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RESEARCH ARTICLE

Exploring Natural Compound Libraries for Breast Cancer Targets: An *In Silico* Study

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Abstract

Background: Breast cancer is a globally prevalent, heterogenous disease affecting both women and men across all ethnic groups. It is a complex disease and is the second most frequently diagnosed solid tumour in women worldwide. The PI3K/AKT/mTOR signalling pathway plays a significant role in breast cancer progression, survival, and drug resistance. It is dysregulated in approximately 20-34% of cases, making it a key target for therapeutic intervention. **Aim:** This study aims to investigate the potential of natural compounds against the PI3K/AKT/mTOR signalling pathway using molecular docking studies to evaluate their binding affinity and interactions with target proteins. **Methods:** Molecular docking was performed to analyse the binding energy, conformational changes, and amino acid interactions of selected natural compounds with PI3K and AKT. **Results:** Our virtual molecular docking studies suggest that the natural compounds ginsenoside (-7.39 kcal/mol), nimbolide (-6.22 kcal/mol), and pristimerin (-6.28 kcal/mol) exhibit strong binding affinities toward PI3K, indicating their potential as pathway inhibitors. Additionally, pristimerin (-5.52 kcal/mol), curcumin (-5.63 kcal/mol), and ginsenoside (-6.07 kcal/mol) demonstrated strong interactions with AKT. **Conclusion:** The docking results suggest these natural compounds exhibit strong interactions with PI3K and AKT, indicating their potential as effective pathway inhibitors. The favourable binding affinities suggest that these compounds may serve as promising candidates for targeted breast cancer therapy, especially for patients with PI3K/Akt pathway dysregulation. Further experimental validation is required to confirm their therapeutic efficacy.

Major Findings: Nimbolide, pristimerin, and ginsenoside demonstrate significant therapeutic potential in breast cancer treatment by modulating the PI3K/AKT signalling pathway.

Keywords: Breast Cancer, Molecular Docking, Natural Compounds, PI3K/AKT/mTOR Pathway

1. Introduction

Breast cancer is a major global health concern and the most frequently diagnosed solid tumour worldwide[1]. In 2024, an estimated 310,720 new cases of invasive breast cancer were reported among U.S. women. According to GLOBOCAN 2022 data, approximately 2.3 million new cases were diagnosed globally, making it the most commonly diagnosed malignancy. It is also the fourth leading cause of cancer-related death due to breast cancer[2,3]. Breast cancer affects women of all ages, with its incidence increasing with advancing age[4]. It is expected that 2,964,197 new cases will be diagnosed in 2040, which equals to an increase by a third over two decades since more than ten hundred thousand women were diagnosed with breast carcinoma for the past ten years, and further growth would only enlarge this number[5]. This rise is attributed to population growth, aging, and increased awareness through screening programs. In low Human Development Index (HDI) countries, breast cancer mortality rates are much higher compared to incidence rates. While approximately 1 in 27 women is diagnosed with breast cancer during their lifetime, 1 in 48 women will succumb to the disease in these regions[4]. Globally, breast cancer is the second most common cancer

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among women, following non-melanoma skin cancer[6]. Being female is a significant risk for developing breast cancer[7]. Approximately 99% of breast cancer cases were diagnosed in the female population[8,9]. However, it is important to note that men are also susceptible to breast cancer, accounting for 0.5-1% of all diagnoses. It is a most prevalent disease that effects all ethnic groups and both genders. The disease develops in the lobules or milk ducts of the breast, and if left untreated, it can spread to other parts of the body, becoming life-threatening.

Breast carcinoma primarily arises from two types of breast tissue: ductal epithelium and lobular epithelium. While most cases originate in the ducts, cancerous cells can also develop in the milk-producing glands. Certain benign breast conditions, such as proliferative and non-proliferative diseases, may increase the risk of developing breast cancer. However, other breast abnormalities, such as sarcomas and lymphomas, are not typically associated with breast carcinoma[10].

Risk factors for breast carcinoma include advanced age, being obese, excessive alcohol consumption, previous exposure to radiation, genetic predisposition, past reproductive history (including age at first pregnancy and onset of menstruation), use of tobacco products, and postmenopausal hormone replacement therapy[11]. Over the past few decades, breast cancer incidence has increased by 30-40%, largely due to the widespread adoption of mammography screening programs that target women in high-risk age groups. These screening efforts have significantly improved early detection and patient prognosis.

Breast cancer is a heterogeneous disease that can be categorized based on molecular and histological characteristics using Immunohistochemistry (IHC)[12]. Histologically, it is categorized into invasive and non-invasive types, which are further divided into lobular and ductal subtypes. Molecular classification is based on the presence of Estrogen/Progesterone receptors (ER/PR) and Human Epidermal Growth Factor Receptor 2 (HER2). Breast cancer is classified into three molecular subgroups: Triple-Negative Breast cancer (TNBC) (15% of cases), HER2 positive (15-20% of cases), and HR positive/ERBB2 negative (70% of patients)[13].

The most common hereditary gene mutations associated with breast cancer include BRCA1, BRCA2, and PALB2, which increase the risk of developing the disease. Other important diagnostic and prognostic markers include estrogen receptors (ERα, ERβ), progesterone receptors (PRα, PRβ), HER2, Ki-67, and serum markers such as CA 15-3 and Bcl-2. Emerging genetic markers and multigene panels are also becoming increasingly relevant for prognosis and treatment selection[14].

Timely diagnosis and comprehensive multidisciplinary treatment plans are critical for improving breast cancer outcomes. Kinases are the vital targets in treating various diseases like tuberculosis, cancer, *etc*[15]. Breast cancer treatment involves a combination of surgery, radiation therapy, cytotoxic chemotherapy, endocrine therapy, and targeted biological therapies. However, additional strategies are needed to reduce breast cancer incidence in high-risk populations.

One promising approach is chemoprevention, which involves the use of natural or synthetic compounds to prevent, delay, or suppress cancer development[16]. Some of the heterocyclic rings have shown therapeutic potential against various types of cancers. Five- and six-membered nitrogen-containing heterocyclic molecules, such as thiazole, pyridine, and pyrimidine derivatives, have gained significant attention in drug discovery due to their diverse biological activities[17-19]. These scaffolds are widely explored for their potential in cancer therapy. The FDA has approved several drugs for breast cancer treatment, including doxorubicin, docetaxel, fulvestrant, lapatinib, eribulin mesylate, trastuzumab, and everolimus. However, challenges such as tumour heterogeneity, DNA repair mechanisms, genetic resistance (*e.g.*, efflux transporters), and the emergence of tumour subclones contribute to chemoresistance. Given the limited treatment options for metastatic breast cancer, chemoprevention may provide an effective alternative. Developing new natural therapeutic agents is essential to achieving selective cancer cell targeting, reducing toxicity, and minimizing side effects.

Computational methods have long been essential in rational drug design and the identification of novel candidates. These resources accelerate the drug discovery process, minimizing both time and costs. In this study, we employed molecular docking to evaluate the binding affinity between the natural compounds and breast cancer targets[20-22].

2. Phytochemicals and Breast Cancer

The use of natural phytocompounds in pharmaceutical studies has been extensive because of their broad spectrum of pharmacological actions and the easy availability of medicinal plants. Phytochemicals are naturally existing chemicals found in plants that have gained attention in the area of research on the prevention and treatment of malignant breast disease. Worldwide, breast cancer is the most prevalent type of malignancy among females and has posed a great threat to our health system; hence, there are many challenges related to implementing successful prevention strategies and curative strategies. In recent studies, a variety of different phytochemicals have been reported to possess potent anti-cancer properties. Phytochemicals and bioactive compounds such as alkaloids, anthocyanins, carotenoids, flavonoids, glycosides, phenols, polyphenols, polysaccharides, saponins, and terpenoids are found abundantly in fruits, vegetables, and medicinal plants and exhibit a range of biological activities that may disrupt crucial pathways linked to the emergence of cancer[23]. These attributes may inhibit cells from adopting malignant fate, prevent carcinogenesis and altogether perform synergistically to improve the prognosis of traditional remedies.

These compounds are also known to alter key steps in inflammation and cell cycle progression, including those that promote the transformation of preneoplastic lesions into carcinomas. Further highlighting their potential use in chemoprevention and therapy, the synergistic effects of phytochemicals, especially when consumed as part of a plant-based diet, have been associated with a reduced incidence of breast cancer[11-13]. In this article, the present data on phytochemicals is investigated in relation to their multi-faceted role as promising anti-cancer agent, including those with reduced toxicity and increased selectivity leading to reduced undesirable effects, offering insights into their potential as crucial components of all-encompassing cancer care. Structures of selected natural compounds are shown in Figure 1 (Table 1).

2.1 Apigenin

Apigenin [2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one] is classified as a part of the flavone class of plant secondary metabolites and is the aglycone of several naturally occurring glycosides. The primary sources of apigenin include wheat sprouts, onions, chamomile, celery, tea, and parsley. It has medicinal qualities like sedative and anti-inflammatory properties. It inhibits angiogenesis and carcinogenesis in melanoma, colon and breast cancer[24]. Dou *et al.*. used a 70% methanol solution to separate the apigenin from *G. veitchiorum* flowers, and then they used High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS) to analyse the constituents of apigenin[25]. Apigenin is a multi-targeted drug that suppresses the growth of breast cancer cell lines by inhibiting the production of growth factors, activating p53, inhibiting transcription factors, and blocking receptor tyrosine kinases[26]. Apigenin mitigates the Akt/FOXM1 signalling pathway by suppressing the levels of FOXM1 (Forkhead box protein M1), a transcription factor essential to the development of the cells. In the MCF-7/AKT clone, apigenin altered the gene expression related to the mitotic cycle that is regulated by FOXM1[27]. Through inducing G2/M phase arrest, raising reactive oxygen species, decreasing Akt phosphorylation, and blocking the PI3K pathway, which is essential for tumour growth, apigenin inhibited the proliferation of MDA-MB-468 cells[28]. Apigenin is classified as a BCS class II drug, meaning it has elevated intestinal permeability with low water solubility. Despite its effective pharmacological properties, its poor solubility limits clinical use. It is essential to develop a new formulation to improve solubility and bioavailability of Apigenin. Nanoparticles have shown promise in improving both its solubility and bioavailability in animal and laboratory studies[29].

2.2 Icaritin

Icaritin is a prenyl flavonoid that has a prenyl group at C-8, Methoxy group at C-4' and Hydroxyl groups at C-3, C-5, and C-7. In Traditional Chinese medicine, icaritin is used to strengthen bones and muscles. In addition to immunological modulation and anti-inflammation, it also provides neuroprotection, cardiovascular protection, and regulation of bone and lipid metabolism. It is present in medicinal plants of the *Epimedium* species (family Berberidaceae). Icaritin can be made by hydrolysing other significant flavonoids found in Epimedium plants, or it can be taken from

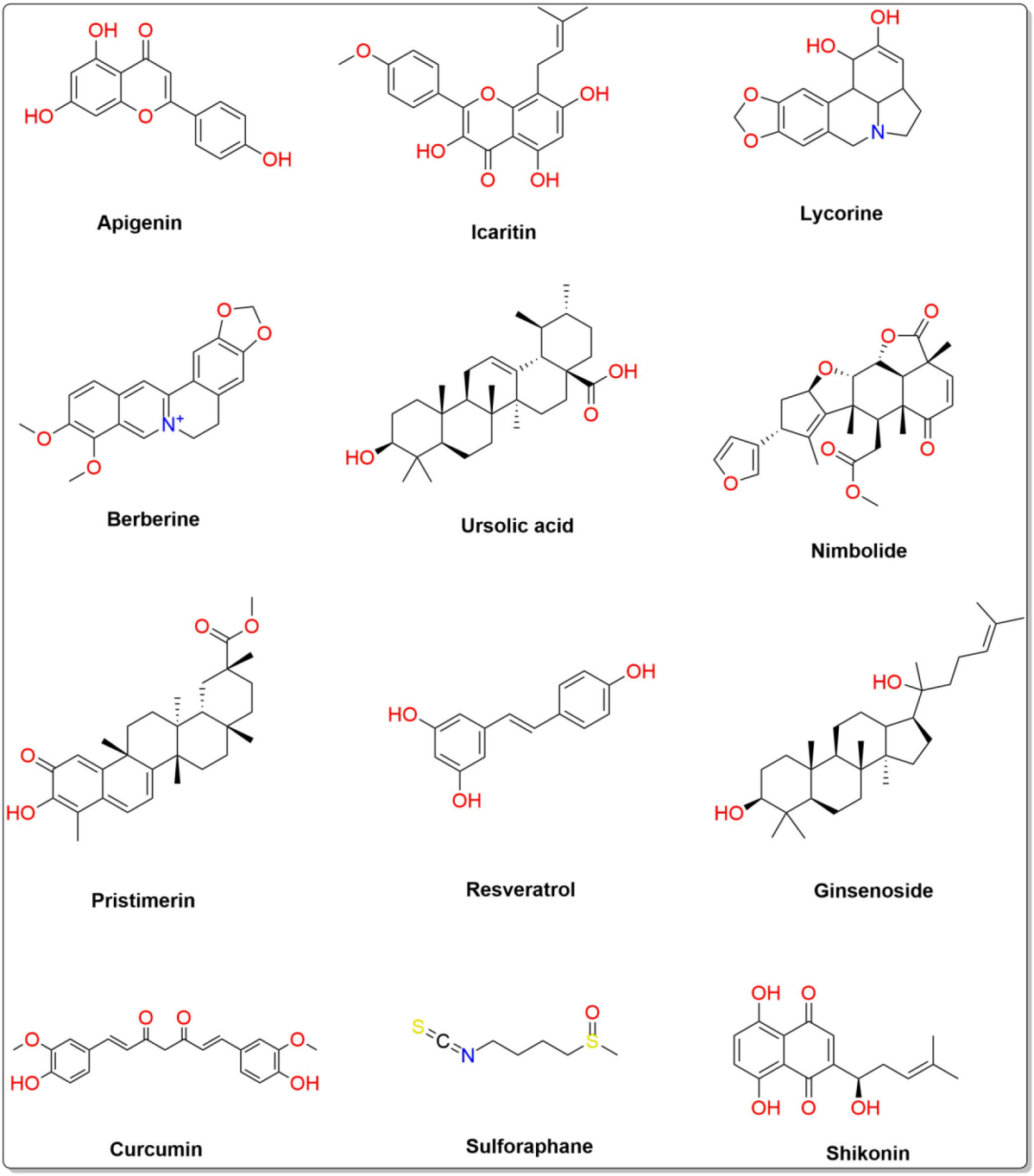


Figure 1. Structures of selected natural compounds.

the stems and leaves. Techniques used in production include hydrolytic reactions to separate the glycone components from icariin and icarisides I and II, chemical synthesis, column chromatography, and acid or enzymatic hydrolysis[30,31]. According to the study, icariin can impede the production of MELK (maternal embryonic leucine zipper kinase), which modulates the PI3K/AKT signalling cascade. In particular, treatment with icariin reduces breast tumour cell migration, increases apoptosis, and decreases cell proliferation[32]. By lowering AKT phosphorylation, P13k activity, and mTOR signalling, icaritin promotes cancer cell apoptosis and reduces proliferation. Icaritin downregulates drug resistance proteins such as permeability glycoprotein and multidrug resistance-related proteins, which are also governed by PI3K/AKT/mTOR cascade. By

Table 1. Collection of natural compounds

S.no	Name of the compound in literature	Source and (Family)	Activity		Mechanism
			MCF-7 (µM)	MDA MB-231 (µM)	
1	Apigenin	wheat sprouts, onions, chamomile, celery, tea, and parsley.	2	14	Induce apoptosis Inhibit proliferation [27]
2	Icaritin	Epimedium species (family Berberidaceae)	2	3	Blocking several pathways including ER-α36, NF-κB, and IL-6/JAK/STAT3, cell cycle arrest and apoptosis [31]
3	Lycorine	*Lycoris radiate* (Family Amaryllidaceae)	7.76±1.16	1.84±0.21	β-catenin signalling suppression and reversed epithelial-mesenchymal transition (EMT) process [36]
4	Berberine	*Berberis* (Family Berberidaceae)	26.53±3.12	4.14±0.35	Decreases E-cadherin expression and HIF-1α protein levels, Anti metastatic to hypoxic tumours [38]
5	Ursolic acid	*Malus pumelia* (Family Rosaceae)	29.0±2.1	24.0±1.8	Promotes PTEN expression, Inactivates FAK/PI3K/Akt/mTOR signalling pathway, Anti-angiogenesis [43]
6	Nimbolide	*Azadirachta indica* (Family Meliaceae)	5.04	1.97	Inhibits mTOR and p62, elevates Beclin 1, inhibits proliferation [46]
7	Pristimerin	*Trypterigium wilfordii* (Family Celastraceae)	0.38 – 1.75 (range)		Anti-angiogenesis, Free radical scavenger [60]
8	Resveratrol	Grapes, peanuts, soybeans, berries	131	25	Apoptosis, blocks pro-carcinogens, Inhibits mTOR pathway [53]
9	Curcumin	*Curcuma longa* (Family Zingiberaceae)	24.5	3.8±0.2	Upregulates Caspase-3 and Caspase-9, induces cell cycle arrest in S+G2/M phase [54]
10	Ginsenoside	*Panax spp* (Family Araliaceae)	16.2	6.2	Blocks NF-κB activation, Reprograms metabolic pathway [48]
11	Sulforaphane	Cruciferous vegetables	14.05	19.35	Blocks G2-M cell cycle, Apoptosis [50]
12	Shikonin	*Lithospermum erythrorhizon* (Family Boraginaceae)	10.3	15	Inhibiting Erα, Metastasis [56]

blocking this signalling cascade, Icaritin may reduce the expression of these proteins and enhance the effectiveness of chemotherapy drugs[33].

2.3 Lycorine

Lycorine, a natural isoquinoline alkaloid derived from the Amaryllidaceae family, has shown significant potential in the treatment of breast cancer. The Amaryllidaceae species most commonly reported to contain lycorine include *Lycoris radiata, Leucojum aestivum, Hymenocallis littoralis, Hippeastrum equestre,* the flowers of *Clivia nobilis, Ammocharis coranica, Brunsvigia radulosa, Crinum macowanii,* and *Leucojum aestivum*. It has anti-inflammatory, antifungal, antiviral, antitumour, and autophagic regulation properties. It also promotes programmed cell death in cancerous cells. The first member of the Lycoris family to be isolated was the pyrrole triphenyl acid alkaloid or lycorine. Its molecular name is 2,4,5,7,12b, 12c-hexahydro-lH-(1,3) dioxolo (4,5-j)-pyrrolo (3,2,1-de) phenanthridine-1-diol[34,35]. It substantially prevented breast tumour metastasis in MDA-MB-231 tail vein mode and MCF 7 cell lines, as well as tumour development and metastasis in xenograft mouse models in both *in vitro* and *in vivo* studies. It has been proven that lycorine inhibits the movement, spread, and progression of breast cancer cells[36].

2.4 Berberine

Berberine is a secondary metabolite belonging to the benzyl isoquinoline class of proto-alkaloids. It is a quaternary ammonium salt found in plant species

of *Berberis*, from which its name is derived. Berberine is present in a few plants, including tree turmeric, phellodendron, goldenseal, Oregon grape, goldthread, and European barberry[37]. Berberine is a naturally occurring alkaloid that has demonstrated encouraging anti-tumour properties against breast cancer via multiple pathways. As a multi-target phytochemical, berberine has been effective in the intervention of numerous cancers and inflammatory ailments. Research has shown that berberine significantly reduces the spread of MDA-MB-231 and MCF-7 strains. By inhibiting AKT, berberine increases the levels of critical regulators of the cell cycle (p21/cip1 and p27/kip1), further improves the cellular localization of these proteins and stabilizes them by changes after translation. These effects increase the blockade of the cell cycle and necrosis in carcinoma cells of breast[38]. The mTOR/AKT signalling pathway depends on SIK3, a part of the AMP-activated protein kinase family for cellular growth. SIK3 is highly expressed in breast malignancies and increases phosphorylation of AKT. By suppressing SIK3 expression and interfering with the AKT pathway, berberine and emodin induce G1/S phase cell cycle senesces and programmed cell death in breast cancerous cells[39].

2.5 Ursolic Acid

Ursolic Acid (UA) is a pentacyclic triterpenoid with hypoglycaemic, anti-obesity, and anti-cancer effects. Ursolic acid, which is abundant in *Malus pumila* Mill peels, arrests the cell cycle, promotes apoptosis, scavenges radicals and modulates both pro- and anti-apoptotic proteins. It also stops the angiogenesis, proliferation and spread of breast cancer cells.

Additionally, Ursolic acid exhibits the potential to reduce tumour invasion and metastasis and sensitizing to chemotherapy and radiation[40]. The *Rosmarinus officinalis* leaves, which contain up to 2.95% of Ursolic Acid, are recognized as the commercial source of the compound. The anti-proliferative effect of ursolic acid was maximal in MDA-MB-231 and MCF-7 cell lines. The proliferation of breast carcinoma cells was significantly suppressed by Ursolic acid in a concentration-time-dependent manner. Ursolic acid has been shown to dramatically slow the onset and progression of breast cancer in zebrafish and mouse xenotransplantation models, all while exhibiting no noticeable hepatotoxicity, nephrotoxicity, or hematotoxicity[41].

In quantitative RT-PCR and Western blot imaging examinations of MDA-MB-231 and MCF-7 breast cancer cell lines, Ursolic acid mechanically promotes PTEN expression to inactivate the FAK/PI3K/Akt/mTOR signalling pathway and the lowered amount of c-Myc. In a mechanism dependent on death receptors, ursolic acid can cleave Caspase-9 and reduce mitochondrial membrane activity. Furthermore, ursolic acid decreased the levels of CDK4, Cyclin D1 and FOXM1, leading to apoptosis in the MCF-7 breast cancer cell line. Due to the low solubility and bioavailability of ursolic acid, liposome administration has been used. In another study, Ursolic acid administered via folate-chitosan nanoparticles led to a reduction in MCF-7 xenograft in mouse models[42-44].

2.6 Nimbolide

Nimbolide is a tetranortriterpenoid limonoid extracted from the Neem tree, *Azadirachta indica*, Meliaceae family. Nimbolide is a primary active ingredient with anti-malarial, anti-cancer, anti-proliferative, and other biological activities. A bioactive substance taken from the neem tree called Nimbolide has demonstrated encouraging anticancer properties, especially against breast cancer[45]. In xenograft models, nimbolide significantly inhibits tumour growth and cell proliferation by exhibiting high cellular toxicity contrary to an array of breast cancer cell lines[46]. Empirical research has demonstrated that nimbolide can effectively decrease tumour volume and mass in various models. By manipulating key signalling pathways, nimbolide causes programmed cell death in breast carcinoma cells. It has been demonstrated to suppress the PI3K/Akt and MAPK cascade controlled by IGF-1, which are necessary for cell vitality and growth and to promote programmed cell death. Nimbolide effectively suppressed the spread of mammary carcinoma cell stains (MDA-MB-231 and MCF-7) by facilitating autophagy signalling through the inhibition of mTOR and p62 and by inducing apoptosis via Bcl-2 inhibition and caspase cascade activation. The application of nimbolide in nanomedicine has been investigated recently, particularly with regard to the creation of nimbolide-encapsulated nanoparticles. By specifically targeting breast carcinoma progenitor cells,

which are frequently resistant to traditional therapies, these formulations seek to improve the distribution and effectiveness of nimbolide[47].

2.7 Ginsenosides

Ginsenosides are extracted from ginseng plants, including *Panax quinquefolium, Panax notoginseng,* and *Panax ginseng*. Ginsenoside is a steroidal glycoside that belongs to the family Araliaceae. The numerous physiological roles of each variety of ginsenoside include immunomodulatory, antioxidant, and anti-inflammatory effects. They show a range of biological activities, such as anti-tumour and neuroprotective characteristics, in addition to anti-diabetic effects. It has been demonstrated that ginsenoside Rd reduces breast cancer metastases[48]. According to research, it could effectively prevent breast cancer from spreading by focusing on particular metastasis-related pathways, which may have a preventive effect on the spread of cancer cells. Ginsenosides slow down the invasive and migratory properties of oncogenic cells by interfering with the PI3K/Akt/mTOR pathway, which may be involved in Epithelial-Mesenchymal Transition (EMT), a critical element in cancer metastasis[49].

2.8 Sulforaphane

Sulforaphane (SFN)is an organosulfur found in cruciferous vegetables like broccoli that shows promise in treating and preventing breast cancer, especially triple-negative breast cancer (TNBC)[50]. Sulforaphane inhibits cell proliferation by upregulating PTEN, a tumour suppressor that leads to the inhibition of the PAM cascade[51].

2.9 Resveratrol

Resveratrol, a flavonoid-free polyphenol found in soybeans, grapes, berries, Japanese knotweed, peanuts and certain fungi, exhibits various health benefits. It reduces inflammatory responses, infections caused by viruses, oxidative damage, accumulation of platelets and malignant cell progression. Advanced extraction methods, like the SPE-UHPLC technique, have improved its isolation from sources such as red wine. Resveratrol induces S phase cell cycle arrest and apoptosis by inhibiting the ERα-dependent PI3K signalling pathway in MCF-7 cell lines. Derivatives of resveratrol having more bioavailability have been produced. Methoxyl substituted resveratrol analogue 3,5,3′,4′,5′-pentamethoxystilbene (MR-5) induces halt in G1 phase cell division in MCF-7 cells by downregulating cyclin D1/D3/E and CDK2/4/6, and upregulating p15, p16, p21, and p27. MR-5 also mediates key cell cycle regulators such as AKT, ERK1/2, p38MAPK, and FAK, contributing to its anti-proliferative effects. Similarly, trans-3,5,4-trimethoxystilbene (TMS) shows 30-100-fold higher capacity than resveratrol in suppressing endothelial cell propagation and intertumoral blood vessel invasiveness[52,53].

2.10 Curcumin

Curcumin, isolated from turmeric *(Curcuma longa*) rhizomes, is a chemical polyphenol used to treat a range of conditions in humans, such as carcinoma, metabolic disorders, inflammatory diseases, and neurological illnesses. It is a natural dietary pigment with properties that reduce inflammation, inhibit growth, and prevent metastasis, thereby hindering tumour formation. It is recognized as a cancer chemo preventive and chemotherapeutic agent. In breast carcinoma cells (MCF-7 and MDA-MB-231), curcumin exerts anti-carcinoma effects by controlling matrix metalloproteinase (MMP)-2, B-cell lymphoma 2 (Bcl-2), Bax, flap endonuclease 1 (Fen1), NF-E2-related factor 2 (Nrf-2), and the PI3K/Akt signalling pathway. Curcumin promotes apoptosis by decreasing Bcl-2 levels and raising Bax levels, and this effect is further amplified when combined with the PI3K inhibitor LY290042. Curcumin inhibits cell proliferation in S and G2/M phases by blocking the basic phosphorylation of AKT, and it also inhibits the phosphorylation of epidermal growth factor receptor (EGFR) and ERK1/2. In MDA-MB-231 cells, curcumin treatment has been reported to significantly lower AKT levels in a manner that depends on both dose and duration, leading to decreased cellular proliferation in TNBC. In MDA-MB-231 cells, curcumin treatment reduced the expression of AXL, β-catenin, slug, and vimentin. AKT activation, which is facilitated by AXL, initiates the PAM pathway. This leads to the invasion of tumour cells targeting downstream epithelial and mesenchymal regulatory markers[54].

2.11 Shikonin

Shikonin, a red naphthoquinone molecule, is widely distributed in the Shikonaceae plant's roots. It is a set of compounds rather than a single substance. 5,8-dihydroxy-1,4-naphthoquinone is the parent nucleus, and hydroxy-substituted isohexenyl is the sidechain of

Shikonin. Research indicates that shikonin may possess anti-inflammatory, antioxidant, antibacterial, and chloride channel inhibitory activities. It may also have antiviral, anti-tumour, and skin-healing abilities. Because of its diverse anticancer effects, shikonin, a naphthoquinone molecule obtained from the root of *Lithospermum erythrorhizon*, family Boraginaceae, has become a prospective candidate for breast cancer treatment. Shikonin extraction techniques that are frequently employed include solvent extraction, ultrasonic extraction, *etc*. Shikonin was extracted from *Arnebia euchroma* using the Ultrasonic-Assisted Extraction (UAE) method. Huang *et al.,* employed the Response Surface Method (RSM) to optimize the UAE method, and they discovered that the ideal extraction conditions for Shikonin were 93 W of ultrasonic power, 87 min, 39°C of temperature, and an 11:1 liquid-solid ratio[55]. Additionally, shikonin inhibited the migration, invasion, and EMT of cancer cells via interacting with Akt and p-Akt (Ser473). Shikonin has an antimigratory action via upregulating GSK-3β and PTEN to reverse EMT. Shikonin affects several important signalling pathways like PI3K-AKT and MAPK signal pathway and also changes the expression of genes linked to cell survival, proliferation and metastasis via modifying these pathways[56].

2.12 Pristimerin

Pristimerin is a quinonoid triterpene extracted from traditional Chinese medicinal herbs belonging to the *Celastraceae* and *Hippocrateaceae* families that halted the proliferation of chronic myelogenous leukaemia cells and induced cells to undergo apoptosis. Pristimerin has exhibited antioxidant, anticancer, antibacterial, and antifungal properties[57]. Pristimerin reduced the migration and invasion of breast cancer cells by lowering the expression of critical molecules in the PI3K/AKT/mTOR pathway and its effectors, P70S6K and 4E-BP1[58]. The study found that Pristimerin exhibited a severe cytotoxic impact on multidrug-resistant MCF-7/ADR breast carcinoma cell lines. This activity was ascribed to suppression of Akt signalling results in the downregulation of antiapoptotic effectors and an increase in apoptosis[59].

3. Major Signalling Pathway in Breast Cancer

Multiple signalling pathways interact with one another, responsible for the onset, spread, and resistance to treatment of breast carcinoma, making it a complex and heterogeneous disease. PI3K/AKT/mTOR is a significant signalling framework that affects the migration, metabolism, survival, and proliferation of cells. According to studies, PTEN and PIK3CA are two of the most commonly mutated tumour suppressor genes and oncogenes, and the phosphoinositide 3-kinase cascade is the most frequently altered mechanism in human carcinoma. Overall, the PAM pathway has been overactivated in 70% of breast cancer. Comprehending this signalling pathway is crucial for creating tailored treatments and enhancing therapy results for individuals with breast carcinoma.

3.1 PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR pathway is a cell communication framework triggered by Receptor Tyrosine Kinases (RTK) and Guanine-Protein-Coupled Receptors. It is essential for cell growth, progression, proliferation, survival, motility, metabolism, immunological response, and apoptosis. Diseases such as neurodegenerative disorders, vascular conditions, and tuberous sclerosis are associated with disruptions in this pathway. In breast cancer, 20-40% of instances have shown mutations in PI3K/AKT/mTOR cascade, making it a commonly altered cascade in the disease. This dysregulation contributes to aggressive tumour behaviour and resistance to treatment. This includes resistance to cytotoxic chemotherapy, HER-2-directed therapy, and endocrine therapy. Estrogen Receptor-positive (ER+) breast carcinoma is specifically linked to PI3K mutation[60].

The key player in this cascade is the PI3K (Phosphatidylinositol-4,5-bisphosphate 3-kinase) heterodimer, which is made up of two smaller units: regulatory subunit (p85) and the catalytic subunit (p110). The regulatory subunit (p85) regulates whether

growth factor Receptor Tyrosine Kinases (RTKs) activate the catalytic subunit (p110) upstream. When a cellular receptor is activated, p85 attaches itself to phosphorylated residues, releasing p110 to catalyse the conversion of PIP2 to PIP3. PTEN, a major tumour suppressor, can revert PIP3 to PIP2, negatively regulating the PAM pathway. INPP4B, another tumour suppressor, has a similar function. Proline-rich inositol polyphosphates and Phosphatase-Tensin homolog (PTEN) are two proteins that inhibit PI3K signalling. Suppression of PTEN and mutations in INPP4B (Inositol Polyphosphate 4-Phosphatase B) are notably more common in triple-negative breast cancer (TNBC), with such mutations reported in up to 66% of TNBC cases[61].

Protein phosphatases RTK, Ras, PI3K, PTEN, p53, PP2A, and PHLPP mutations cause AKT (Protein kinase B) to remain activated in the cell membrane, potentially leading to cancer. Activated by PIP3, AKT phosphorylates various downstream targets, influencing processes such as glucose metabolism, cell survival, and growth. AKT inhibits apoptosis by targeting proteins like Bcl-2 and FOXO while also regulating downstream effectors like mTOR, which integrates signals from growth factors to control protein synthesis and cell proliferation. PI3K/Akt pathway activation and its downstream signalling are shown below in Figure 2.

AKT is overexpressed in 38% of invasive breast cancers and 33% of Ductal Carcinoma In Situ (DCIS), which are premalignant and aggressive forms of breast cancers. Furthermore, enhanced AKT1 activity is observed in 40% of breast malignancies, and phosphorylation of AKT at Ser473 may promote breast cancer metastasis[62]. By reducing the expression of AKT and HER-2, curcumin suppresses cellular aggression, replication, and the spread of cancer in hormone receptor-negative breast cancer. Resveratrol (RES), a non-flavonoid phytoalexin molecule found in plants like berries, peanuts, grapes, and plums, is another substance that has been shown to suppress the PI3K/Akt pathway in a dose-dependent manner, which decreases cell migration. Resveratrol also alleviates the PI3K/Akt/mTOR signalling cascade by increasing PTEN expression and reducing Akt phosphorylation (pAkt). Ginsenosides RK1 and luteolin suppressed the PI3K-mediated signalling pathway and modulated autophagy, which affected the development of breast carcinoma cells[53].

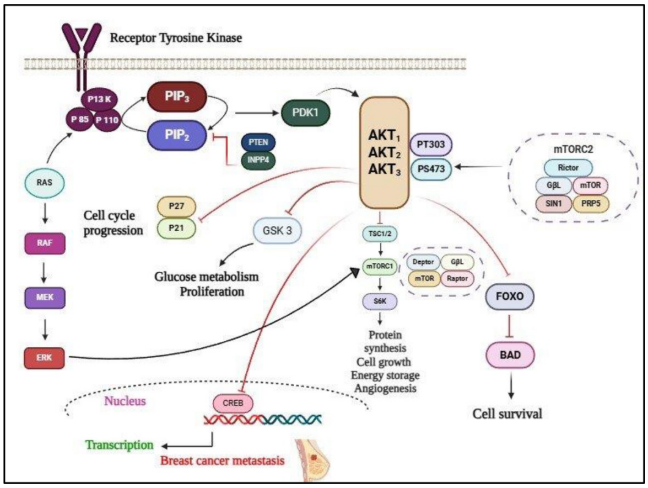


Figure 2. PI3k/AKT/mTOR cascade.

4. Methodology

4.1 Selection of Target

Drug discovery for Breast cancers has identified the PI3K/AKT/mTOR signalling pathway as an exciting target due to its vital involvement in tumour formation, survival and resistance to treatment. The pathway of PI3K/AKT/mTOR is frequently dysregulated in breast cancer, which results in uncontrolled cell proliferation and escapes apoptosis.

The PIK3CA gene, responsible for encoding a PI3K subunit, is mutated in 20-32% of cases of breast cancer. These mutations enhance signalling through this pathway, which in turn promotes carcinogenesis. One intriguing option to improve therapy efficacy and overcome drug resistance in breast cancer is to target the PAM cascade via combination treatments and selective inhibitors[63,64].

This study reveals the complex molecular interactions between active compounds and the mTOR protein during the docking analysis. Given the complex regulatory nature of the PI3K/AKT/mTOR pathway, some considerations suggest that targeting mTOR may not be the most effective approach. Blocking PI3K and AKT suppresses the PI3K/AKT/mTOR cascade, potentially rendering additional mTOR inhibition unnecessary for therapeutic benefit. mTOR inhibition

triggers a compensatory feedback loop that can lead to the overexpression and hyperactivation of PI3K and AKT. This activation signals downstream pathways that may contribute to disease progression and potentially counteract the intended therapeutic effect[65].

mTOR exists in two complexes, namely mTORC1 and mTORC2. Selective inhibition of mTORC1 (*e.g.*, by everolimus and its analogs) disrupts negative feedback control and increases AKT activation via mTORC2. In contrast, mTOR kinase inhibitors block both complexes but may have broader consequences. Inhibiting mTOR is associated with significant toxicities, including stomatitis, fatigue, hyperglycemia, and hyperlipidemia. Avoiding mTOR inhibition may help mitigate these side effects. Since PI3K and AKT are the key regulators of the pathway, their inhibition may be sufficient to achieve the desired therapeutic outcome[66]. However, there may be instances in which combining a PI3K/AKT inhibitor with an mTOR inhibitor would offer a more encompassing blockade of the pathway.

4.2 Molecular Docking Studies

Molecular docking was performed to assess the binding affinities and interactions of natural compounds with target proteins. A comprehensive literature review was conducted to identify the most promising lead compounds for docking studies. The selection criteria for lead compounds were based on their reported IC_{50} values from previous investigations. Molecular docking is an essential computational approach for lead optimization and drug discovery, allowing for the prediction of ligand interactions within an active site of target protein. Over the past three decades, docking algorithms have evolved significantly, incorporating diverse search strategies and scoring functions to enhance accuracy and reliability[67-69].

The structures of selected natural compounds were drawn using ChemDraw Professional 16.0. These structures underwent geometry optimization using an Amber 10: EHT forcefield to ensure their stability for docking studies. The 3-dimensional crystal structure of the protein was retrieved from the Protein Data Bank, selected based on parameters such as R-value, Ramachandran outlier and resolution.

The retrieved target protein is prepared using a quick prep module in Molecular Operating Environment (MOE) software. To optimize the protein for docking, water molecules and heteroatoms were removed to prevent interference in the binding site. The protein was then subjected to 3D protonation to enhance its interaction potential with electronegative atoms.

The optimized compounds underwent rigid docking using the GOLD score within the MOE 2022, Schrodinger LLC suite. For each ligand, the triangle matcher algorithm generated thirty possible conformations to identify the optimal binding mode. The best conformations were selected based on S-scores and had RMSD values, ensuring that the final conformations had RMSD values of less than 3 Å. The interactions between ligands and the target protein were visualized in both Two-Dimensional (2D) and Three-Dimensional (3D) formats. These representations facilitated the interpretation of binding interactions, including hydrogen bonding, hydrophobic interactions and π-π stacking. Specific amino acid residues involved in key interactions were identified to provide mechanistic insights into ligand binding[70,71].

5. Results

5.1 Molecular Docking Studies

Molecular docking studies were conducted to evaluate the binding interactions and affinities of selected natural compounds with two key proteins of the PI3K/AKT/mTOR signaling pathway: PI3K (PDB ID: 8TSC) and AKT (PDB ID: 3E87). These proteins play essential roles in cell survival, proliferation, and drug resistance, making them crucial targets in breast cancer therapy.

To ensure accurate docking predictions, the ligand structures of Apigenin, Icaritin, Lycorine, Berberine, Nimbolide, Pristimerin, Resveratrol, Curcumin, Ginsenoside, Sulforaphane, and Shikonin were drawn using ChemDraw Professional 16.0. Prior to docking, ligand structures were optimized by assigning appropriate protonation states and charges to reflect their biologically active forms. The docking simulations were carried out using MoE's Dock module, which employs a combination of scoring functions and algorithms to predict binding affinities. The docking parameters allowed flexibility in the ligands while maintaining the rigidity of the protein targets to ensure realistic binding interactions.

5.2 Docking Results for PI3K (PDB ID: 8TSC)

The docking scores for natural compounds against PI3K were analyzed to assess their binding affinity and potential inhibitory effects. The docking results showed that Ginsenoside exhibited the highest binding affinity (-7.39 kcal/mol), comparable to the standard PI3K inhibitor Alpelisib (-7.94 kcal/mol). Pristimerin demonstrated a docking score of -6.28 kcal/mol, indicating a strong interaction with PI3K. Nimbolide had a docking score of -6.22 kcal/mol, suggesting potential inhibition of PI3K activity.

These compounds formed significant interactions with key residues in the PI3K binding pocket, contributing to their stability and potential inhibitory effects. The binding interactions of the highest-scoring compound (Ginsenoside) with PI3K are illustrated in Figure 3, showing the hydrogen bonding and hydrophobic interactions within the active site.

5.3 Docking Results for AKT (PDB ID: 3E87)

To explore broader modulation of the PI3K/AKT/mTOR pathway, docking studies were also performed on AKT (PDB ID: 3E87). The results revealed that ginsenoside, Nimbolide, and Pristimerin exhibited moderate binding affinity toward AKT, indicating their potential to regulate AKT activity in addition to PI3K inhibition.

The docking poses revealed multiple hydrogen bonding interactions and hydrophobic contacts with key active-site residues in AKT, suggesting stable ligand-protein interactions.

The binding interactions of the best-scoring compound with AKT are illustrated in Figure 4, highlighting key interactions that contribute to its binding stability.

Table 2. Docking score and ligand residue interaction of natural compounds

Compound name Target- PI3K (8TSC)	Docking score kcal/mol	Interacting Amino acid	Type of Interaction
Apigenin	-4.60	Gln 682 Glu 135, Thr 679	Side chain donor Backbone donor
Icaritin	-5.96	Lys 678 Gln 682 Arg 683, Glu 135	Backbone donor Side chain donor Side chain acceptor
Lycorine	-4.72	Asp 133 Glu 135 Thr 462	Side chain donor Backbone donor
Berberine	-5.48	Thr 303 Asn 114 Glu 689	Back bone acceptor
Ursolic acid	-5.71	Lyn 132 Thr 462	Sidechain Acceptor
Nimbolide	-6.22	Trp 414 His 209 Phe 211	Arene- arene Back bone acceptor Backbone donor
Pristimerin	-6.28	Phe 219	Arene- arene
Resveratrol	-5.35	Asp 133	Sidechain donor
Curcumin	-6.03	Lys 678 Glu 127	Backbone donor Sidechain donor
Ginsenoside	-7.39	His 408 Met 104	Backbone donor Backbone acceptor
Sulforaphane	-4.40	Thr 462 Arg 683	Sidechain Acceptor
Shikonin	-5.32	Gln 682	Side chain donor
Standard drug (Alpelisib)	-7.94	Glu 186	Sidechain Acceptor

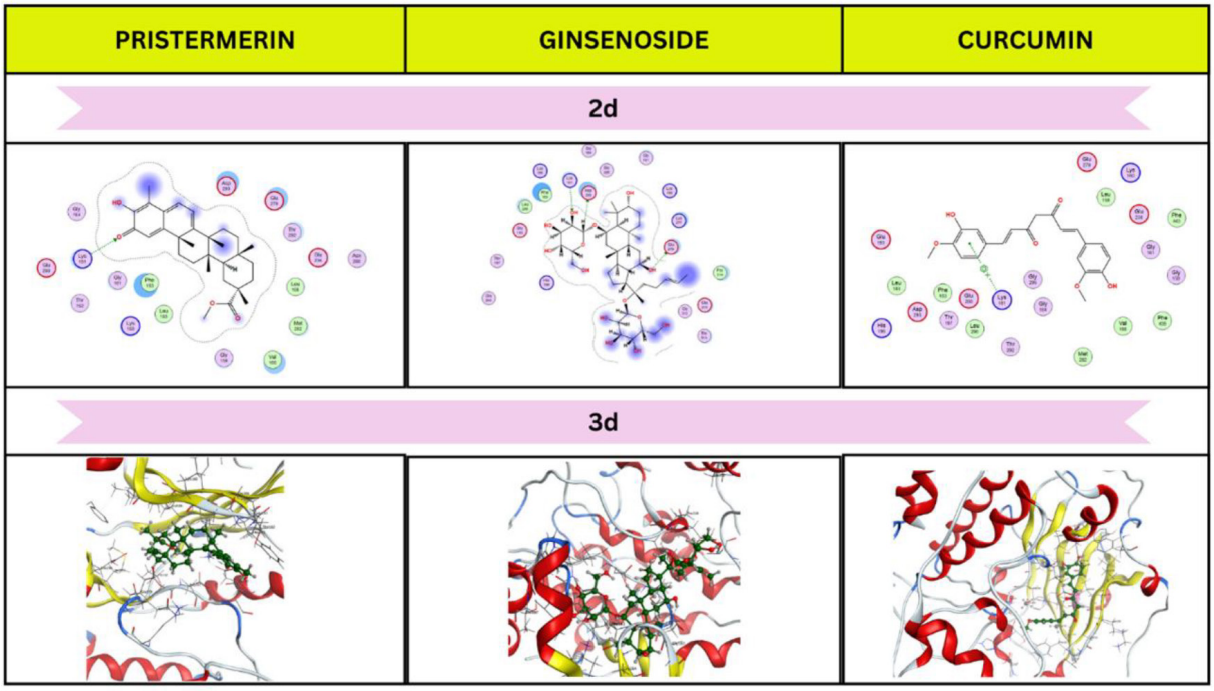


Figure 3. 2D and 3D Interaction diagram of Protein (PI3K) - natural compound complex.

Table 3. Docking score and ligand residue interaction of natural compounds

Compound name Target- AKT (3E87)	Docking score kcal/mol	Interacting Amino acid	Type of Interaction
Apigenin	-4.61	His222	Backbone acceptor
Icaritin	-5.50	His 469 Lys 146 Arg 467	Sidechain donor
Lycorine	-4.62	Glu 186	Sidechain acceptor
Berberine	-4.85	His222 Pro 453	Arene - H
Ursolic acid	-4.79	Lys277 Glu279	Sidechain acceptor Sidechain donor
Nimbolide	-5.31	Gln 466 Lys 146 His 469	Backbone acceptor Sidechain acceptor Arene - H
Pristimerin	-5.52	Lys 181	Sidechain acceptor
Resveratrol	-4.83	Glu 186	Sidechain acceptor
Curcumin	-5.63	Lys 181	Arene - H
Ginsenoside	-6.07	Lys 181 Asp 293	Sidechain acceptor Sidechain donor
Sulforaphane	-4.41	His 469 Lys 185 Lys 146	Sidechain donor
Standard drug (Capivasertib)	-6	Glu 264 Trp 414	Sidechain acceptor Arene - Hydrogen

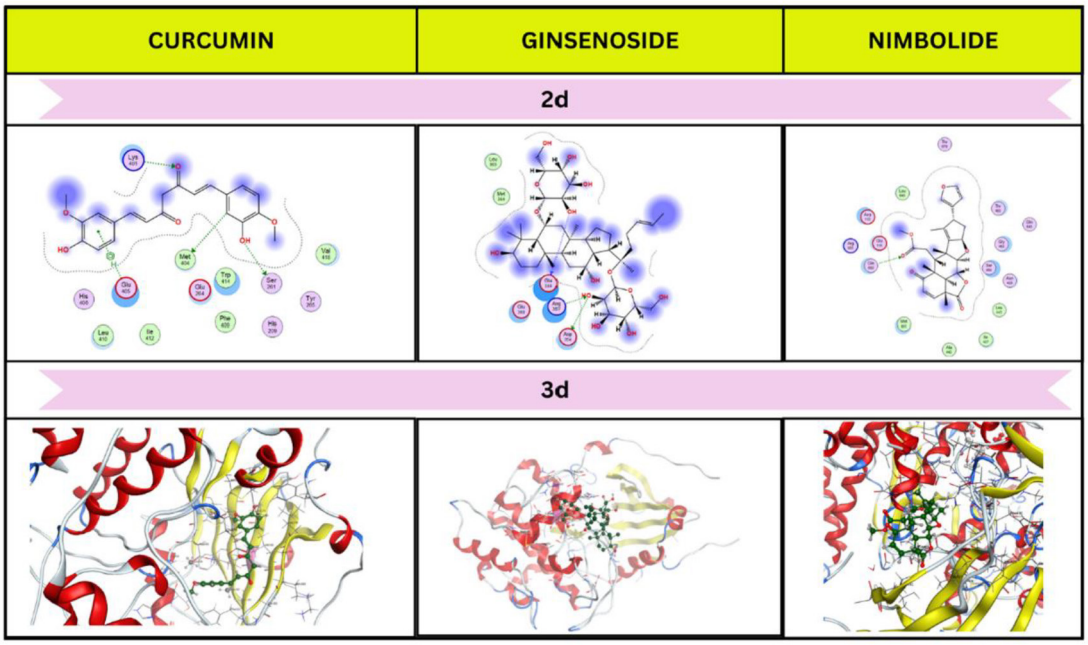


Figure 4. 2D and 3D Interaction diagram of Protein (AKT) - Natural Compound Complex.

5.4 Comparison with Standard Inhibitor

To evaluate the effectiveness of the selected natural compounds, their docking scores were compared with Alpelisib, a clinically approved PI3K inhibitor used in breast cancer therapy. The binding affinity of Ginsenoside (-7.39 kcal/mol) was close to that of Alpelisib (-7.94 kcal/mol), suggesting that this compound may serve as a promising lead molecule for PI3K inhibition. Similarly, Nimbolide and Pristimerin showed favourable binding scores, indicating their potential to interfere with PI3K/AKT signaling.

The docking results revealed that several compounds exhibited strong binding affinities, suggesting their potential as inhibitors. The docking scores, hydrogen bonding interactions, and other molecular interactions were analyzed to identify key compounds for further evaluation. Tables 2 and 3 present the docking scores of the natural compounds with target proteins PI3k and Akt, respectively. The binding interactions of the best-scoring compound with each target are illustrated in Figures 3 and 4.

6. Discussion

The PI3K/AKT/mTOR signaling pathway plays a pivotal role in breast cancer progression, cell survival, and drug resistance. Dysregulation of this pathway, particularly through PIK3CA gene mutations, enhances PI3K signaling, promoting carcinogenesis. Consequently, targeting this pathway represents a promising therapeutic strategy in breast cancer treatment.

The results of molecular docking studies suggest that Nimbolide, Pristimerin, and Ginsenoside exhibit significant inhibitory potential against PI3K. The docking scores for these compounds were -6.22 kcal/mol, -6.28 kcal/mol, and -7.39 kcal/mol, respectively, indicating their ability to bind effectively to PI3K and potentially inhibit its activity. Among these, Ginsenoside displayed the highest binding affinity (-7.39 kcal/mol), closely comparable to the standard PI3K inhibitor Alpelisib (-7.94 kcal/mol). Given the role of PI3K in breast cancer cell proliferation, metabolism, and angiogenesis, these findings suggest that these compounds may influence key cellular processes involved in tumor progression. Notably, targeting PI3K has demonstrated significant therapeutic benefits, particularly in tumors with PI3K pathway dysregulation, reinforcing the potential of these compounds in breast cancer therapy.

In addition to PI3K inhibition, Nimbolide, Pristimerin, and Ginsenoside also exhibited moderate binding affinity towards AKT, suggesting

their potential for broader modulation of the PI3K/AKT/mTOR pathway. The docking scores for Curcumin, Ginsenoside, and Pristimerin with AKT were -5.63 kcal/mol, -6.07 kcal/mol, and -5.52 kcal/mol, respectively. Comparatively, the standard AKT inhibitor Capivasertib had a docking score of -6.00 kcal/mol. Interestingly, ginsenoside demonstrated a slightly higher binding affinity for AKT (-6.07 kcal/mol) than Capivasertib (-6.00 kcal/mol), indicating its potential as a potent AKT inhibitor. These compounds were observed to form hydrogen bonding interactions with key residues in the AKT binding site, further supporting their potential role in AKT inhibition. Given that AKT hyperactivation is a major driver of drug resistance in breast cancer, these compounds may help counteract resistance mechanisms and enhance therapeutic efficacy.

The dual inhibition of PI3K and AKT offers distinct advantages over single-target inhibition strategies. While mTOR inhibitors, such as Everolimus, have been used clinically, they are often limited by compensatory feedback mechanisms that lead to PI3K and AKT hyperactivation. This paradoxical effect can diminish therapeutic efficacy and contribute to metabolic toxicities such as hyperglycemia and hyperlipidemia. In contrast, direct inhibition of PI3K and AKT by compounds such as ginsenoside, pristimerin, and nimbolide could provide a more effective therapeutic approach with potentially fewer side effects.

These findings highlight the therapeutic potential of nimbolide, pristimerin, and ginsenoside in breast cancer treatment through modulation of the PI3K/AKT signaling pathway. However, experimental validation through *in vitro* and *in vivo* studies is essential to confirm these computational predictions.

7. Conclusion

The present study explored the interactions between several natural chemicals and important proteins (PI3K, Akt) involved in the PI3K/Akt/mTOR signalling cascade, which is key to the development of breast cancer, its treatment and its resistance through Molecular docking studies. All the compounds demonstrated strong binding to the selected proteins with favourable docking scores. The findings show that phytochemicals with substantial binding affinities with PI3K, such as ginsenoside, nimbolide and pristimerin, act as a potential pathway inhibitor. Furthermore, substances including pristimerin, curcumin and ginsenoside revealed strong binding interaction with AKT. These results highlight the potential of natural compounds as therapeutic agents by altering key pathways associated with breast cancer. Depending on the severity of the cancer, these natural compounds can be used alone or in combination, offering better therapeutic outcomes and fewer side effects compared to conventional chemotherapy. These findings support the potential of these natural compounds in drug development for breast cancer, particularly targeting pathways involved in tumour growth and progression.

The selected natural compounds have good IC_{50} values in previous studies and were analysed using computational techniques. The current research reveals strong binding interactions with PI3K and AKT and acts as a potential molecule for breast cancer. In addition, experimental validation and *in vivo* studies are recommended to explore their therapeutic efficacy as monodrug or combined drugs in breast cancer models in future.

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9. Author Contribution

Devakeerthana Mantharachalam: Data curation, methodology, writing – original draft; Bharath Kumar Chagaleti: Writing and editing; Anjana GV: Writing and editing; Shantha Kumar B: Writing – review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

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