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Phytochemicals for Breast Cancer Therapeutic Intervention: Exploratory In Silico Molecular Docking Study

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Abstract: Globally, cancer is a major contributor to the disease burden. A poor lifestyle and exposure to potentially dangerous environmental elements are the main causes of cancer development. Out of all cancer forms, breast cancer is most prevalent in women and has emerged as a global public health concern. Based on molecular profiles, breast cancer is often categorized into three basic subtypes: triple-negative tumors, human epidermal growth factor receptor (EGFR), and hormone estrogen receptor-positive tumors. To treat breast cancer, there has been a great deal of interest in the creation of medications that specifically target hotspot factors for cancer, such as mTOR, estrogen receptor alpha, and progesterone receptor. The main goal of the present research work is to use molecular docking studies to find phytochemical compounds derived from plants that can effectively interact with the targeted proteins that cause the onset and spread of breast cancer. 1064 phytochemical compounds derived from plant sources have been evaluated against five putative hotspot targets: the progesterone receptor, the EGFR kinase domain, the human estrogen receptor alpha ligand-binding domain, the FRB segment of mTOR, and the NUDT5 of breast cancer. According to our findings, out of all the compounds, 25 phytochemicals have potential therapeutic value for the treatment of breast cancer. Our results are in concordance with the literature which shows therapeutic efficacy in both in vitro and in vivo cancer models and further strengthen our findings. While the anticancer properties of baicalein, delphinidin 3,5-diglucoside, and morphine have already been established, more in vitro and in vivo research is necessary to determine the effectiveness of vitexin, isoskimmiwallin, nodifloretin, jaceosidin, and nepetin.

Keywords: breast cancer, phytochemicals, estrogen receptor, molecular docking, therapeutics

1. Introduction

According to the World Health Organization's 2022 report on cancer, 18.1 million individuals worldwide were diagnosed with the disease in 2018, and 9.6 million individuals lost their lives to it. Additionally, it was reported that by 2040, the number of cancer patients will have nearly doubled, with the largest increase occurring in low- and middle-income countries, where the disease accounts for more than two-thirds of all premature deaths among adults aged 30–69 worldwide [1]. According to the National Breast Cancer Statistics report in 2020, an estimated 276,480 new cases of invasive breast cancer (BC) would be diagnosed in women in the United States as well as 48,530 new cases of non-invasive (in situ) BC till the year 2040. BC is most common among women and remains a health issue worldwide [2, 3]. BC is expected to be 40% of all cancers diagnosed in female [4, 5]. The high mortality rate due to BC ignites scientific interest in the search for novel anticancer molecules from natural plant sources [6]. Numerous genetic traits, ethnicity, race, and family history of the disease are among the well-established risk factors for BC identified by epidemiologic studies. Other risk factors include variable exposure to alcohol, exogenous hormones, physical

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inactivity, and certain factors related to female reproduction [7]. Human epidermal growth factor receptor (EGFR) 2 (HER2)-positive, triple-negative tumors, and hormone receptor-positive (ER) tumors are the three primary subtypes of BC based on the molecular background [8, 9]. At first, it was thought that estrogen receptors were what caused BC to spread [10]. Progesterone receptors (PR) have been linked to BCBC and are crucial for the healthy development of the breast during puberty. They can also cause the breast to swell excessively, which can result in BC [11]. EGFR is a member of the ErbB family of tyrosine kinase receptors that promote growth. This protein is expressed in many carcinomas, and overexpression of EGFR is a characteristic shared by human tumors that exhibit the malignant phenotype [12, 13]. The most dangerous type of BC is triple-negative breast cancer (TNBC). The unique characteristic of TNBC, which is derived from epithelial cells, is the overexpression of EGFR-2/HER-2 and the lack of estrogen receptors and PR [8, 14, 15]. This TNBC accounts for about 15% of female BC cases [16].

According to numerous studies, exposure to environmental and/or lifestyle factors is the primary cause of cancer, with hereditary abnormalities accounting for only 10% of cases overall [17]. Cancer may be brought on by an excess of reactive oxygen species (ROS) brought on by inflammatory diseases and stress. ROS-induced DNA instability sets off several harmful metabolic pathways that result in the transformation of healthy cells into cancerous ones. Moreover, ROS may potentially be in charge of base modifications that result in mutations that start tumor development. Agents derived from plants called phytochemicals may be able to lessen these oxidative stressors. Dietary phytochemicals may hinder the carcinogenic process by interfering with one or more cellular pathways, according to recent research in the field of drug development. As a result, they are crucial for cancer chemoprevention [18–21]. These phytochemicals have therapeutic value because they influence the molecular targets of cancer. Preclinical studies are presently being conducted to determine the anti-cancerous effect of tyrosine kinase inhibitors and other medications with anti-angiogenic characteristics [2].

The formation and progression of BC are caused by a variety of intracellular molecules, which are now utilized in the detection and therapy of BC. Over 60% of all cases of BC are ER-positive [3, 10]. For BC targeting, the most widely used molecular marker is estrogen receptor alpha (ER-α). The receptor belongs to the nuclear receptor family, which regulates several physiological and biological functions [22]. Under normal physiological conditions, estrogen functions as a ligand and activates this receptor; however, the development of BC is caused by overexpression of ER-α [23]. This receptor is essential to the growth and development of breast malignancies and is strongly linked to both hormone-dependent and hormone-independent tumors [24]. In conjunction with ER-α, the Jab1 (Jun activation domain-binding protein 1) plays a significant role in the onset and progression of BC. A study conducted in British Columbia using immunohistochemistry to investigate 283 ERα-positive breast tissues revealed a strong positive connection between Jab1 and ERα expression [25]. For hormone-responsive BC, aromatase inhibition-mediated decrease of in situ estrogen synthesis is a viable treatment option. The cytochrome P450 enzyme aromatase (CYP19) limits the pace at which androgens can be converted to estrogens [26].

Progesterone receptor (PR) is another receptor implicated in carcinogenesis and the development of BC [11, 27–29]. Approximately two-thirds of patients with ER-positive (ERþ) BC express this receptor, which is a member of the nuclear receptor family, also referred to as the ER-regulated gene family [30]. Progesterone's part in the genesis of BC is becoming more and more obvious. It is possible that frequent activation of PR and its downstream effectors, such as cyclin D1, WNT4, and RANKL, during recurrent menstrual cycles, promotes the development of BC [31]. The substantial correlations shown between BC and women's reproductive characteristics, including age at menarche, age at menopause, and parity, further underscore the importance of endogenous estrogens and progesterone in the development of BC [32]. Consider these known risk factors for BC as measurements of the total "dose" of progesterone and estrogen that the breast epithelium is subjected to throughout time [33].

BC is one of the many human cancers that have been linked to the main oncogene EGFR-2/HER-2 (EGFR) [34]. Through various signaling pathways crucial to cellular proliferation and differentiation, this receptor is involved in regulating cell growth, survival, and differentiation. Multiple transmembrane glycoproteins make up this receptor. The four primary members of the receptor family are referred to as HER-1, HER-2, HER-3, and HER-4, or ErbB1, ErbB2, ErbB3, and ErbB4, in that order [35]. Mutations that activate EGFR cause unchecked cell survival and proliferation. Numerous human cancer types, including lung adenocarcinoma, head and neck squamous cell carcinoma, colorectal carcinoma, and BC, have been linked to EGFR mutations. Consequently, there has been a lot of interest in employing medications with possible EGFR inhibitory effects to target EGFR [36]. Recently, phytonutrients from plant-based sources with promising medicinal properties have been investigated for the treatment of BC due to the negative effects of currently employed chemotherapy medicines.

The term "phytochemicals" denotes the bioactive compounds found in plant-based products. These compounds give foods their color, taste, and aroma while containing a variety of nutrients, minerals, vitamins, and fibers. Studies have shown that these molecules have anti-carcinogenic and anti-mutagenic characteristics [37]. Based on their chemical structure, origin, and biological properties, phytochemicals have been classified into several categories. The most common phytochemicals are phenolics, alkaloids, carotenoids, organosulfur compounds, and nitrogen-containing compounds; of these, carotenoids, phenolics, and organosulfur compounds have been studied for their potential therapeutic benefits [38]. It has been noted that phytochemicals that are ingested and their derivatives found in plants offer potential ways to increase cancer patients' treatment effectiveness and reduce side effects from chemotherapy [39–41]. More than 5000 phytochemicals have been found in food derived from plants and plants themselves so far [42]. Many of these phytochemicals are physiologically active, naturally occurring substances with strong anticancer potential [18, 19, 43, 44]. Moreover, it was noted that phytochemicals strongly prevent BC cells from migrating [45]. Certain phytochemicals are effective in preventing BC because they have antioxidant properties and cause cancer cells to undergo apoptosis [46, 47]. Natural phytochemicals prevent BC by increasing the effectiveness of treatment and reducing the negative side effects of chemotherapy and radiation alone [48, 49]. The increased interest in the role of phytochemicals in BC prevention and treatments is attributed to more recent observations of phytochemicals in diet across a spectrum of tumor forms and incidence [20, 38, 50]. The current therapeutic approaches for BC, including endocrine therapy, chemotherapy, and phytochemical supplements, target several locations and processes involved in the progression of BC. Moreover, combinatorial treatment techniques improved therapeutic efficacy, reduced side effects, and ultimately solved clinical issues [48]. Researchers screen specific therapeutic

molecules and develop a treatment that selectively interacts with the target protein that causes cancer to progress thanks to recent developments in the field of computational biology. A potent computational method for structure-based drug discovery is molecular docking [51]. These days, it is most usual to perform in silico prediction, characterization, molecular docking, and dynamic investigations on natural constituent chemicals as a novel target for the search for promising anti-disease drugs [21, 52, 53]. This is useful for determining the atomic-level binding affinities of medications to targets as well as important details regarding the pharmacological characteristics of particular pharmaceuticals [54]. The molecular docking method was used to investigate the anticancer potential of several compounds, including 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone (ChalcEA) [55], furanocoumarins [56], 5-mercapto-1,2,4-triazole derivatives [57], quercetin, quercitrin and salanin [58], pyridoacridines [59], abemaciclib [60], magnoflorine [61]. These compounds have been validated, and both *in vitro* and *in vivo* models have demonstrated their therapeutic efficacy. Because they are quicker and less expensive than traditional methods, computational approaches to drug development have several advantages [52]. The present work uses molecular docking studies to search plant-based phytochemical compounds that can effectively interact with the targeted proteins involved in the initiation and progression of BC. Approximately 1064 phytochemical compounds derived from plant sources have been evaluated against five putative hotspot targets: the progesterone receptor, the EGFR kinase domain, the human ER-α ligand-binding domain, the FRB segment of mTOR, and the NUDT5 of BC. The outcome suggests that out of all the compounds, 25 phytochemicals have encouraging therapeutic potential for treating BC. The literature that is now accessible and demonstrates the lead compounds' prospective therapeutic efficacy in both in vitro and in vivo cancer models further supports our observation.

2. Methodology

2.1. Data collection

The drug structures from PubChem were downloaded to create a library of 1064 phytochemicals. The potential anti-chemotherapeutic efficacy of these compounds was investigated using an in silico docking tool, the Maestro 12.4 version of the Schrodinger Suite-2020-1 in reaction to specific target hotspot proteins of BC. A molecular docking investigation was conducted to determine these drugs' potential effectiveness for the chosen respective targets. The RCSB Protein Data Bank (RCSB-PDB) was used to download crystal structures of five putative target proteins: EGFR kinase domain (PDB ID-2J6M), Human ER-α Ligand-Binding Domain (PDB ID-3ERT), FRB fragment of mTOR (PDB ID-4DRH), Progesterone receptor (PDB ID-4OAR), and NUDT5 (PDB ID-5NWH).

2.2. Protein and ligand preparation

Using Maestro's protein preparation wizard, the structures of five putative target proteins were prepared: the EGFR kinase domain (PDB ID-2J6M), the human ER-α ligand-binding domain (PDB ID-3ERT), the FRB fragment of mTOR (PDB ID-4DRH), the progesterone receptor (PDB ID-4OAR), and NUDT5 (PDB ID-5NWH). In a nutshell, this wizard inserts improved loops, missing residues, and hydrogen atoms. Moreover, it adds disulfide bonds and fixes side-chain positions. Additionally, the protonation states of amino acids at pH 7.4 were generated using the PROPKA module, which replicates physiological circumstances [62, 63]. Subsequently, the OPLS-3e force field of the Schrodinger suite was utilized to minimize the protein structures. For each protein target, a grid box was created using the important active site amino acid residues that were chosen from the literature [64–67].

The phytochemicals' 3D structures in SDF format were obtained from PubChem and created with the LigPrep module of the Schrodinger suite [68] which produces enantiomers and tautomers of compounds automatically. Additionally, it uses the Epik module to do protonation of molecules at pH 7.4 [69]. The glide HTVS docking procedure (high throughput virtual screening) was used in the initial step of the multistep docking process to screen the phytochemicals' potential for binding to their particular targets. Subsequently, the in silico docking approach glide standard protocol was applied to the top 100 compounds. The top 20 phytochemicals were then docked using the Glide XP (extra precision) module, which yields precise binding efficacy [52, 70, 71]. The schematic of the methodology used is shown in Scheme 1.

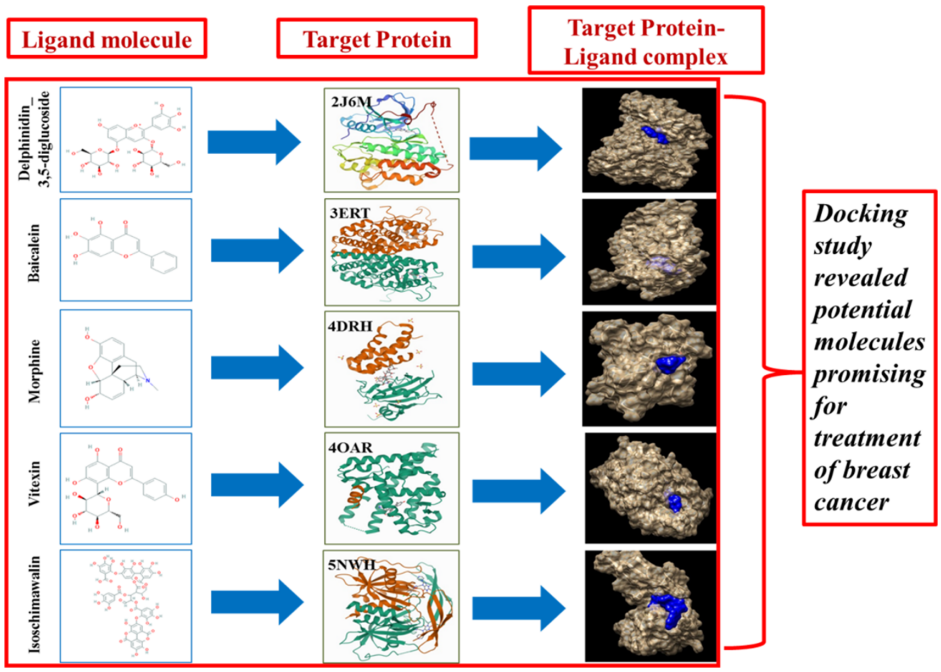
2.3. Binding free energy (MMGBSA) calculation

In the solvation condition of the Schrodinger suite's Prime module, the binding free energy (MMGBSA) of specific phytochemical compounds that exhibit a high docking score with the corresponding protein target was computed using the OPLS 3e force field. The Δ G bind between protein and ligand complexes has been calculated using the prime module [72]. After docking, Δ G calculations were performed on the top 5 complexes, which are listed in Table 1.

3. Results and Discussion

Consumption of dietary phytochemicals is protective against the development of BC and contributes to reported variations in BC incidence [73]. Numerous phytochemicals, including carotenoids, chlorophyll, flavonoids, indole, isothiocyanate, polyphenolic compounds, protease inhibitors, sulfides, and terpenes, have been shown in studies to have anti-carcinogenic potential [39]. It has been demonstrated that a natural active flavonoid found in a variety of plants has a range of biological effects, including anti-apoptotic, anti-inflammatory, antioxidant, and anticancer properties [74]. Most phytochemicals appear to have a variety of particular mechanisms of action, some of which are still unclear in their protection against cancer [75]. It may be quite challenging to evaluate the interaction effects of dietary phytochemicals on cancer risk due to their vast and diverse variation. By scavenging DNA reactive substances, limiting the aberrant growth of early, preneoplastic lesions, blocking certain characteristics of the cancer cell, and inhibiting phase I and phase II enzymes, phytochemicals can reduce carcinogenesis [39]. A multistep strategy aimed at maximizing the complementary effects of several agents may eventually be developed as a result of the variety of phytochemical responses at various stages in the carcinogenesis process.

The compounds from plant sources are screened in the present study using the molecular docking approach. ERα, PR, EGFR, and mTOR were chosen as targets for phytochemical screening based on the literature because they are implicated in the initiation and progression of cancer. EGFR kinase domain protein shows the highest affinity (docking score −13.381) and binding energy



Scheme 1. Schematic representation of methodology used for identifying potential lead phytochemical molecules using molecular docking method for the treatment of breast cancer

Table 1. List of potential lead molecules against selected breast cancer proteins along with binding energy (MMGBSA) and binding affinity (XP G score)

Protein PDB ID	Pubchem ID	Name	XP G score	MMGBSA
EGFR kinase domain (2J6M)	10100906	Delphinidin 3,5-diglucoside.1	−13.381	−124.9
	42607742	Palasitrin.1	−11.912	−112.37
	101685135	Arborside D.1	−10.86	−95.67
	44257997	Parthenosin.1	−10.092	−96.45
	21310440	Kaempferol 3-O-beta-D-xyloside.1	−10.086	−89.08
Human estrogen receptor alpha ligand-binding domain (3ERT)	5281605	Baicalein.1	−10.542	−66.23
	145826	34dihydroxy flavonol.1	−10.612	−57.01
	92775	Butin.1	−9.686	−61.02
	6293	Alizarin.1	−9.526	−69.6
	591830	Isovestitol.1	−9.436	−80.64
FRB fragment of mTOR (4DRH)	5288826	Morphine.1	−9.444	−61.28
	167718	Tembeterine.1	−9.171	−71.58
	442106	Hypaphorine.1	−8.74	−51.4
	5280805	Rutin.1	−8.685	−101.54
	442169	Armepavine.1	−8.382	−63.21
Progesterone receptor (4OAR)	5280441	Vitexin.1	−12.827	−89.64
	5320181	Nodifloretin.1	−11.277	−67.55
	10100906	Delphinidin 3,5-diglucoside.1	−10.89	−115.69
	5379096	Jaceosidin.1	−10.809	−78.98
	5317284	Nepetin.1	−10.255	−81.01
NUDT5 (5NWH)	16130370	Isoskimmiwallin.1	−12.059	−104.78
	11972472	Isoflavone.1	−7.91	−98.45
	68245	Delphinidin.1	−6.945	−63.45
	65084	Casuarin.1	−5.797	−70.2
	5280647	Gossypetin.1	−5.21	−58.08

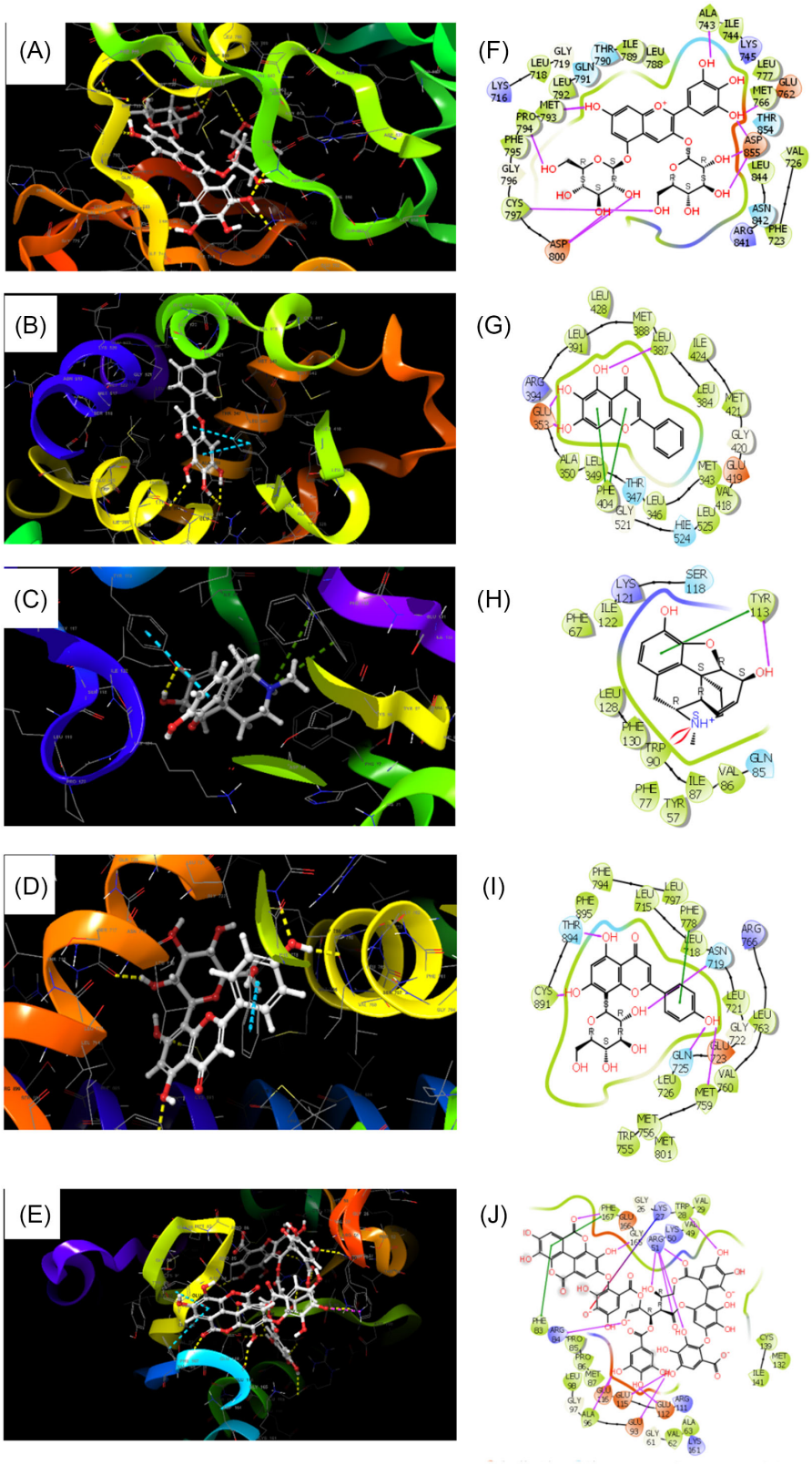


Figure 1. The representative docking poses (A) EGFR kinase domain (2J6M)-Delphinidin 3,5-diglucoside. (B) Human ER alpha ligand-binding domain (3ERT)-Baicalein. (C) FRB fragment of mTOR (4DRH)-Morphine. (D) PR (4OAR)-Vitexin. (E) NUDT5 (5NWH)-Isoskimmiwallin. Interactions between the protein with respective docked ligands. (F) 2J6M-Delphinidin 3,5-diglucoside. (G) 3ERT-Baicalein. (H) 4DRH-Morphine. (I) 4OAR-Vitexin. (J) 5NWH-Isoskimmiwallin.

Table 2. The anticancer properties reported so far for proposed lead phytochemical molecules for the treatment of breast cancer

PDB ID	Phytochemical name	Chemical name/ synonyms	Study (In vitro/ In vivo)	Type of cancer	Target	Ref.
2J6M	(EGFR kinase domain)					
	Delphinidin 3,5-diglucoside	Delphinidin 3,5-diglucoside DELPHIN delphinidin 3-O-beta-D-glucoside-5-O-beta-D-glucoside	In vitro & In vivo	Lung Cancer	EGFR/ VEGFR2 signaling pathways	[76]
			In vitro Human Caucasian breast adenocarcinoma (MCF7) cells	Breast cancer	MCF7 cell proliferation inhibition	[77]
			In vitro Human breast epithelial cells MCF10A cell line	Breast cancer	Akt/ HOTAIR	[78]
	Palasitrin	Palasitrin				
	Arborside D	Arborside D	–	–	–	–
	Parthenosin	Quercetin 3-(6”-n-butylglucuronide)	OV2008, A2780, and GM9607 cells	Ovarian cancer	p53-dependent endoplasmic reticulum stress pathway	[79]
			Human colorectal HT29 cancer cell line	Colorectal cancer	Cell cycle arrest and apoptosis	[80]
			In vitro HCCLM3 cells	Various human Cancer	Suppressing the Expression of p-Akt1	[81]
	Kaempferol 3-O-beta-D-xyloside	kaempferol 3-O-beta-D-xyloside Kaempferol-3-O-alpha-L-arabinoside Kaem-3-Ara Kaempferol 3-xylopyranoside	Human acute leukemia Jurkat T cell clones	Leukemia	G_2-Arrest and Mitochondria-Dependent Apoptosis	[82]
			In vitro MCF-7 cell line	Breast cancer	Inhibition of MCF-7	[83]
			In vitro K562 and U937 cell lines	Leukemia cancer	Akt Inactivation & Bax and SIRT3 Activation	[84]
3ERT	(Human estrogen receptor alpha ligand-binding domain)					
	Baicalein	Baicalein 5,6,7-Trihydroxyflavone 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one Noroxylin Biacalein BaiKalein 5,6,7-trihydroxy-2-phenylchromen-4-one Baicelein		Pancreatic cancer cells	Bcl-2 proteins	[85]
				Melanoma	mTOR-HIF-1a signaling pathway	[86]
			In vitro and in vivo	lung cancer	VEGF, FGFR-2, and RB-1	[87]
			In vivo	Bladder cancer	pGSK; p-ERK	[88]
			In vivo	Lung cancer	Src/Id1 Pathway	[89]
			In vitro	Bladder cancer	CDC2 kinase	[90]
			In vivo	colon cancer	MAPK ERK and p38 signaling pathways	[91]
	3’ 4’-dihydroxy-flavonol	3’,4’-dihydroxyflavonol 3,3’,4’-TRIHYDROXYFLAVONE	In vivo & In vitro	Breast cancer	Epigenetically regulating miR-34a and miR-21	[92]
	Butin	Butin (-)-Butin 7,3,4-trihydroxydihydroflavone)	Chinese hamster lung fibroblasts (V79-4 cells)	Cancer	Protect oxidativedamage of DNA by activation of the PI3K/Akt/OGG1 pathway	[93]

	Alizarin	Alizarin Alizarin Red 1,2-dihydroxyanthracene-9,10-dione Mordant Red 11	In vitro	Bone Tumors	ERK Signaling	[94]
	Isovestitol	Isovestitol Isobestitol	In vitro	Cancer cells	Inhibit cell growth	[95]
4DRH	(FRB fragment of mTOR)					
	Morphine	morphine Morphia Morphinum Morphium Morphin	In vivo	Breast, Colon, Lung, Pancreas, Gallbladder, and Melanoma cancer	NA	[96]
			In vivo	Breast Cancer	MAPK) signaling pathway	[97]
	Tembeterine	Tembetarine (S)-tembetarine (+)-Tembetarine (S)-(+)-tembetarine				
	Hypaphorine	Hypaphorine Lenticin Tryptophan betaine Glyyunnanenine	In vitro	Cancer	PTP1B inhibition and cytotoxic activity	[98]
	Rutin	RUTIN	In vitro	Breast Cancer	G2/M and G0/G1 phases of Cell cycle	[99]
		rutoside	In vitro	Cervical cancer	ability to induce cellular apoptosis	[100]
		Phytomelin Quercetin 3-rutinoside	In vitro	Breast Cancer Cells	arresting the G2/M phase	[101]
			In vitro	Neuroblastoma	G2/M Cell Cycle Arrest	[102]
	Armepavine	Armepavine (-)-Armepavine Evoeuropine	–	–	–	–
4OAR	(Progesterone receptor)					
	Vitexin	Vitexin	In vitro	Colon Cancer	Apoptosis induction	[103]
		Apigenin 8-C-glucoside Vitxein	In vitro	Bladder cancer	induction of extrinsic apoptosis	[104]
	Nodifloretin	Batatifolin Nodifloretin	cell lines (Hep G2, COLO 205, MCF-7, and HL-60)	Leukemia	Anti-inflammatory and growth inhibitory	[105]
	Jaceosidin	Jaceosidin	In vivo & In vitro	Bladder cancer	cell cycle arrest	[106]
			In vivo & In vitro	Cervical cancer	Inhibitory effect E6 and E7 oncoproteins	[107]
			In vitro	Oral cancer	Inhibits the Akt Pathway	[108]

(*Continued*)

Table 2. (*Continued*)

PDB ID	Phytochemical name	Chemical name/ synonyms	Study (In vitro/ In vivo)	Type of cancer	Target	Ref.
			In vitro	Ovary cancer	Induces Apoptosis	[109]
	Nepetin	6-Methoxyluteolin Nepetin Eupafolin	In vivo & In vitro	Prostate cancer	Akt signaling	[110]
5NWH	(NUDT5)					
	Isoskimmiwallin	Isoschimawalin A	–	–	–	–
	Isoflavone	5,7-Dihydroxy-6-methoxy-4'-(6-O-beta-D-glucopyranosyl-beta-D-glucopyranosyloxy)isoflavone	In vivo	Breast cancer	NA	[111]
			In vivo & In vitro	Breast & Prostate cancer	NF-κB and Akt signaling pathway	[112]
	Delphinidin	Delphinidin chloride	In vitro	Lung Cancer	EGFR/VEGFR2 Signaling Pathways	[76]
		Delphinidin Delphinidine	In vitro	Breast cancer	radical scavenging activity, inhibit cell proliferation, and increase apoptosis	[77]
		Delphinidol	In vitro Human breast epithelial cells MCF10A	Breast cancer	Akt/ HOTAIR signaling pathway	[78]
	Casuarin	(+)-Gallocatechin Gallocatechin (+)-gallocatechol Gallocatechol	In vivo	Prostate cancer	Anti-histone acetyltransferase activity	[113]
	Gossypetin	Gossypetin	In vitro	Prostate cancer	Inducer of apoptotic	[114]
		Articulatidin Equisporol	In vitro & In vivo	Esophageal cancer	MKK3 and MKK6 inhibitor	[115]

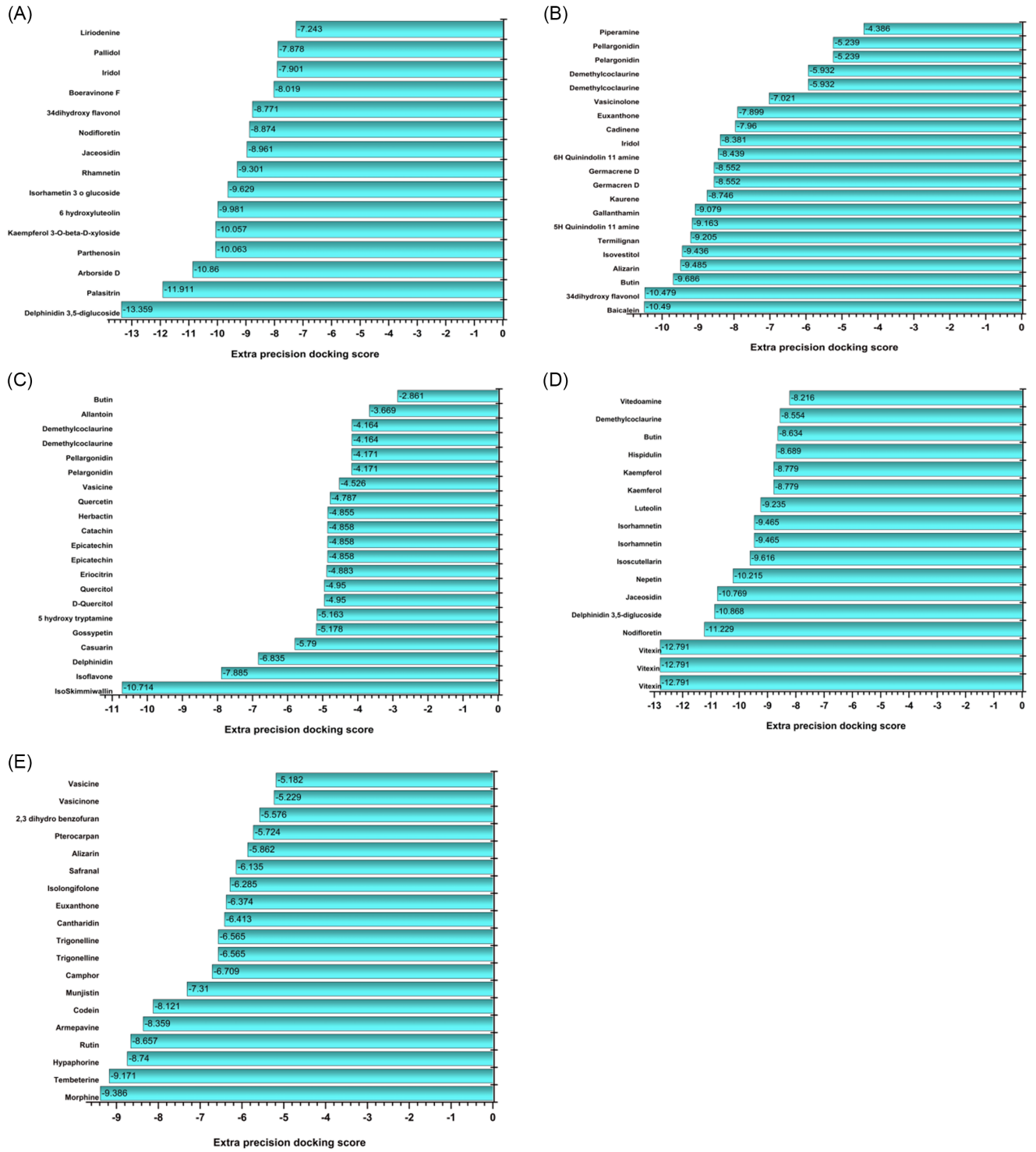


Figure 2. The ranking histogram of each target with top ligands based on docking score. (A) EGFR kinase domain. (B) ER alpha-binding domain. (C) FRB fragment of mTOR. (D) PR. (E) NUDT5.

(−124.9) with delphinidin 3,5-glucoside. The amino acid in the binding pocket ALA 743, MET 766, MET 793, PRO 794, CYS 797, ASP 800, and ASP 855 forms a total of 10 hydrogen bonds with atoms of delphinidin 3,5-diglucoside (Figure 1 A&F). The human ER-α ligand-binding domain (3ERT) interacted with higher affinity (docking score −10.542 and binding energy −66.23) with baicalein. Here GLU 353, and LEU 387 form 4 hydrogen bonds, and PHE 404 forms 2 pi-pi stacking interactions with the target protein (Figure 1 B&G). The morphine is completely intercalated in the binding pocket of the FRB fragment of mTOR (4DRH) (docking score −9.444 and binding energy −61.28). The amino acid TYR 113 is involved in 1 hydrogen bond and one pi interaction where TRP 90 forms 2 pi cation interaction with the morphine molecule (Figure 1 C&H).

Table 3. The inhibitory constant (pKi) and ligand efficiency against a respective protein target

Compound name	Affinity (kcal/mol) XP G Score	Kipred	pKi	pKi	Non-H-atom	LE
Delphinidin 3,5-diglucoside	−13.381	0.977669	0.009808	9.80834	22	0.608227
Palasitrin	−11.912	0.980096	0.008732	8.731556	42	0.283619
Arborside D	−10.86	0.981837	0.00796	7.960435	37	0.293514
Parthenosin	−10.092	0.983111	0.007397	7.397487	38	0.265579
Kaempferol 3-O-beta-D-xyloside	−10.086	0.983121	0.007393	7.393089	30	0.3362
Baicalein	−10.542	0.982365	0.007727	7.727339	20	0.5271
34dihydroxy flavonol	−10.612	0.982248	0.007779	7.778649	20	0.5306
Butin	−9.686	0.983785	0.0071	7.099887	20	0.4843
Alizarin	−9.526	0.984051	0.006983	6.982606	18	0.529222
Isovestitol	−9.436	0.9842	0.006917	6.916635	20	0.4718
Morphine	−9.444	0.984187	0.006922	6.922499	21	0.449714
Tembeterine	−9.171	0.98464	0.006722	6.722389	32	0.286594
Hypaphorine	−8.74	0.985357	0.006406	6.406464	25	0.3496
Rutin	−8.685	0.985448	0.006366	6.366149	43	0.201977
Armepavine	−8.382	0.985952	0.006144	6.144048	23	0.364435
Vitexin	−12.827	0.978583	0.009402	9.402255	31	0.413774
Nodifloretin	−11.277	0.981147	0.008266	8.266098	23	0.490304
Delphinidin 3,5-diglucoside	−10.89	0.981788	0.007982	7.982425	21	0.518571
Jaceosidin	−10.809	0.981922	0.007923	7.923051	24	0.450375
Nepetin	−10.255	0.98284	0.007517	7.516967	23	0.44587
Isoskimmiwallin	−12.059	0.979852	0.008839	8.839308	90	0.133989
Isoflavone	−7.91	0.986738	0.005798	5.79807	44	0.179773
Delphinidin	−6.945	0.988347	0.005091	5.09072	32	0.217031
Casuarin	−5.797	0.990263	0.004249	4.24923	22	0.2635
Gossypetin	−5.21	0.991245	0.003819	3.818956	23	0.226522

Progesterone receptor (4OAR) protein binds exhibit higher affinity (docking score −12.827 and binding energy −89.64) for vitexin and amino acid CYC 891, THR 894, ASN 719, GLN 725, and MET 759 forms hydrogen bond and PHE 778 forms pi interactions with vitexin molecule (Figure 1 D&I). The docking score of −12.059 and binding energy of −104.78 were observed when isoskimmiwallin interacted with binding pocket residues of NUDT5 (5NWH). The key amino acids involved here are ARG 51, LYS 50, LYS 27, TRP 28, PHE 167, PHY 83, ARG 84, ALA96, GLU 93, GLU, 116, GLU 115, which forms hydrogen bonds and PHE 83 and PHE 167 forms pi-pi interaction with the atoms of isoskimmiwallin (Figure 1 E&J). The protein targets with a docked ligand which shows the highest affinity have been shown in Figure and the list of top 20 potential lead molecules against their respective target BC proteins along with binding energy (MMGBSA) and binding affinity (XP G Score) has been presented in Table 1. Our observation is further strengthened by the available literature, which revealed the anti-chemotherapeutic potential of these phytochemicals against BC. For the treatment of BC, delphinidin, parthenosin, and kaempferol be efficacious in both in vitro and in vivo models [76–82]. In vivo investigations have shown that rutin and morphine, which target mTOR, are potent anti- BC medications [83–88]. A small number of in vitro and in vivo studies have already been conducted for different human malignancies, including colon, bladder, and cervical cancer. Nodifloretin, jaceosidin, nepetin, and vitexin targeting PR have not yet been investigated for their effectiveness in treating BC [89–96]. Although our study shows that isoskimmiwallin exhibits higher binding towards NUDT5, its therapeutic efficacy in BC treatment has not been evaluated so far. However, other phytochemicals such as isoflavone, delphinidin, casuarin, and gossypetin have already been shown to be therapeutically effective both in vitro and in vivo for a variety of cancer types [76, 77, 97–99]. Table 2 lists the anticancer characteristics of lead phytochemical compounds that have been suggested for the treatment of different cancer types that have been documented in the literature to date. Our research revealed that delphinidin interacted with multiple BC risk factors, such as EGFR and NUDT5; thus, it would be advantageous to assess its therapeutic efficacy in the BC model. The ranking histogram of each target with top ligands based on the docking score has been shown in Figure 2. The inhibitory constant (pKi) and ligand efficiency against respective protein targets have been calculated and presented in Table 3. Further, isoskimmiwallin, vitexin nodifloretin, jaceosidin, and nepetin show higher binding interaction with hotspot proteins responsible for BC, but they are not evaluated for BC treatment yet and therefore need further study.

4. Conclusion

Naturally occurring phytochemicals have been known for their therapeutic efficacy since a long back, since then there is considerable interest to search for potent molecules for the treatment of cancer. Being cost-effective with fewer adverse effects and ease of availability, the use of phytochemicals as an anticancer agent could have the potential to revolutionize the cancer treatment regime. In our study, phytochemicals delphinidin 3,5-diglucoside, baicalein, morphine, vitexin, and isoskimmiwallin showed high interaction affinity and binding energy with target proteins EGFR, ER, PR, and NUDT5; therefore, these molecules may be explored for their anti- BC treatment efficacy. However, the in vitro and in vivo validation of their effectiveness is warranted. The literature supports our findings by demonstrating that these lead molecules have promising therapeutic efficacy in both an in vitro and an in vivo cancer model. While the anticancer properties of baicalein, delphinidin 3,5-diglucoside, and morphine have already been established, more in vitro and in vivo research

is necessary to determine the effectiveness of vitexin, isoskimmiwallin, nodifloretin, jaceosidin, and nepetin.

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Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contribution Statement

Arpana Parihar: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Nidhi Puranik:** Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Ashok Kumar Nadda:** Resources, Writing – original draft. **Vikas Kumar:** Resources, Writing – original draft. **Keun Woo Lee:** Resources, Writing – original draft. **Raj Kumar:** Resources, Writing – original draft. **Rekha Khandia:** Validation. **Raju Khan:** Validation, Writing – review & editing.

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