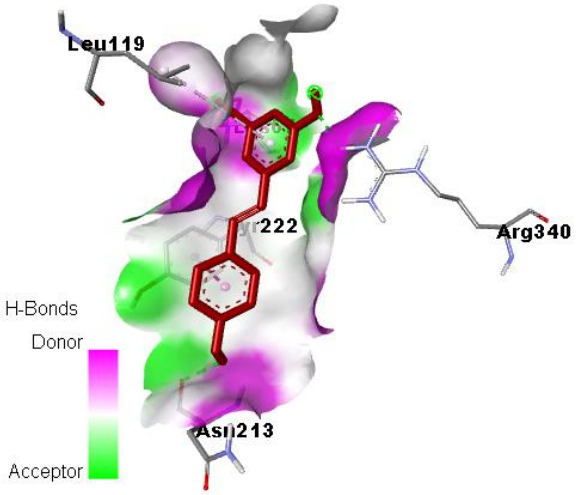
Fig 4: 2D visualization of ligand-receptor interactions, emphasizing hydrogen, Van der Waals, and hydrophobic bonds at the receptor's active site.

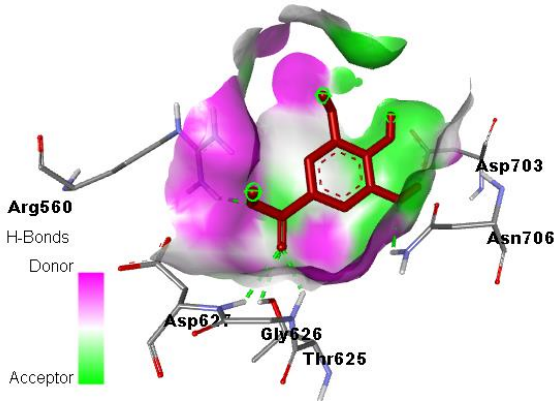


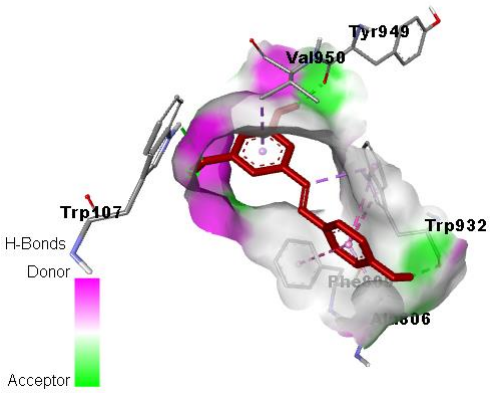

Cys1847
H-Bonds
Donor
Arg1758
Acceptor


H-Bonds
Donor
Acceptor
Thr1799


Gln242
Pro122
Phe134
Ala118
H-Bonds
Donor
Acceptor
Asn116


Leu119
Arg340
H-Bonds
Donor
Acceptor
Asn213


Asp703
Arg560
H-Bonds
Donor
Asn706
Gly626
Thr625
Acceptor


Tyr949
Val950
Trp107
Trp932
H-Bonds
Donor
Acceptor

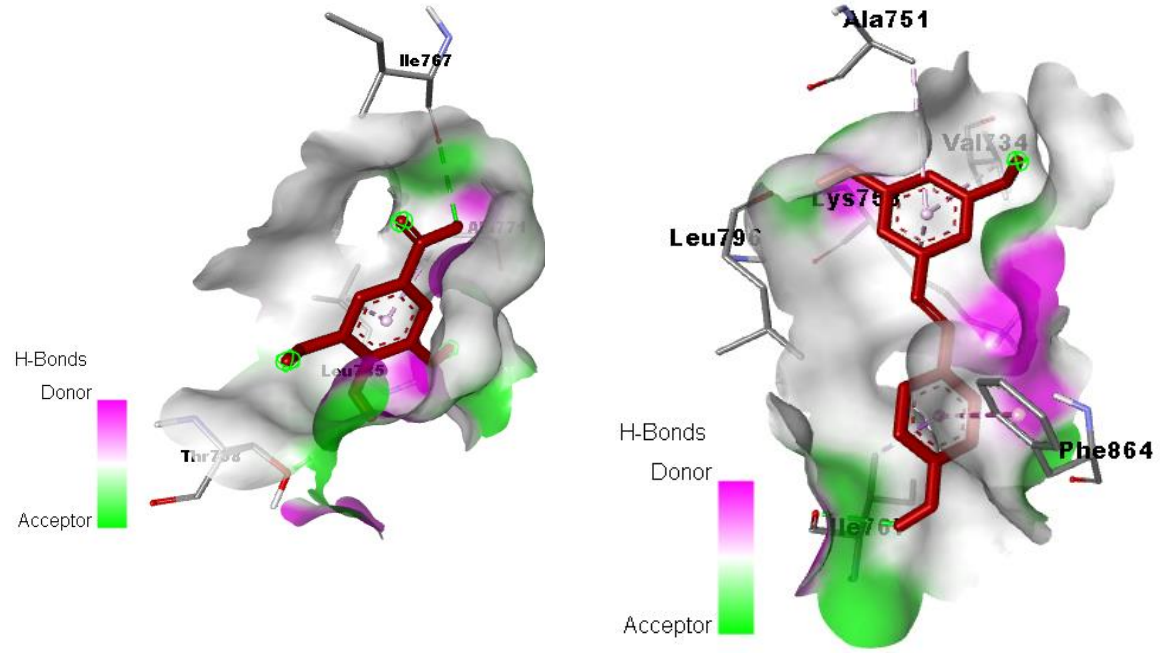


Table 3.2 Amino Acid binding sites of gallic acid and resveratrol in different cancer proteins.

	PDBID 5WAC	**PDBID 1ZUC**	**PDBID 7PCD**	**PDBID 1JNX**	**PDBID 1VFP**
	Estrogen Receptor	**Progesterone Receptor**	**HER-2**	**BRCA1**	**VEGF**
GA	**Asn116** gln242 Gln120 Ala118 Phe134 Pro122	Leu718 Met759 Gln725 Phe778 Met801	**Ile767** Thr798 **Leu785** **Ala771**	Arg1758 **Cys1847** Ile1760	Asn706 Asp703 Arg560 Gly626 Thr625 Asp627

Res	Arg340	Tyr753	Leu796	Thr1799	Tyr949
	Lys67	Ser757	**Ile767**	Pro1806	Trp107
	Asn213 Tyr222	His881	Val734	**Cys1828**	Trp932
	Leu119	His888	Lys753		Val950
		Val884	**Ala751**		Ala806
			Phe864		Phe809

When analyzing the interactions of Gallic Acid (GA) and Resveratrol with different receptors, several similarities in their binding sites emerge. Both GA and Resveratrol interact with the Estrogen Receptor (PDBID 5WAC) although they engage different residues. GA binds with Asn116 and Gln242, while Resveratrol interacts with Arg340, Lys67, Asn213, Tyr222, and Leu119. Despite differences in specific residues, both compounds target critical regions of the estrogen receptor, contributing to their potential effects on breast cancer.

For the Progesterone Receptor (PDBID 1ZUC), GA binds with Gln120, Ala118, Phe134, and Pro122, while Resveratrol interacts with Tyr753, Ser757, His881, His888, and Val884 Though the binding residues differ, both compounds engage with key areas of the receptor that are crucial for its function. In the HER-2 Receptor (PDBID 7PCD), both GA and Resveratrol share the residue Ile767, showing a common binding point. Additionally, GA interacts with Leu718, Met759, Gln725, Phe778, and Met801, while Resveratrol binds to Leu796, Val734, Lys753, Ala751, and Phe864. This shared interaction at Ile767 may contribute to similar biological effects on HER-2, which is particularly relevant in breast cancer treatment. For BRCA1 (PDBID 1JNX), GA interacts with Phe212, Arg174, Ser183, and Glu180, whereas Resveratrol binds with Thr1799, Pro1806, and Cys1828, showing distinct binding residues for

each compound. Similarly, in VEGF (PDBID 1VFP), GA interacts with Arg1758, Cys1847, and Ile1760, while Resveratrol binds with Tyr949, Trp107, Trp932, Val950, Ala806, and Phe809.Overall, the similarities in binding residues, particularly Ile767 in HER-2 and Leu and Arg residues in various receptors, suggest that both GA and Resveratrol may exert similar biological effects on these receptors, despite their differing chemical structures. These shared interactions highlight their potential as therapeutic agents in targeting multiple pathways in cancer.

Choosing two different compounds like Gallic Acid (GA) and Resveratrol to interact with a variety of receptors offers several advantages, particularly when studying their combined or synergistic effects. Despite some differences in the amino acids they bind to in receptors like the Estrogen Receptor, Progesterone Receptor, HER-2, BRCA1, and VEGF, the dual use of these compounds provides a broader range of biological activity. Each compound has distinct binding affinities and modes of action. For instance, Gallic Acid binds to specific residues such as Asn116 in the Estrogen Receptor and Leu718 in HER-2, while Resveratrol binds to different residues like Arg340 in the Estrogen Receptor and Ile767 in HER-2. These differences in binding sites can result in complementary or additive effects, where both compounds enhance each other's efficacy in modulating these receptors. This approach of combining GA and Resveratrol targets multiple pathways simultaneously, potentially inhibiting cancer cell growth more effectively.

Gallic Acid is known for its strong antioxidant and anti-inflammatory properties, which can reduce oxidative stress and prevent cancer cell proliferation. Resveratrol, on the other hand, is recognized for its ability to modulate multiple signaling pathways, including NF-κB, PI3K/Akt, and mTOR, influencing cancer cell survival, apoptosis, and metastasis. The synergistic effect of these two compounds arises from their ability to target different parts of the same receptor or different receptors altogether, creating a multi-pronged attack on cancer cells. For example, while Gallic Acid may inhibit certain growth factors or inflammatory pathways, Resveratrol might block estrogen signaling or HER-2 activity. Together, they can more effectively downregulate pathways involved in cancer progression, offering a more comprehensive therapeutic strategy. In summary, the combination of Gallic Acid and Resveratrol allows for targeting a wider range of receptors and pathways, increasing the likelihood of

disrupting cancer cell growth. Their distinct but complementary modes of interaction create a synergistic effect that could enhance the overall efficacy of cancer treatment.

3.3 Results of ADMET analysis

The ADMET analysis of Gallic Acid and Resveratrol provides insight into their drug-likeness, absorption, metabolism, and toxicity, revealing significant differences that impact their potential therapeutic application in cancer treatment.

Table 3.3 Drug Likeness by SwissADME

Sr No	Compound	Molecular Weight	Nortb	HBD	HBA	Log	A	Violation
1	Gallic acid	170.12	1	4	5	0.50	39.47	0
2	Resveratrol	228.24	2	3	3	2.76	67.88	0

Table 3.4 ADMET profiling of top selected Compounds by AdmetSar

Absorption		
Models	**Gallic acid**	**Resveratrol**
Blood-Brain Barrier	-	+
Human Intestinal Absorption	+	+
Caco-2 Permeability	-	+
P-glycoprotein Substrate	-	-
P-glycoprotein Inhibitor	-	-
GI absorption	+	+

skin permeation	-	-
Metabolism		
CYP1A2 inhibit ion	-	+
CYP2C19 inhibition	-	+
CYP2C9 inhibition	-	+
CYP2C9 substrate	-	-
CYP2D6 inhibition	-	-
CYP2D6 substrate	-	-
CYP3A4 inhibition	-	+
CYP3A4 substrate	-	-
CYP inhibitory promiscuity	-	+
Toxicity		
AMES mutagenesis	-	-
Carcinogenicity (binary)	-	-

The ADMET analysis reveals that gallic acid and resveratrol have promising profiles, with distinct advantages and some limitations. Both compounds are well absorbed in the gastrointestinal (GI) tract, indicating their potential for oral administration. However, resveratrol stands out for its ability to cross the blood-brain barrier (BBB) and its Caco-2 permeability, which allows it to pass through intestinal

cell membranes, enhancing its bioavailability. Gallic acid, in contrast, does not cross the BBB, limiting its effects on the central nervous system (CNS) but potentially reducing off-target CNS effects.

In terms of metabolism, resveratrol shows a higher potential for interaction with other drugs due to its inhibition of key cytochrome P450 (CYP) enzymes, such as CYP1A2, CYP2C19, CYP2C9, and CYP3A4. This enzyme inhibition can lead to drug-drug interactions, affecting the metabolism of other medications. Gallic acid, however, does not inhibit these enzymes, making it a safer option in combination with other therapies as it poses a lower risk of metabolic interactions. Both compounds have favorable toxicity profiles. Neither gallic acid nor resveratrol is mutagenic, as shown by AMES testing, meaning they do not induce genetic mutations that could lead to cancer. Additionally, they are non-carcinogenic, which is beneficial for long-term use as it minimizes the risk of secondary cancer development. Overall, gallic acid offers a safer metabolic profile, while resveratrol provides enhanced permeability, especially for targeting areas beyond the GI tract, making them both viable therapeutic agents with complementary strengths.

Conclusion

This study showcases the promising synergy of gallic acid and resveratrol as a novel approach in breast cancer treatment, particularly for combating aggressive subtypes like triple-negative breast cancer. By demonstrating potent binding affinities with multiple cancer-related receptors (ER, PR, HER-2, BRCA1, and VEGF) and favorable pharmacokinetic profiles, this dual-compound therapy offers a multi-targeted strategy to tackle cancer's complex resistance mechanisms. Their shared interactions with key receptor sites underscore a unique potential to inhibit tumor growth, reduce metastasis, and circumvent chemotherapy's harsh side effects. With further research, this plant-based combination could pave the way for safer, more effective, and sustainable treatments, marking a significant advance in cancer therapeutics.

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