Original Research Article

Integrative Computational Identification of Non-Toxic Flavonoid Derivatives as MMP-1 Inhibitors in Breast Cancer

Abstract

Aims: This study aims to identify non-toxic, flavonoid-derived phytochemicals as potential inhibitors of Matrix Metalloproteinase-1 (MMP-1), a key enzyme involved in breast cancer invasion and metastasis.

Study Design: An integrative in silico approach was adopted, incorporating structure-based drug design (SBDD), network pharmacology, ADMET profiling, and toxicity prediction to discover phytochemical-based MMP-1 inhibitors.

Methodology: A total of 125 phytochemicals were selected based on their reported anti-cancer and anti-metastatic properties from Dr. Duke's and PubChem databases. Biological activity prediction was performed using PASS software, while Molinspiration and Lipinski's Rule of Five were used to evaluate drug-likeness. Toxicity prediction was carried out using DeepToxLab. Gene-disease association and protein interaction networks were constructed via DisGeNET, STRING, and Cytoscape. Molecular docking studies were performed using CB-Dock2, and ligand-receptor interactions were visualized in Discovery Studio.

Results: PASS analysis identified several flavonoids with high potential for anti-cancer and anti-MMP activity (Pa > 0.5). Docking studies revealed that Isohamnetin showed the strongest binding affinity to MMP-1 with a Vina score of -9.0 kcal/mol, followed by Kaempferol and Diosmetin (both -8.5 kcal/mol), and Hispidulin (-8.4 kcal/mol). Geneistein showed the weakest binding at -7.8 kcal/mol. Visualization confirmed that all compounds bound within the active site of MMP-1, forming stable interactions. These lead compounds also exhibited favorable ADME profiles and the selected compounds, including kaempferol, hispidulin, and genistein, demonstrate low carcinogenicity with high-confidence predictions, while varying levels of developmental toxicity uncertainty were observed across the compounds.

Conclusion: Flavonoid compounds like Isohamnetin, Kaempferol, and Diosmetin have shown potential as non-toxic MMP-1 inhibitors, paving the way for their preclinical development as novel breast cancer agents.

Keywords: *Flavonoids, Matrix Metalloproteinase-1 (MMP-1), Breast Cancer Metastasis, In Silico Drug Design, Network Pharmacology, Phytochemicals, Molecular Docking, ADMET Profiling*

I INTRODUCTION

Breast cancer is a prevalent malignant tumor globally, with 2.3 million new cases in 2022. It ranks fourth in mortality rate and is a heterogeneous disease with various molecular subtypes. HER2-positive and triple-negative breast cancer (TNBC) are highly prone to metastasis and high recurrence rates. Treatment strategies have evolved from single surgical resection to comprehensive therapies including chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Despite advancements, recurrence and metastasis remain the primary causes of treatment failure (Jiang et al., 2025). The World Health Organization (WHO) has performed a study based on the GLOBOCAN database to examine the worldwide burden of female breast cancer. According to the study, in 2022, 2.3 million new cases and 670,000 deaths occurred globally. Annual rates were elevated by 1–5% in half of the countries reviewed, with 29 high Human Development Index (HDI) countries seeing mortality rates fall. Seven nations, such as Denmark and Belgium, are already fulfilling the Global Breast Cancer Initiative target of a 2.5% annual decline. In 2050, new instances and fatalities will grow by 38% and 68%, respectively (Kim *et al.,* 2025). Breast cancer is determined by environmental, lifestyle, and genetic factors worldwide. An increase in population and adoption of a Western lifestyle are contributing to rising cases of breast cancer in developing nations. Death rates will decrease with enhanced access to prevention services, early diagnosis, and medical care (Xiong *et al.,* 2025).

Cancer is the fifth most common cause of death in India, and it accounts for 5.7% of total deaths. The age-specific incidence rate of breast cancer differs, with 32.8% in the age group 25-49 years, 27.9% in the age group 50-69 years, and 23.4% in the age group 70 years and above. In 2020, breast cancer was found in 25.8 per 100,000 women and had a mortality rate of 12.7 per 100,000 women. The southern and northern parts of the country had the highest burden at 685.5 and 677.6 DALYs per 100,000 women, respectively (Pillai *et al.,* 2025).

Metalloproteinases (MPs) play important roles in cancer progression, hemostasis, and development. MP inhibitors have limited therapeutic action owing to their multifunctionality in various cell types and normal conditions. Epigenetic regulators of MP expression have emerged as indirect targets, and epigenetics-targeting drugs are more developed than MP inhibitors (Seehawer & Polyak, 2025). Matrix metalloproteinases (MMPs) are a group of zinc-binding endopeptidases that play an important role in breast cancer development by remodeling the extracellular matrix (ECM) and basement membrane, thereby promoting tumor invasion, angiogenesis, and metastasis. Various MMPs, such as MMP-1, MMP-2, MMP-7, MMP-9, and MMP-14, have been found to be involved in breast cancer, with each of them playing different but overlapping roles. Among these, MMP-1, also referred to as interstitial collagenase, holds special significance. MMP-1 directly degrades interstitial collagens—types I, II, and III—important structural elements of connective tissue. Through the degradation of these collagens, MMP-1 facilitates cancer cell invasion through tissue barriers and into adjacent stroma, thus enhancing metastatic dissemination to distant organs like the lungs and bones. Besides its proteolytic activity, MMP-1 is also able to bind to the protease-activated receptor-1 (PAR-1), activating downstream signaling pathways leading to enhanced tumor cell migration, proliferation, angiogenesis, and survival. Clinical reports have shown that increased MMP-1 expression is associated with more aggressive tumor phenotypes, higher metastatic capabilities, and worse patient prognosis in breast cancer (Duffy *et al.,* 2007; Overall & Kleifeld, 2006; Gong *et al.,* 2014). Recent studies also emphasize MMP-1 as a good therapeutic target, with approaches aimed at blocking its activity demonstrating effectiveness in preclinical models of breast cancer (Winer *et al.,* 2018).

Flavonoids, a structurally diverse group of polyphenolic compounds from plants, have been noted for their therapeutic potential, including their activity as matrix metalloproteinase (MMP) inhibitors. MMPs are a group of enzymes responsible for the degradation of the extracellular matrix and are involved in tumor growth and metastasis, especially in breast cancer (Lin et al., 2013). A number of flavonoid derivatives that are not toxic, like kaempferol, genistein, and hispidulin, were found to be inhibitors of MMP activity without being cytotoxic in nature, suggesting them as lead compounds for the treatment of cancer (Mannello et al., 2005) (Patil et al., 2023). They are safer in comparison to other chemotherapeutic drugs because of their low toxicities and efficacy to modulate important signaling pathways involved in the development of cancer . By molecular docking and in vitro testing, flavonoid derivatives have shown the ability to bind the active sites of MMP-1, inhibiting its enzymatic activity and perhaps metastasis suppression . This renders them promising candidates for the creation of new, less toxic MMP-targeted therapies for cancer. (Cabral-Pacheco et al., 2020) (Fatima et al., 2021).

This research will identify new and non-toxic phytochemical derivatives, more specifically flavonoid-derived compounds, as possible chemotherapeutic agents against Matrix Metalloproteinase-1 (MMP-1) in breast cancer. MMP-1 is important in tumor invasion and metastasis by breaking down interstitial collagens and hence is an appealing molecular target for anticancer therapy. The research utilizes structure-based drug design (SBDD) methods to screen thousands of ligands and estimate their binding affinity against chosen disease targets. Structure-based virtual screening is well known as an effective tool to identify potential hits efficiently while removing non-complementary compounds from large chemical libraries. Here, the emphasis is placed on the design and screening of new flavonoid derivatives, the identification of high-binding-score ligands, and their ADMET profiles to ascertain drug-likeness and safety. Besides virtual screening, a network pharmacology approach is incorporated to elucidate the multi-target interaction of the hit compounds, charting their activity across major signaling pathways and biological processes implicated in breast cancer development. This dual approach maximizes the discovery of candidate drugs by factoring in both molecular binding affinity and systemic therapeutic effect. Overall, the study presents new findings and a starting point for the development of new anticancer agents with improved efficacy and fewer side effects.

2 Result

2.1 Collection of phytochemicals used in the study

Phytochemicals for the study were chosen from Dr. Duke's Botanical Database (https://phytochem.nal.usda.gov/phytochem/search), review and research articles using the keywords anti-breast cancer property, anti-metastatic property, and anti-matrix metalloproteinase property. The structures of these compounds were downloaded from the National Institute of Health's PubChem online database, which is freely available for virtual screening (Hanessian et al., 2001). A total of 125 compounds, including terpenes, flavonoids, flavones, flavanones, chalcones, isoflavones, alkaloids, aldehydes, and anthocyanidins, were identified and further screened using the PASS online software.

2.2 Prediction of Ligand Activity

The PASS (Prediction of Activity Spectra for Substances), an online software, predicts the result based on structure, which is equal to its activity, and is used to compare the function of unknown biologically active compounds (Filimonov and Poroikov 2008). It estimates the probability of a particular substance's characteristic to the active and inactive subsets of a substance from the Structure Activity Relationship Base (SAR Base). Using the Bayesian

approach implemented in the computer program PASS compared the function of selected structures (Geronikaki et al., 2002). The SMILES (Simplified Molecular-Input Line-Entry System) format for the phytochemical's structure was given as input. Four activities—antineoplastic, anti-breast cancer, anti-metastatic, and anti-MMP expression—were considered. For each ligand, Pa (probable activity) and Pi (probable inactivity) values for all four activities were obtained. Only the activities with Pa>Pi and Pa>0.5 were considered as experimentally possible for a particular compound (Poroikov et al., 2002).

2.3 DEG Analysis and Construction of the PPI Network

DisGeNET is a discovery platform integrating information on gene-disease associations (GDAs) from several public data sources and the literature (Piñero et al., 2016). Online tool STRING (http://string-db.org) was used to identify the potential interaction networks of protein products of these genes (Szklarczyk et al., 2016). Further, the protein-protein interactions (PPI) network was constructed and visualized by Cytoscape version 3.0.1 (Institute of Systems Biology, Seattle, WA, USA) (Majeed & Mukhtar, 2023). Nodes were screened out in the PPI network with degree ≥ 1, and 'degree' represented the connections with other nodes. According to the top pathways with their corresponding targets, the diseases, and the co-expressed protein network were constructed using Cytoscape version 3.8.0 (Guo et al., 2017).

2.4 ADME Prediction

Molinspiration offers a variety of software tools for drug design, such as molecule manipulation, normalization, tautomer generation, fragmentation of molecules, and molecular modeling. The tools facilitate substructure and similarity searching, fragment-based virtual screening, prediction of bioactivity, and data visualization. Molinspiration's Java-based tools are available on any computer platform, and this improves the comprehension of molecular properties (*Calculation of Molecular Properties and Bioactivity Score*, n.d.). Lipinski's rule of five requires that an orally active drug should not contain more than five hydrogen bond acceptors and donors, have a molecular weight of less than 500 g/mol, have a partition coefficient log P of five, and have fewer than four violations. The two drug-like molecules were analyzed by Molinspiration, a Physicochemical Properties Calculator, which determines partition coefficient, molecular weight, number of heavy atoms, hydrogen donors, acceptors, and violations (Zashumo *et al.,* 2022).

2.5 Toxicity Prediction

The DeepToxLab platform utilizes a deep learning-based predictive model to assess the toxicity potential of small molecules, particularly focusing on carcinogenicity. It integrates chemical structure input, typically provided in SMILES format, and converts it into graph-based and physicochemical descriptors that feed into pre-trained deep neural networks. These networks have been trained on extensive public datasets such as Tox21 and PubChem BioAssay, enabling robust quantitative structure-activity relationship (QSAR) modeling. The model assigns a toxicity probability score between 0 and 1, where lower values indicate reduced toxic potential. A key feature of DeepToxLab is its inclusion of a confidence label for each prediction—classified as either high or low. High-confidence predictions, denoted by a blue "H" icon, signify strong model reliability and are particularly valuable during early-stage drug development. In this study, only compounds with both low carcinogenicity scores (below 0.2) and high-confidence predictions were considered for further analysis, aligning with the platform's guidelines for reliable toxicity interpretation (DeepToxLab, 2025).

2.6 Docking and visualization

CB-Dock2 is a blind docking server that makes predictions of ligand binding sites and conducts molecular docking without knowing the binding pocket in advance. It uploads three-dimensional structures of the receptor protein and the ligand in PDB format, detects possible binding cavities, ranks the top five, and establishes suitable docking boxes. Molecular docking is conducted by an integrated AutoDock Vina engine, producing binding poses and Vina scores. The optimal receptor-ligand complexes are additionally examined using Discovery Studio Visualizer, which investigates important molecular interactions such as hydrogen bonds, hydrophobic contacts, pi-pi stacking, and electrostatic interactions. Visualization of 2D interaction plots and 3D complex structures is possible through the software, giving further insight into the binding between protein active site residues and the ligand.

3 Methodology

3.1 Selection of phytochemicals

For the present study, 125 phytochemical compounds were taken. The phytocompounds were chosen based on an intricate search in research and review articles that satisfied any one of the following conditions: (1) anti-breast cancer and (2) anti-metastatic properties. Since choosing all phytocompounds from all plants with the specified properties was not practically possible, the number of phytocompounds taken for the study was restricted to 150. Consequently, after removal of duplicate entries (owing to more than one common name for a single flavonoid), the final number of phytocompounds was 125. The phytochemicals list with the above-said properties was obtained from Dr. Duke's Botanical Database (https://phytochem.nal.usda.gov/phytochem/search) (Hanessian et al., 2001) and grouped into flavonoids and non-flavonoids.
The two-dimensional unique structures of 125 phytocompound ligands were obtained from the National Institute of Health's PubChem online database (http://pubchem.ncbi.nlm.nih.gov/). The list of phytocompounds with their PubChem ID used in the study is provided inAppendix 1 (Table 1).

3.2 Classification of chosen Phytochemicals

Based upon the structure, phytochemicals chosen for the study were classified precisely. Table 1 shows the distribution of the 125 established phytochemicals with respect to the number of constituent phytocompounds in the chosen data set. The 125 ligands were classified into several classes (flavonoids and non-flavonoids) of phytochemicals, as shown in Figure 1.

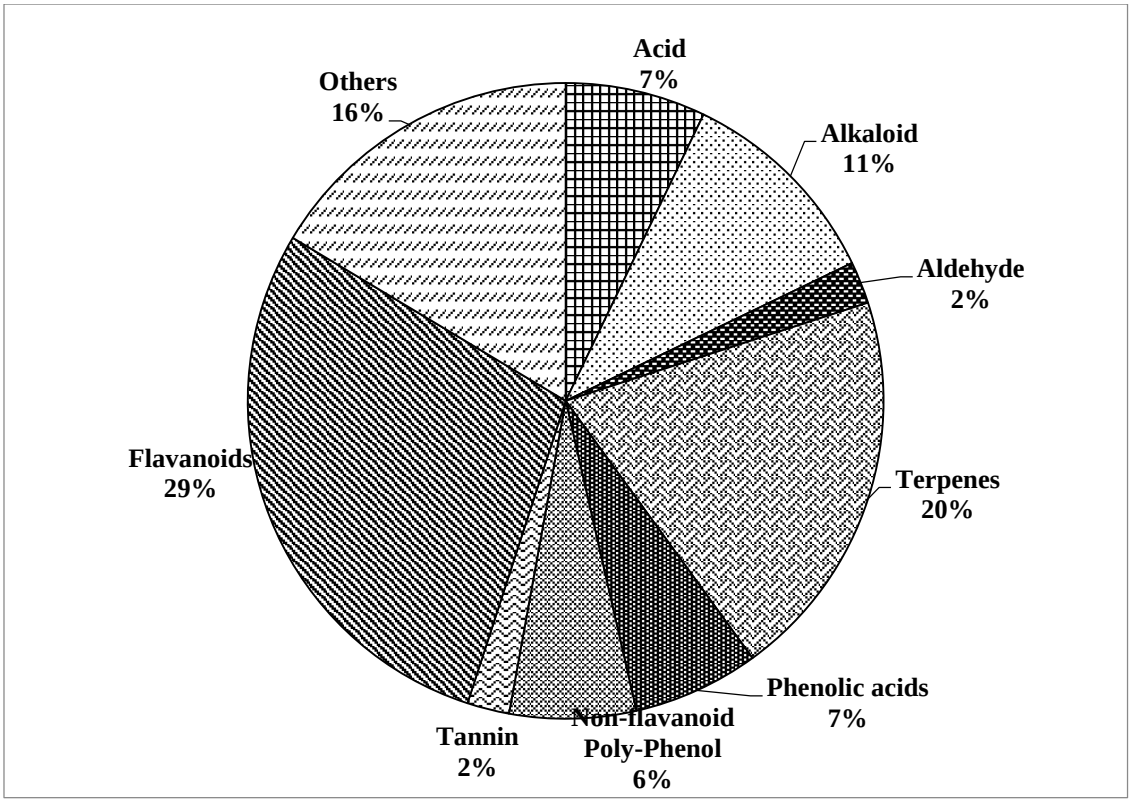

Figure 1: Pie Chart Illustrating the Percentage Composition of Phytochemicals Employed in this Research. This pie chart presents the distribution of different phytochemical classes that have been encompassed in this work, with a general view of their comparative percentages. The pie chart demonstrates the choice of multiple phytochemical types, e.g., flavonoids, alkaloids, and terpenoids, as per their bioactive interest and potential utility against breastcancer.This spread reflectsthe wi derange screening methodology followed for surveying a diverse group ofphytochemi cals to ascertain theirtherapeutic value for intervention against cancer mechanisms, v iz., metastasis and MMP control.

From the results, the major percentage of phytochemicals was represented by (i) non-flavonoids, with 71% distribution, and (ii) flavonoids, with 29%. In the non-flavonoids category, terpenes were in higher distribution with 20% representation. All the acids, including benzoic acid, cinnamic acid, carboxylic acid, and caffeic acid, were grouped into one category as acids, and their distribution is 7%. Also, phenolic acids share 7% distribution, and polyphenols other than flavonoids have 6% distribution. Alkaloid distribution was 11%. Other groups, such as tannin, aldehyde, polysaccharides, phytosteroid, and organosulfur compounds, demonstrated very low distribution.

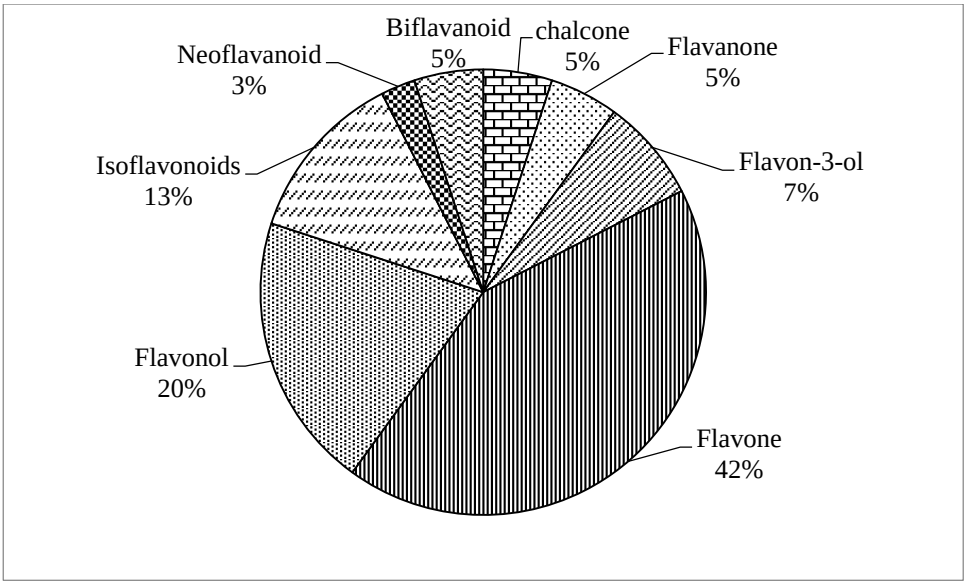

Figure 2: Pie chart showing the percentage distribution of flavonoid compounds used in this study.This pie chart demonstrates the comparative distribution of various flavonoid compounds chosen for study in the present study. The chart is indicative of the variety of flavonoid structures and their comparative representation, pointing to the diversity of phytochemicals considered for investigation for their anti-cancer and MMP-inhibitory activities. This distribution reflects the criteria chosen at the screening phase of the compounds.

The flavonoids (as represented in Figure 2) were further subdivided into flavones (42%), flavonols (20%), flavan-ols (7%), flavanones (5%), isoflavonoids (13%), neoflavonoids (3%), bi-flavonoids (5%), and chalcones (5%).

Earlier studies on MMP-1 inhibition by phytochemicals are only very few in number. Those studies suggested that the inhibition of MMP-1 is frequently restricted to only a few compounds, either plant-specific or compound-specific ligands (Maity et al., 2011) (Lim & Kim, 2007). Hence, in this study, we have attempted to investigate the inhibition activity of MMP-1 through more than a hundred phytocompounds based upon their classification. More assortment groups used here are considered important, as it will increase the probability of eventually identifying the best compound that has more possibility to inhibit MMP-1.

3.3. Prediction of Ligand Activity

All the 125 phytocompounds were tested for their anti-cancer-related activity in the PASS tool. Based upon the combined properties such as MMP inhibition, anti-metastatic, anti-neoplastic, and anti-breast cancer properties, the compounds predicted with more than one property with Pa > Pi were only considered. The filtered 56 compounds (out of 125) based on Pa and Pi values are given in Table 1, and they have been grouped into flavonoids (41 compounds) and non-flavonoids (25 compounds).

Table 1: Flavonoid Compounds Shortlisted Based on Multi-Target Activity Predictions Using the PASS Online Tool

Sl.No	Phytocompounds	Class	Pa	Pi	Activity
1.	Apigenin	Flavone	0.780	0.004	MMP expression inhibitor
			0.775	0.015	Anti-neoplastic
			0.669	0.007	Anti-neoplastic (breast cancer)

2.	Baicalein	Flavone	0.789	0.013	Anti-neoplastic
			0.588	0.011	Anti-neoplastic (breast cancer)
			0.681	0.008	MMP expression inhibitor
3.	Chrysin	Flavone	0.654	0.007	Anti-neoplastic (breast cancer)
			0.766	0.004	MMP expression inhibitor
4.	Diosmetin	Flavone	0.682	0.006	Anti-neoplastic (breast cancer)
			0.814	0.003	MMP expression inhibitor
5.	Eupatilin	Flavone	0.698	0.005	Anti-neoplastic (breast cancer)
			0.724	0.005	MMP expression inhibitor
6.	Genkvannin	Flavone	0.798	0.003	MMP expression inhibitor
			0.634	0.008	Anti-neoplastic (breast cancer)
7.	Hispidulin	Flavone	0.752	0.004	MMP expression inhibitor
			0.694	0.005	Anti-neoplastic (breast cancer)
8.	Isothymonin	Flavone	0.622	0.009	Anti-neoplastic (breast cancer)
			0.673	0.009	MMP expression inhibitor
9.	Luteolin	Flavone	0.672	0.007	Anti-neoplastic (breast cancer)
			0.777	0.004	MMP expression inhibitor
10.	Nobiletin	Flavone	0.579	0.018	MMP expression inhibitor
			0.698	0.005	Anti-neoplastic (breast cancer)
11.	Rhamnazin	Flavone	0.767	0.004	MMP expression inhibitor
			0.586	0.012	Anti-neoplastic (breast cancer)
12.	Rhoifolin	Flavone	0.53	0.014	Anti-metastatic
			0.504	0.019	Anti-neoplastic (breast cancer)
13.	Tangeretin	Flavone	0.604	0.015	MMP expression inhibitor
			0.679	0.006	Anti-neoplastic (breast cancer)
14.	Fisetin	Flavonol	0.514	0.018	Anti-neoplastic (breast cancer)
			0.638	0.012	MMP expression inhibitor
15.	Galangin	Flavonol	0.722	0.005	MMP expression inhibitor
			0.550	0.014	Anti-neoplastic (breast cancer)
16.	Isohamnetin	Flavonol	0.777	0.004	MMP expression inhibitor
			0.599	0.010	Anti-neoplastic (breast cancer)
17.	Kaempferol	Flavonol	0.738	0.005	MMP expression inhibitor
			0.565	0.013	Anti-neoplastic (breast cancer)

18.	Myricetin	Flavonol	0.578	0.012	Anti-neoplastic (breast cancer)
			0.842	0.008	Anti-neoplastic
			0.696	0.007	MMP expression inhibitor
19.	Quercetin	Flavonol	0.576	0.012	Anti-neoplastic (breast cancer)
			0.734	0.005	MMP expression inhibitor
20.	Rutin	Flavonol	0.536	0.016	Anti-neoplastic (breast cancer)
			0.522	0.015	Anti-metastatic
21.	Diadzein	Isoflavonoid	0.864	0.002	MMP expression inhibitor
			0.506	0.018	Anti-neoplastic (breast cancer)
22.	Formononetin	Isoflavonoid	0.887	0.002	MMP expression inhibitor
			0.526	0.016	Anti-neoplastic (breast cancer)
23.	Geneistein	Isoflavonoid	0.571	0.013	Anti-neoplastic (breast cancer)
			0.908	0.001	MMP expression inhibitor
24.	Glycitein	Isoflavonoid	0.564	0.013	Anti-neoplastic (breast cancer)
			0.888	0.002	MMP expression inhibitor
25.	Epigallocatechin	Flavan-3-ol	0.722	0.005	MMP expression inhibitor
			0.500	0.020	Anti-neoplastic (breast cancer)
26.	Epigallocatechin-3-Gallate	Flavan-3-ol	0.562	0.020	MMP expression inhibitor
			0.540	0.015	Anti-neoplastic (breast cancer)
27.	Hesperitin	Flavanone	0.656	0.007	Anti-neoplastic (breast cancer)
			0.801	0.003	MMP expression inhibitor
28.	Naringenin	Flavanone	0.639	0.008	Anti-neoplastic (breast cancer)
			0.764	0.004	MMP expression inhibitor
29.	Dalbergin	Neoflavonoid	0.599	0.010	Anti-neoplastic (breast cancer)
			0.745	0.004	MMP expression inhibitor
30.	Ginkgetin	Biflavonoid	0.666	0.007	Anti-neoplastic (breast cancer)
			0.750	0.004	MMP expression inhibitor
31.	Butein	Chalcone	0.591	0.011	Anti-neoplastic (breast cancer)
			0.770	0.004	MMP expression inhibitor

From 100 non-flavonoids initially chosen for the study, 25 compounds have been filtered through PASS online tool and the list is given in Table 1.

3.4 Network Pharmacology

The network pharmacology approach was used to narrow down the MMP inhibitor as a useful target for arresting the metastasis stage of breast cancer because prediction of potential proteins is of more importance towards the analysis of therapeutic identification in biological systems (Vyas et al., 2023). The constructed network system can predict the main active protein and its corresponding proteins by 'network targets.' In the current study, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and Cytoscape (version 10.0) were applied to investigate target expression data in the framework of biological network information. Cytoscape has successfully given the valuable clue that exposes the interactions of MMP-1 in metastasis of breast cancer.

3.5 Predicting protein targets

The functional interactions between MMP-1 and other key proteins can provide a framework for the molecular mechanism of cellular processing of metastasis. When the relationships between protein interactions are significantly high, then the protein/gene is declared as differentially expressed genes (DEGs). These encoding genes have direct interactions with their respective proteins and will finally affect the expression level of protein. The significant related proteins to breast cancer were extracted from the STRING database as per the protocol of Cai et al. (2017). In our study, a total of 438 DEGs that correlated to breast cancer metastasis in association with MMP-1 were screened by protein query of the STRING database. The DEGs were screened by weighted correlation analysis with $|logFC| > 0.58$ and $P < 0.05$. The top 20 DEGs and their functional enrichment pathway are shown in Table 2. As can be seen, IL-6 was the upregulated DEG with the highest fold change of 0.999 in the current study. Besides, the DEGs of the MMP family, MMP-2 and MMP-9, were also upregulated with the combined scores of 0.993 and 0.997, respectively.

Table 2: Most Significant Differentially Expressed Genes (DEGs) Associated with Breast Cancer Pathogenesis and Progression

Symbol	geneid	Symbol	Combined Score	Pathway
MMP1	3569	IL6	0.999	Cellular responses to stress; Immune System; Signal Transduction
MMP1	4318	MMP9	0.997	Developmental Biology; Extracellular matrix organization; Signal Transduction
MMP1	7422	VEGFA	0.995	Cellular responses to stress; Developmental Biology; Hemostasis; Signal Transduction
MMP1	7040	TGFB1	0.994	Developmental Biology; Extracellular matrix organization; Hemostasis; Signal Transduction
MMP1	7157	TP53	0.994	Cell Cycle; Cellular responses to stress; DNA Repair; Gene Expression; Hemostasis; Programmed Cell Death; Signal Transduction
MMP1	5743	PTGS2	0.993	Metabolism
MMP1	4313	MMP2	0.993	Developmental Biology; Extracellular matrix organization; Metabolism of proteins
MMP1	3553	IL1B	0.992	Immune System
MMP1	3576	CXCL8	0.991	Cellular responses to stress; Metabolism of proteins; Signal Transduction
MMP1	4314	MMP3	0.991	Extracellular matrix organization; Signal Transduction
MMP1	596	BCL2	0.99	Immune System; Programmed Cell Death

MMP1	6347	CCL2	0.99	Metabolism of proteins; Signal Transduction
MMP1	1029	CDKN2A	0.99	Cell Cycle; Cellular responses to stress; Metabolism of proteins
MMP1	3458	IFNG	0.989	Immune System
MMP1	4524	MTHFR	0.989	Metabolism
MMP1	3552	IL1A	0.989	Cellular responses to stress; Immune System
MMP1	1636	ACE	0.987	Metabolism of proteins
MMP1	1956	EGFR	0.986	Developmental Biology; Immune System; Signal Transduction
MMP1	7099	TLR4	0.985	Immune System; Programmed Cell Death

Table 2 also shows the co-expression of MMP-1 with BCL-2 and VEGF-A proteins. Based on the STRING database, protein network was constructed.

Analysis of these data revealed that, out of 438 proteins that interacted with MMP-1 during metastasis of breast cancer, 161 proteins were upregulated.

3.6 PPI Network Construction

A network is made up of nodes (the points of communication or redistribution of proteins/genes) and edges (the lines of communication or relation joining the proteins/genes) (Raman, 2010). The interaction network of 161 MMP-1-related upregulated proteins was analyzed using the STRING database. Most of the upregulated proteins derived using this tool are overexpressed in breast cancer cells and involved in the survival of those cells. The co-expression network of MMP-1-regulated genes was established by setting the Pearson correlation coefficient at >0.7. There were 483 edges in the PPI network for 161 DEGs (Figure 3). Degrees > 10 were set as the cutoff criterion, and the average node degree was found to be 14.2. Among the 161 nodes, BCL2, p53, EGFR, VEGF-A, IL-6, IL-10, TNF, MMP-2, and MMP-9 were identified as hub genes with higher degrees. Moreover, it clearly shows that MMP-1 had interactions with proteins that are significantly correlated with metastasis of BC. Interestingly, our study is in correlation with a previous study, which showed a relationship between MMP-1 and the aggressiveness of breast cancer cells (Chen et al., 2015). It also depicts the ability of MMP-1 to instruct immune cells to modify the expression of other proteins, such as MMP-2 and MMP-9.

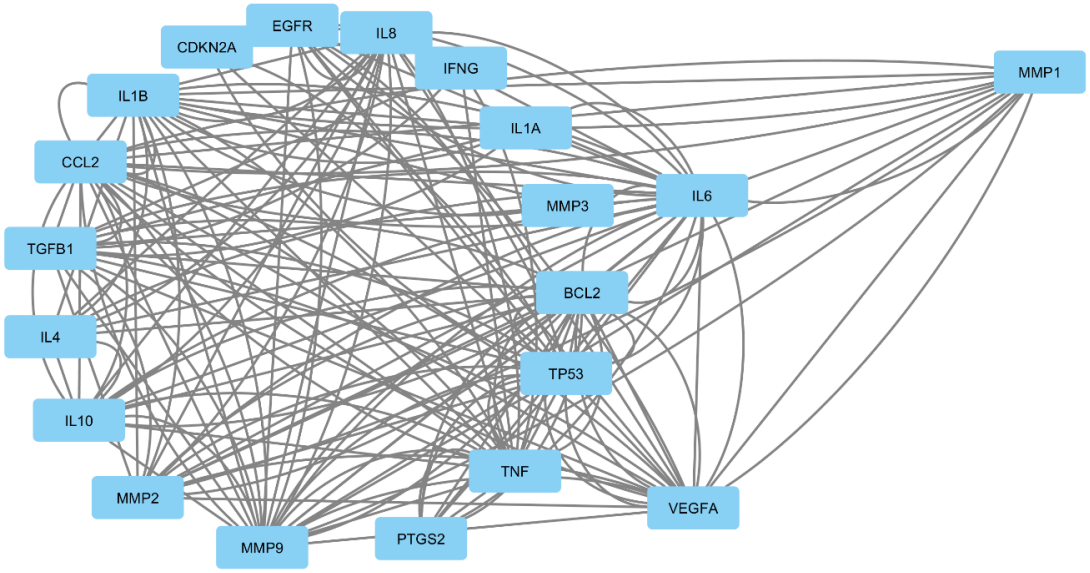

Figure 3: Cluster-1 of the Protein–Protein Interaction (PPI) Network Highlighting MMP-1 and Its Functionally Associated Proteins in the Context of Breast Cancer.This graph represents Cluster-1 from the PPI network, with special focus given to matrix metalloproteinase-1 (MMP-1) and its tightly interacting proteins, designated as significantly co-expressed or functionally interacting in breast cancer pathways. The network was removed and graphically represented to designate molecular interactions responsible for tumor invasion, metastasis, and remodeling of the extracellular matrix. MMP-1's central localization in this cluster reflects its implication as a potential target and suggests further examination of flavonoid-based inhibitors under this molecular environment.

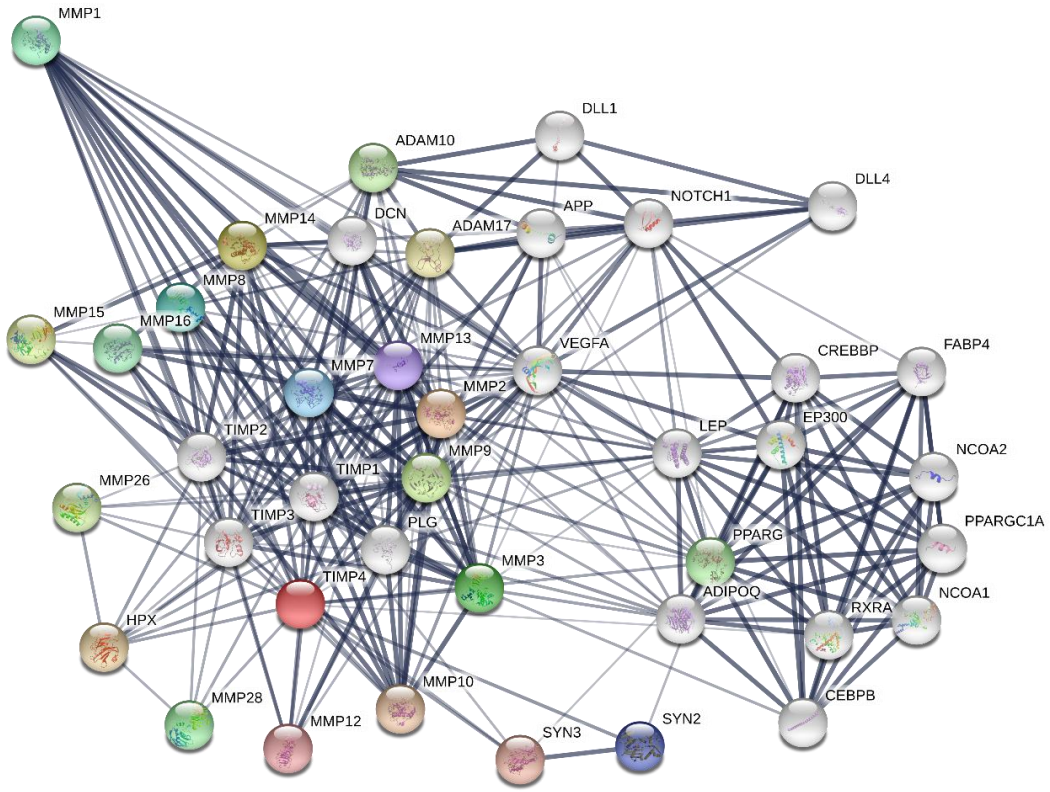

Fig. 4: Protein–Protein Interaction (PPI) Network of 161 Proteins Generated Via STRING Database and Visualized Via Cytoscape (Version 10.0). This figure displays the PPI network built from 161 uniquely expressed proteins in breast cancer, retrieved from the STRING database using a confidence cut-off. The network was visualized and examined with Cytoscape version 10.0, which identified significant hub proteins and interaction clusters that may play a role in tumor development, metastasis, and MMP-related pathways. The analysis lends validity to the identification of essential molecular targets, such as MMP-1, to be further subjected to docking and interaction with phytochemicals.

In the sub-network of MMP-1 that was involved in the metastasis process, BCL-2 (B Cell Lymphoma-2), TN53 (Tumor Protein), TNF (Tumor Necrosis Factor), MMP-2 (Matrix Metalloproteinase-2), MMP-9 (Matrix Metalloproteinase-9), IL-1 (Interleukin-1), IL-6 (Interleukin-6), VEGF-A (Vascular Endothelial Growth Factor-A), and EGFR (Epidermal Growth Factor) genes were identified to exhibit a higher degree of interaction in breast cancer (Figure 4).

Proteins that are co-expressed with MMP-1 are important in breast cancer survival. BCL-2 inhibits apoptosis in different cell systems, promoting survival. MMP-1 interacts with MMP-2 and MMP-9, which are important regulators of degrading extracellular matrix proteins and tissue repair. TGFB1 signaling in breast cancer is associated with seeding lung metastasis by interfering with cell-cell interaction. Cytokines such as IL-1A, IL-1B, IL-4, IL-6, IL-8, and IL-10 are involved in inflammation, initiation of tumors, promotion of tumors, angiogenesis, and metastasis. IL-1 is implicated in poor prognosis of breast tumors and causes metastasis. IL-6 and IL-10 enhance tumor invasion and angiogenesis. CCL2 initiates a chemokine cascade and enhances metastatic sites of breast cancer cells. VEGF-A is postulated to be the regulator of signaling tumor cells, controlling cancer cells. These proteins are more connected than 0.9wddx with MMP-1 protein.

3.7 ADME Prediction

In the present investigation, a set of 24 phytochemicals, mostly flavonoids and structurally related polyphenols, were screened for their drug-likeness-relevant physicochemical attributes. Some of the crucial parameters, like molecular weight (Mol Wt), lipophilicity (Log P), aqueous solubility (Log S), polar surface area (MolPSA), molecular volume (Mol Vol), drug-likeness score, hydrogen bond acceptors (HBA), and donors (HBD), were considered.

With the exception of baicalein (446.08 Da) and epigallocatechin-3-gallate (458.08 Da), most ligands had molecular weights in the optimal range (<500 Da) and were according to Lipinski's Rule of Five (Lipinski et al., 2001). All compounds expressed moderate to very good drug-likeness values (>0.1), except for naringenin (1.13), which had the highest value along with quercetin (0.93) and was indicated to have considerable potential for the development of a drug.

Lipophilicity, as indicated by Log P values, varied from 0.13 (baicalein) to 3.45 (formononetin), implying differential membrane permeability for the ligands. Substances such as formononetin, apigenin, and chrysin, with log P values >3.0, may exhibit greater cellular uptake but at the potential cost of poor solubility or off-target activity.

Water solubility (Log S) throughout the ligands was generally moderate (-3 to -4 range), with camptothecin as an outlier at a Log S of -4.84, indicating its lower aqueous solubility. In drug development, such low-solubility molecules usually need special delivery systems.

MolPSA values also validated the permeability profiles, with all but two of the compounds being within optimal PSA ranges (<140 Å²). Epigallocatechin-3-gallate (158.72 Å²) and

baicalein (143.74 Å²) were higher than this, indicating possible problems in passive diffusion, especially through the blood-brain barrier.

Hydrogen bond donors and acceptors (HBD/HBA) were in agreement with reported structural characteristics of flavonoids. Ligands like epigallocatechin-3-gallate and baicalein contained a high donor and acceptor count, which can improve targeted interactions with biological targets but at the same time compromise membrane permeability if in excess.

Amongst the candidates, naringenin, quercetin, hesperetin, and epigallocatechin were identified as lead scaffolds for drug development, with a balance of drug-likeness, solubility, and permeability attributes. Highly hydrophilic or large molecules such as epigallocatechin-3-gallate, on the other hand, would need formulation approaches to enhance bioavailability.

Therefore, the physicochemical profiling indicates that most of the flavonoids studied have good drug-like characteristics, warranting their further assessment in in-silico docking.

Table 3: Filtered Phytochemical Ligands Based on Drug-Likeness and Bioactivity Scores Using Molinspiration Software Analysis

Sl.No	Ligands	Mol Wt	Log P	Log S	MolPSA	Mol Vol	Drug likeness	HBA	HBD
1.	Naringenin	272.07	2.30	-3.69	71.98	251.12	1.13	5	3
2.	Quercetin	302.04	2.11	-3.87	102.61	281.71	0.93	7	5
3.	Epigallocatechin	290.08	1.88	-3.24	90.45	261.13	0.92	6	5
4.	Hesperitin	302.08	2.27	-3.81	78.55	283.84	0.88	6	3
5.	Luteolin	286.05	2.68	-4.07	89.05	272.86	0.86	6	4
6.	Butein	272.07	2.60	-3.67	80.48	265.25	0.82	5	4
7.	Kaempferol	286.05	2.49	-3.96	87.13	268.99	0.77	6	4
8.	Apigenin	270.05	3.06	-4.16	73.57	260.14	0.77	5	3
9.	Fisetin	286.05	2.49	-3.93	86.06	271.53	0.76	6	4
10.	Baicalein	446.08	0.13	-3.15	143.74	397.35	0.73	11	6
11.	Hispidulin	300.06	2.91	-3.47	79.15	291.88	0.72	6	3
12.	Geneistein	270.05	2.72	-3.89	72.64	260.69	0.71	5	3
13.	Formononetin	268.07	3.45	-4.36	46.02	271.80	0.70	4	1
14.	Eupatilin	344.09	3.23	-3.79	76.79	344.60	0.69	7	2
15.	Isohamnetin	316.06	2.46	-3.98	93.69	301.71	0.67	7	4
16.	Rhamnazin	330.07	2.81	-4.20	83.62	323.01	0.65	7	3
17.	Diadzein	254.06	3.10	-3.95	56.09	250.50	0.56	4	2
18.	Glycitein	284.07	3.07	-4.01	62.66	283.22	0.47	5	2
19.	Diosmetin	300.06	3.03	-4.28	80.13	292.86	0.42	6	3

20.	Epigallocatechin-3-gallate	458.08	2.58	-3.76	158.72	404.49	0.39	11	8
21.	Isothymonin	360.08	2.73	-3.00	92.04	354.36	0.33	8	3
22.	Chrysin	254.06	3.32	-4.30	55.95	249.59	0.18	4	2
23.	Galangin	270.05	2.75	-4.10	69.51	258.44	0.13	5	3
24.	Camptothecin	348.11	1.58	-4.84	62.92	387.50	0.11	5	1

3.8 Toxicity Prediction

Table 4: Non-Carcinogenic Phytochemical Compounds Identified Through DeepToxLab Screening with High-Confidence Predictive Scores Indicating Favorable Toxicological Profiles

Compounds	Carcinogenicity	Carcinogenicity_uncertainty	developmental_toxicity	developmental_toxicity_uncertainty
Kaempferol	0.160004	High-confidence	0.050185	High-confidence
Hispidulin	0.063692	High-confidence	0.790232	Low-confidence
Geneistein	0.127951	High-confidence	0.279911	Low-confidence
Isohamnetin	0.101123	High-confidence	0.112059	Low-confidence
Rhamnazin	0.14471	High-confidence	0.192321	Low-confidence
Diosmetin	0.161894	High-confidence	0.60431	Low-confidence
Isothymonin	0.038897	High-confidence	0.094264	High-confidence

Among seven compounds identified as non-carcinogenic based on an initial screen of 24 compounds on the DeepToxLab platform, backed by low scores for carcinogenicity (all ≤0.2) and high-confidence predictive output, kaempferol, hispidulin, genistein, isohamnetin, rhamnazin, diosmetin, and isothymonin were all recognized as potential candidates. The predictive model singled out Isothymonin and Hispidulin as particularly compelling with scores of 0.039 and 0.064, respectively. All seven compounds are flavonoid derivatives, a structurally diverse family of compounds well characterized for their anti-inflammatory, antioxidant, and chemoprotective activities. Their safe toxicity profiles, as reliably predicted in silico, concur with past literature reports of the low carcinogenic risk posed by naturally occurring flavonoids. DeepToxLab's high-confidence predictions provide a good-quality early-screening option, enabling prioritization of lead molecules toward development. But as is the case with any computational screen, these results deserve experimental confirmation using in vitro and in vivo toxicity studies prior to considering them viable drugs or therapeutic agents.

3.9 Docking

Molecular docking experiments were performed to assess the binding efficiencies of several flavonoid molecules with the active site of matrix metalloproteinase-1 (MMP-1) (active cavaties). Docking scores are presented in Table 5.

Table 5: Molecular Docking Scores of Selected Flavonoid Compounds Against MMP-1, Highlighting Binding Affinities and Potential Inhibitory Interactions

COMPOUNDS	DOCKING SCORE
Kaempferol	-8.5
Hispidulin	-8.4
Geneistein	-7.8
Isohamnetin	-9.0
Rhamnazin	-8.0
Diosmetin	-8.5
Isothymonin	-8.1

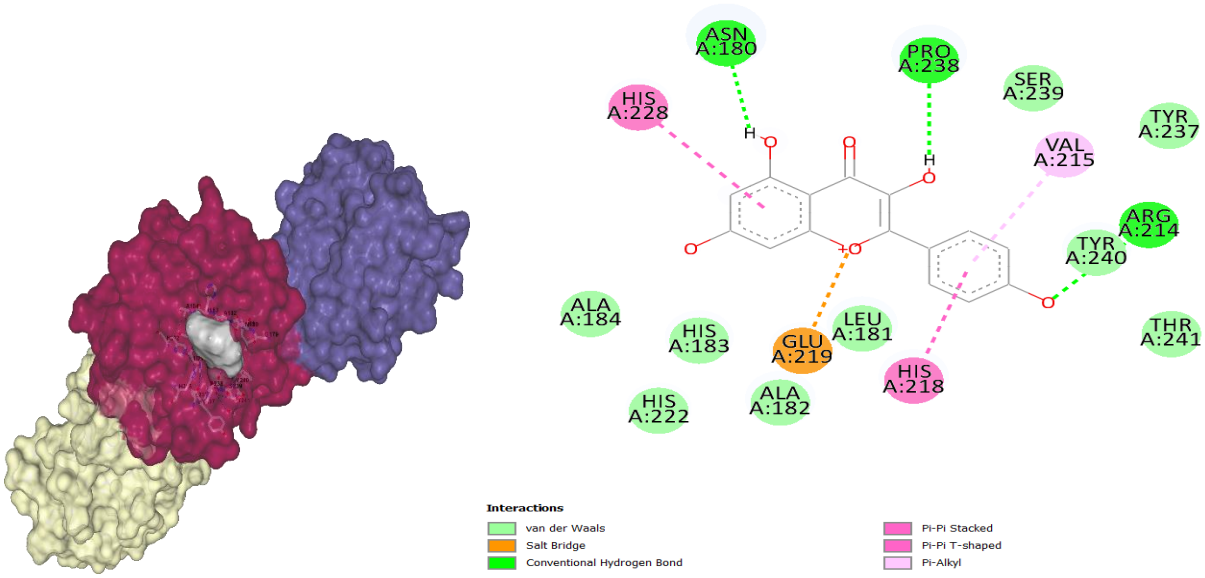

Figure 5 ; Kaempferol binds with the active site of MMP-1

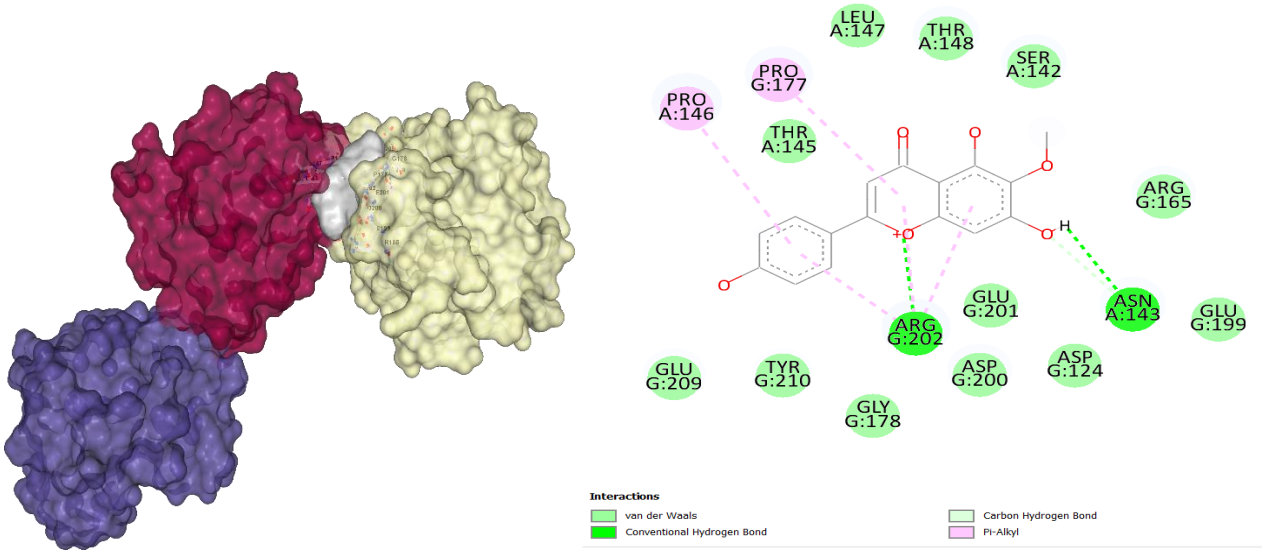

Figure 6 ; Hispidulin binds with the active site of MMP-1

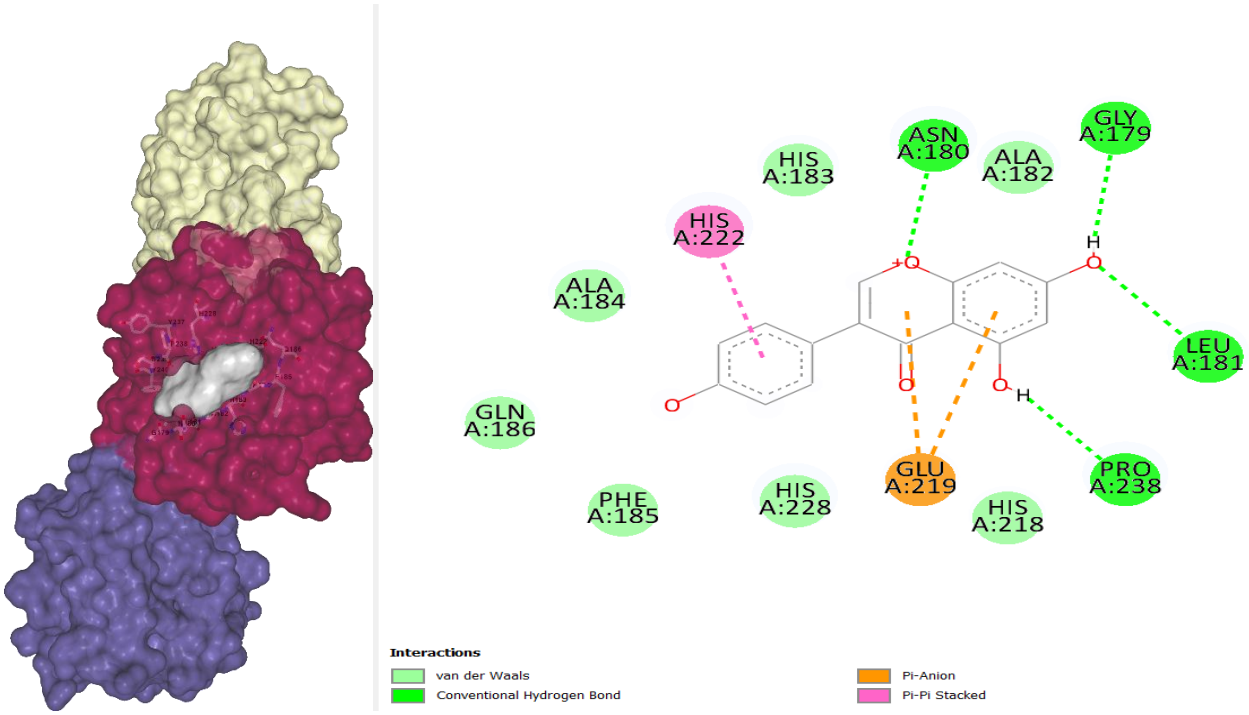

Figure 7 ; Geneistein binds with the active site of MMP-1

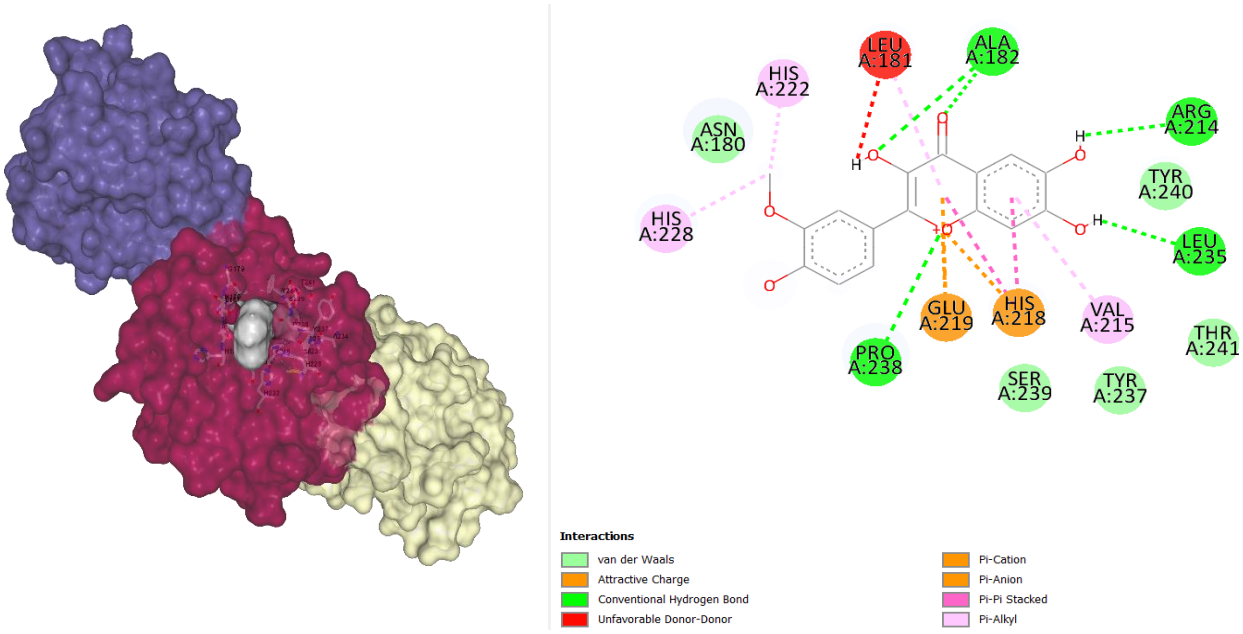

Figure 8 ; Isohamnetin binds with the active site of MMP-1

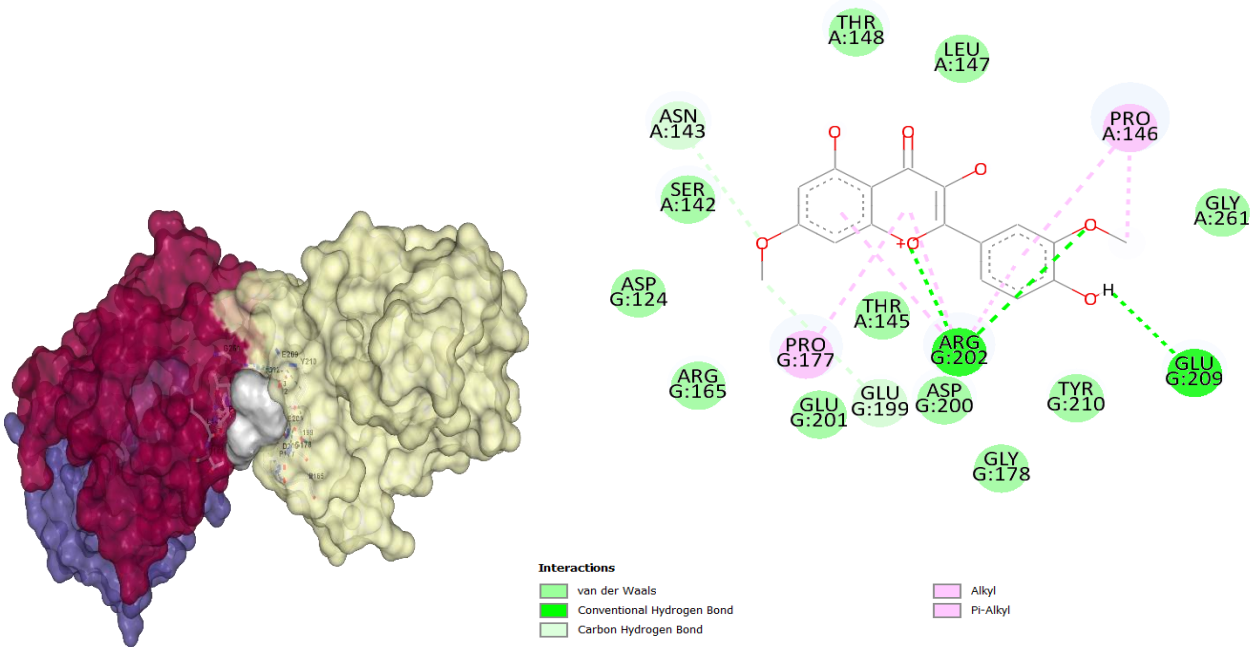

Figure 9 ; Rhamnazin binds with the active site of MMP-1

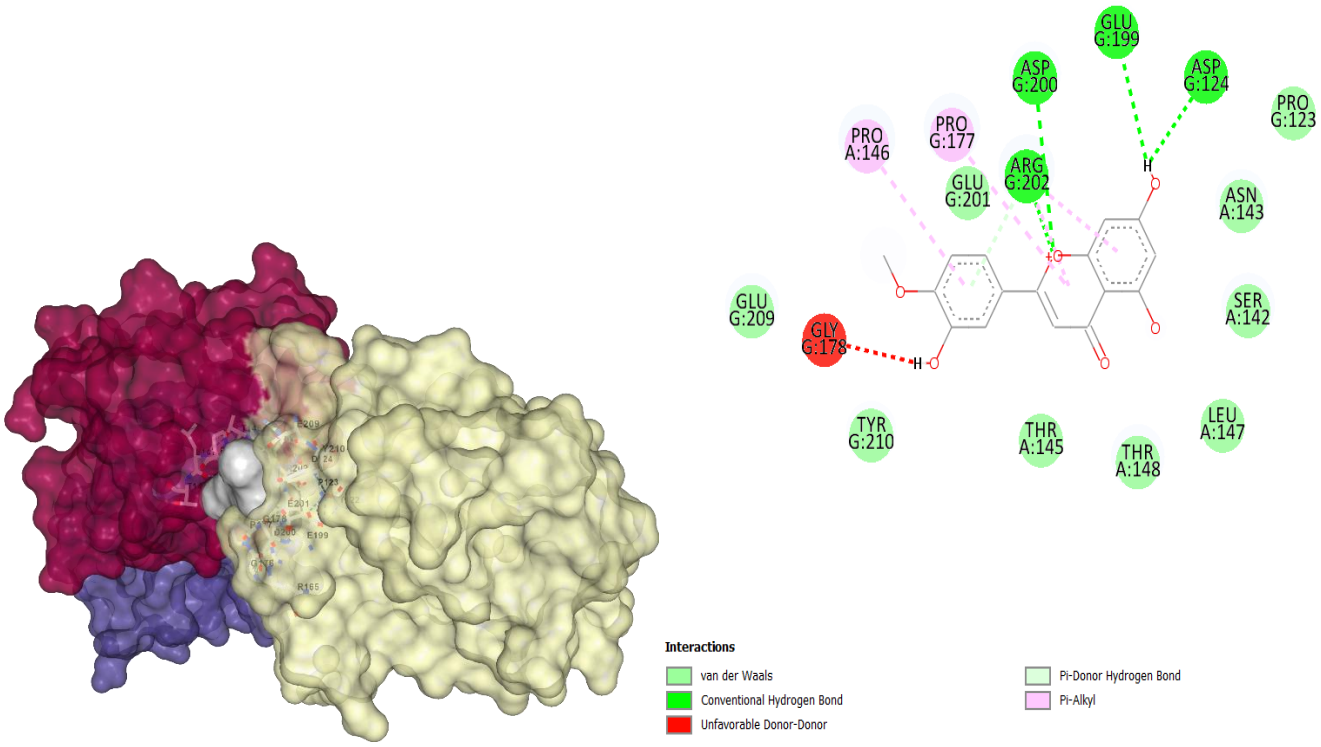

Figure 10 ; Diosmetin binds with the active site of MMP-1

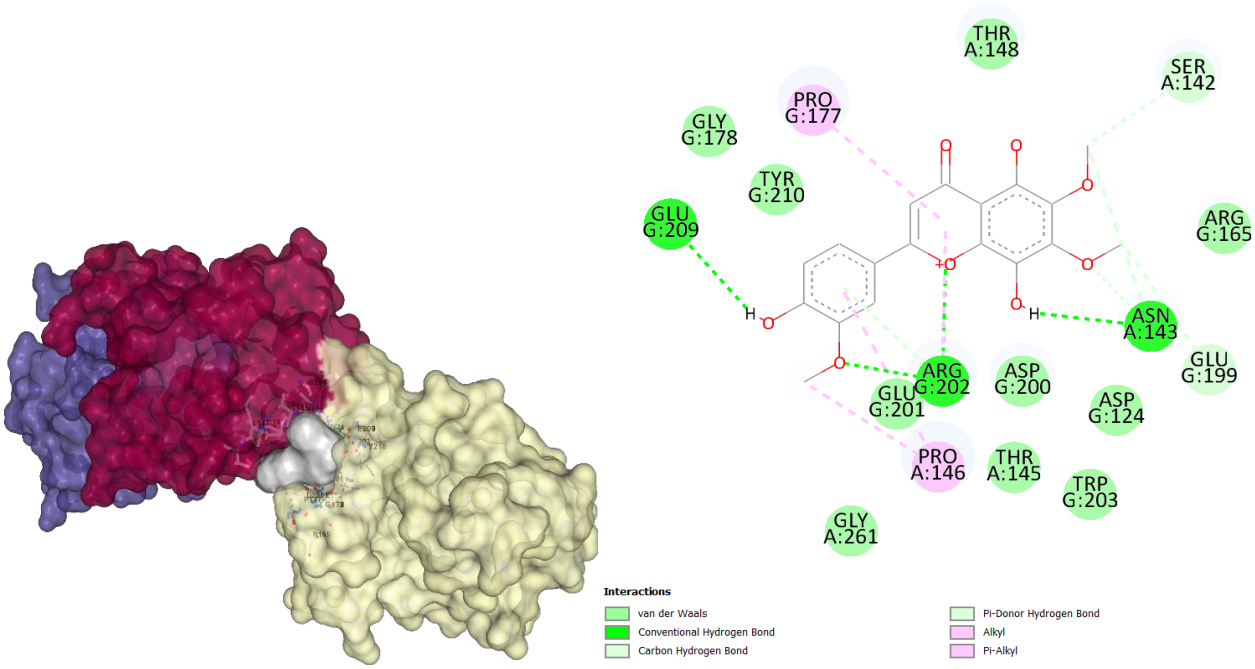

Figure 11 ; Isothymonin binds with the active site of MMP-1

Figures 5–11: Molecular Docking Images Depicting the Binding of Chosen Flavonoids (Kaempferol, Hispidulin, Genistein, Isohamnetin, Rhamnazin, Diosmetin, and Isothymonin) to MMP-1 Active Site.These structures represent the molecular docking interactions between matrix metalloproteinase-1 (MMP-1) and seven bioactive flavonoid compounds isolated by virtual screening. All the compounds showed a good binding affinity to the active site of MMP-1, establishing crucial interactions with catalytic or substrate-binding residues. The docking conformations indicate the promise of these flavonoids as natural MMP-1 inhibitors, making them worthy of further investigation in breast cancer therapy against MMP-mediated metastasis.

Among the compounds tested, isohamnetin had the highest binding activity to MMP-1 with a docking score of -9.0 kcal/mol, suggesting it as a highly potent MMP-1 inhibitor. Kaempferol and diosmetin had high binding activities as well, both with docking scores of -8.5 kcal/mol. Geneistein, on the other hand, had the poorest interaction with a docking score of -7.8 kcal/mol.

Visualization of the docking poses indicated that all compounds were able to bind within the active site of MMP-1 and form multiple interactions likely to be responsible for their binding stability. The results indicate that some flavonoids, notably isohemnetin, can be good lead compounds for further development as MMP-1 inhibitors.

4 Discussion

Better understanding of proteins involved in breast cancer pathogenesis is warranted to develop novel therapeutic methods for treating this disease (Gao et al., 2016). In the end, we selected the top upregulated candidate proteins by the STRING database and then performed visualization using the Cytoscape tool. Molecular interaction networks of proteins can be proficiently studied using network visualization software. Cytoscape can make a reputed protein-protein interaction network for a target protein. Its vital arrangement principle is a network diagram, with biological entities (genes/proteins) represented as nodes and biological interactions represented as edges (Doncheva et al., 2012).

Visualization results illustrated that most of the top-ranked candidate genes involved in metastasis of breast cancer are directly connected to either breast cancer or known breast cancer-associated proteins. The results of the current study indicated that MMP-1 may have key functions in the progression of breast cancer by interaction networks.

So far, prognostic targets were screened based on co-expression modules analysis for prostate cancer (Li et al., 2008), pancreatic cancer (Bahadorimonfared et al., 2024), and breast cancer (Place et al., 2011). However, our study is the first of its kind to validate the involvement of MMP-1 and its co-expressed proteins in breast cancer. Thus, based on the literature (Nagel, 2019) (Wang et al., 2017). indicating the role of MMP-1 in the metastasis of breast cancer, and based on network analysis indicating the interactions of various proteins with MMP-1 in breast cancer, MMP-1 was chosen as the target molecule, and its inhibition by phytocompounds using *in-silico* studies was done.

5 Conclusion

A pre-filter screening of 125 phytochemicals by Prediction of Activity Spectra for Substances (PASS) online program resulted in 56 candidate compounds showing good multi-target potential, especially against metastasis, breast cancer, and matrix metalloproteinase (MMP) expression. Selection criteria used considered compounds with more than a moderate chance of activity against at least two relevant pharmacological actions such that only compounds with strong therapeutic potential were being left behind. Importantly, the resultant subset demonstrated enrichment in flavonoid structures, consistent with existing literature highlighting the varied biological activities of flavonoids, including their well-established anticancer, anti-metastatic, and MMP-inhibitory effects.

The initial virtual screening thus sets a solid basis for the remainder of the research pipeline. The combination of differentially expressed gene (DEG) analysis with the construction of protein-protein interaction (PPI) networks will enable the contextual positioning of MMP-1 in the wider molecular context of breast cancer. The systems biology strategy will enable the screening for potential synergistic interactions between chosen phytochemicals and key nodes in the cancer-related signal transduction pathways, providing greater mechanistic understanding of their therapeutic value.

After the identification of lead compounds, absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling will be used to evaluate their drug-likeness and safety profiles. Only candidates with desirable pharmacokinetic and toxicological profiles will be progressed to the molecular docking stage, where their binding affinities and modes of interaction with primary molecular targets will be extensively explored. This stringent, stepwise process integrating in silico predictive modelling , network pharmacology, and structure-based drug design seeks to optimize the identification of novel phytochemical-derived therapies that are not merely efficacious but also safe and tolerable. Ultimately, this integrated approach possesses great potential for the creation of innovative and targeted therapeutic interventions against breast cancer, especially those treating metastatic progression and MMP dysregulation.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

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Author'shereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Author Contributions Statement

I, V.Shunmuga Priya conceived the study, designed the experiments and supervised the project. I conducted the literature review and selected the phytochemicals for screening. Mr.C Vijay Aadhithya performed toxicity prediction and molecular docking studies. Ms.S Vanaja, analyzed and interpreted the results. All authors contributed to manuscript writing, reviewed the final version, and approved it for submission.

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