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Active Phytochemicals of Indian Spices Target Leading Proteins Involved in Breast Cancer: An in Silico Study

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ABSTRACT. Indian spices are well known for their numerous health benefits, flavour, taste, and colour. Recent Advancements in chemical technology have led to better extraction and identification of bioactive molecules (phytochemicals) from spices. The therapeutic effects of spices against diabetes, cardiac problems, and various cancers has been well established. The present in silico study aims to investigate the binding affinity of 29 phytochemicals from 11 Indian spices with two prominent proteins, BCL3 and CXCL10 involved in invasiveness and bone metastasis of breast cancer. The three-dimensional structures of 29 phytochemicals were extracted from PubChem database. Protein Data Bank was used to retrieve the 3D structures of BCL3 and CXCL10 proteins. The drug-likeness and other properties of compounds were analysed by ADME and Lipinski rule of five (RO5). All computational simulations were carried out using Autodock 4.0 on Windows platform. The proteins were set to be rigid and compounds were kept free to rotate. In-silico study demonstrated a strong complex formation (positive binding constants and negative binding energy ΔG) between all phytochemicals and target proteins. However, piperine and sesamolin demonstrated high binding constants with BCL3 (50.681×10^3 mol^{-1}, 137.76×10^3 mol^{-1}) and CXCL10 (98.71×10^3 mol^{-1}, 861.7×10^3 mol^{-1}), respectively. The potential of these two phytochemicals as a drug candidate was highlighted by their binding energy of -6.5 kcal mol^{-1}, -7.1 kcal mol^{-1} with BCL3 and -6.9 kcal mol^{-1}, -8.2 kcal mol^{-1} with CXCL10, respectively coupled with their favourable drug likeliness and pharmacokinetics properties. These findings underscore the potential of piperine and sesamolin as drug candidates for inhibiting invasiveness and regulating breast cancer metastasis. However, further validation through in vitro and in vivo studies is necessary to confirm the in silico results and evaluate their clinical potential.

Key words: Breast cancer, BCL3, CXCL10, Indian spices, Phytochemicals

INTRODUCTION

Breast cancer (BCa) is the most frequent malignancy among women and the 5th cause of cancer related deaths worldwide.[1] Based on 1980-2010 data, an annual increase of 3.1% in BCa cases was estimated, which still is supported by BCa cases in recent years.[2,3] Over the last decade, various treatment modalities have been discovered to combat the heterogeneity of BCa and reduce the adverse effects of aggressive treatments. This has led to increased chances of cure in 70-80% of patients. However, metastatic BCa is still a big challenge. Several mutations in tumor suppressor genes and other genes have been linked to a high frequency of BCa.[4] Among metastasis in several tissues and organs (lungs, liver and brain, in addition to lymph nodes) in the body, bone is one of the most common sites of invasion in BCa.[5] Metastasis to bone typically results in a poor prognosis, reducing life expectancy to 2–3 years post-diagnosis. The tumor cell mass exerts mechanical pressure that can contribute to bone pain. Pain may also occur due to release of inflammatory cytokines from the tumor cells themselves or by altering the bone

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microenvironment and bone homeostasis in adjacent areas.[6] An elaborate network of signaling pathways orchestrates the communication between cancer cells and the surrounding stroma of bone. Chemotherapy, radiation therapy, and surgical removal of tumors are standard methods implemented to treat BCa. Singh and co-workers found that the protein encoding genes, BCL3 and CXCL10 were upregulated in BCa bone metastasis samples.[7] Both genes are well known for their crucial role in BCa. Overexpression of B-cell-lymphoma-3 (BCL3) protein was first reported in hematological cancers. The oncogenic activity of BCL3 in these cancers was due to its influence on cyclinD1 and p53 expression.[8–10] BCL-3 expressed in mammary adenocarcinomas, can promote tumorigenesis and survival signaling, and has a key role in tumor metastasis. BCL3 is involved in regulating NF-κB-mediated transcription of genes involved in apoptosis and proliferation, such as BCL2, Cyclin D1, and STAT3. BCL3 has been implicated in maintaining BCa cell survival after DNA damage via both p53-dependent and -independent pathways. These findings suggest that BCL3 plays a crucial role in the intricate balance between cell survival and death, which is often dysregulated in cancer.[11] BCl3-mediated upregulation of ceruloplasmin expression promoted ovarian cancer progression.[12] A significant reduction in tumour growth was reported upon suppression of BCL3 in prostate cancer xenografts.[13] CXCR3, a receptor for CXCL10, is overexpressed in a variety of cancers and promotes tumour growth. The CXCL10/CXCR3 axis can enhance the migration and invasion of colorectal cancer. Upregulation of CXCL10 expression increases the incidence of lung metastases via PI3K/AKT/GSK-3β signalling pathway.[14]

Spices have been widely used as condiments for thousands of years because of their flavour, taste, and colour. Their roles as therapeutic agents have also been evidenced since ancient times through various forms such as tinctures, teas, powders, poultices, etc. without any knowledge of the active ingredients. However, with the advances in organic chemistry and chemical analysis, analytical investigations of medicinal plants and herbal remedies have identified, purified and characterized numerous active components (therapeutic molecules).[15] For example, salicylic acid, the precursor of aspirin (from plant Salix sp.) – the famous pain-killer, quinine (Cinchona officinalis) – anti-malarial drug, digitoxin (Digitalis purpurea and Digitalis lanata) – for cardiac problems, and many others with pharmaceutical compounds with clinical potential have been well identified.[15]

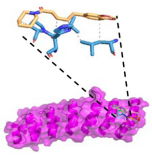
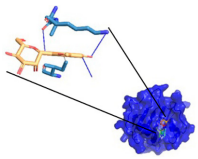
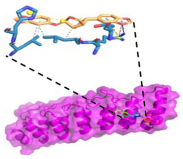
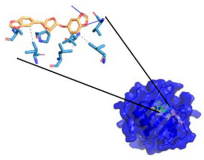
For example, some antioxidants from spices, such as curcumin (from turmeric), eugenol (from cloves), and capsaicin (from red pepper), were experimentally evidenced to control cellular oxidative stress due to their antioxidant properties. They also have the capacity to block the production of reactive oxygen species and interfere with signal transduction pathways.[16] In addition to reducing oxidative stress, other roles of spices such as reducing inflammation, elevating immune response, modulating cellular enzymes, etc. have been reported. Therefore, reports on spices, which could be used to prevent several human diseases such as cancers, have been increasing day by day. In fact, epidemiological and experimental evidences have shown that certain spices might lower risks of some cancers.[15–18] Bioactive compounds of garlic have been reported for their anticancer potential in a vast range of cancers such as breast, gastric, lung, colorectal, and bladder cancers in several in vitro and in vivo studies.[19] Sesamolin could attenuate Endometrial cancer by disrupting the interplay between Myosin Heavy Chains (MYH)- 9 and 14 and repressing MYH9-regulated Wnt/β-catenin signalling.[20] Piperine treatment resulted in substantial cellular death in the prostate cancer cell line (PC-3) via inhibition of the RAC-alpha serine/threonine-protein kinase (AKT1) protein.[21] In the present study, the active ingredients (phytochemicals) of common Indian spices were investigated for their interaction with BCL3 and CXCL10, two well-known proteins involved in bone metastasis and invasiveness of BCa.[7] Any strong interactions will indicate anti-cancer role of those spices and can further be validated in in vitro and in vivo studies and used as therapeutics molecules.[22]

EXPERIMENTAL

Literature Survey and Ligands Selection

Indian spices from the southern region with potent anticancer properties were screened using an extensive literature survey performed on Google scholar and PubMed platforms. Shortlisting of 29 compounds from 11 species was done based on the literature survey for in silico validation in our study. PubChem web repository was exploited to retrieve three-dimensional (3D) structures of the active biomolecules and their 3D structures were downloaded in SDF format and listed with the effects of the spice along with the PubChem ID (*Table* 1 and Supplementary Table S1). To prepare ligands for molecular docking, the retrieved ligand 3D structures were converted from SDF format to PDB file format utilizing the Open Babel program.[23]

***Table* 1.** Molecular Docking interaction of phytochemicals with BCL3 and CXCL10. The docking interaction of phytochemicals and proteins are amplified from protein-phytochemical complexes for a better view. Complete data for every phytochemical with both proteins is given in Supplementary *Table* S1

S. No.	Indian Spices (botanical names)	Com-pounds	PubChem ID	Docking interactions with BCL3	Binding energy (kcal mol^{-1})	Binding constant ($\times10^3$) mol^{-1}	Docking interactions with CXCL10	Binding energy (kcal mol^{-1})	Binding constant ($\times10^3$) mol^{-1}
1	Pepper (*Piper nigrum)*	Piperine	CID_638024		-6.5	50.68		-6.9	98.71
2	Sesame (*Sesamum indicum*)	Sesamolin	CID_101746		-7.1	137.76		-8.2	861.7

Preparation of BCL3 and CXCL10 for Docking

The 3D crystal structure of the ankyrin repeat domain (seven repeats) of BCL3 at a resolution of 1.86 Å (PDB ID:1K1A) spanning from residues 119 to 359 and CXCL10 (PDB ID: 1O7Z_1) were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB)-Protein Data Bank in PDB format.[22,24] Removal of nonspecific molecules and water was done to prepare the macromolecules for docking with the help of UCSF Chimera.[25,26] The addition of polar hydrogen atoms to the 3D structures of BCL3 and CXCL10 was done for allowing intramolecular interactions through hydrogen bonds. Further energy minimization and structure optimization was performed by Swiss-Pdb Viewer while the side chain angles correction was done utilizing clean geometry module embedded in the Discovery Studio package.[26,27]

Molecular Docking

Molecular docking studies of shortlisted compounds with BCL3 and CXCL10 were performed to identify potential inhibitors of BCL3 and CXCL10, respectively, using AutoDock Vina. Molecular docking studies and conformational analysis were performed using a stand-alone version of Autodock Vina. For docking studies, the X, Y, and Z grid box centres were set to 19 Å, 71 Å, and 111 Å, respectively, while the X, Y, and Z box size-was set to 116 Å, 81 Å, and 93 Å cover the entire length of the protein. We adopted a blind docking approach to allow drugs to bind anywhere on the protein. Molecular docking was performed using standard precision protocols with default parameters. Out of the stimulated interactions, the top 10 poses were selected based on docking energies. The complex with the lowest binding energy was considered the best complex and filtered out.

Drug Likeness

The traditional approach in pharmacokinetics (*i.e.*, the fate of a therapeutic compound in the organism) is to break down the various effects that impact the access to the target into individual parameters. These parameters include absorption, distribution, metabolism, and excretion (ADME) and their calculations are among the most crucial aspects of drug designing as early estimation of ADME in the discovery phase can significantly reduce the chances of pharmacokinetics-related failure in the clinical phases.[28,29] The SWISSADME tool was utilized to analyze ligands and predict their drug likeness and pharmacokinetic properties. The predictive absorption for molar refractivity (MR), skin permeability coefficients (log Kp), total polar surface area (TPSA), number of rotatable bonds (nRotB), gastrointestinal (GI) absorption, and CYP1A2 inhibitor were evaluated in addition with the Lipinski's Rule of 5 (RO5), which predicts drug-likeness of the design derivatives were also considered. Lipinski's RO5 states that compounds in excesses of 5 H-bond donors, 10 H-bond acceptors, molecular weight more than 500 Daltons, and the calculated Log P (MLogP) greater than 5 likely had poor absorption or permeation of the molecular entities. Hence, molecules will unlikely to become orally bioavailable as a drug if they pose properties greater than the desired number.[24]

RESULTS AND DISCUSSION

Identification of drug targets with the help of molecular

docking approach has gained significant prominence in ligand-based computer-aided drug discovery. In the present scenario, the analysis and annotation of a large amount of data from drug libraries can be achieved quickly saving immense amount of energy, time, and costs related to drug discovery.[26,30,31] Indian spices such as garlic, clove, coriander, ginger, turmeric, and black pepper are part and parcel of every kitchen and have been known for their medicinal value since ancient times. Recent studies have highlighted their potential role in treating different cancers.[32,33] Based on traditional knowledge and an extensive literature survey of current research conducted on Pubmed and Google Scholar on role of spices in cancer therapy, we shortlisted 11 spices and their 29 prominent phytochemicals for evaluating their role in targeting two crucial proteins BCL3 and CXCL10 involved in breast cancer progression.

The molecular docking results showed that all twenty-nine phytochemicals could bind with both proteins under investigation with different affinities. The binding constants (Ka) for BCL3 and CXCL10 ranged from 0.1 to 162.75 kcal/mol and 0.2 to 861.7 kcal/mol and binding energies (ΔG) ranged from -2.8 to -7.2 $\times 10^3$ $mole^{-1}$ and -3.2 to -8.2 $\times 10^3$ $mole^{-1}$, respectively. Four out of 29 phytochemicals investigated, sesamolin (from sesame), capsanthin (from red chilli), piperine, and piperidine (both from pepper) showed highest binding affinities with both proteins (*Table* 1, Supplementary *Table* S1). Moreover, the 2-aminoisobutyric acid (from fenugreek) showed highest affinity towards CXCL10 only. The diallyl sulphide, diallyl trisulfide, and allicin (all three from garlic) showed least affinity towards both proteins (Supplementary *Table* S1).

Drug Likeness

Drug likeness was calculated using Lipinski's RO5. As per Lipinski's rule, for any ligand to be considered as drug-like, a molecule should pass the following criteria: molecular weight ≤500, number of H-bond donors ≤5, number of H-bond acceptors ≤10 and LogP ≤5, and ≤5 and molar refractivity from 40 to 130. As evident in Table 2, Capsanthin with two violations (MW>500, MLOGP>4.15) was the only compound not following Lipinski's rule.

Top four compounds based on the binding affinity have a molar refractivity of piperidine (30.75), sesamolin (91.52), capsanthin (187.17), piperine (85.47).

The TPSA is recognized as a good indicator of drug absorption in the intestine (TPSA less than 140 Angstroms squared [$Å^2$]) and blood-brain barrier penetration (TPSA < 60 $Å^2$).[34] Out of the 29 compounds screened, only one compound i.e, isobiflorin had a higher TPSA than the recommended limit (*Table* 2). The lower TPSA value of piperidine (12.03 $Å^2$), sesamolin (64.61 $Å^2$), capsanthin (57.53 $Å^2$), piperine (38.77 $Å^2$) is indicative of good drug absorption in the intestine (*Table* 2). The top four molecules viz., piperidine, sesamolin, capsanthin, and piperine present 3, 1, 3, and 3 hydrogen bond acceptors respectively (*Table* 2). All other compounds tested except isobiflorin, which had six hydrogen bond donors, followed the Lipinski range criteria of H-bond acceptors and H-bond donors (*Table* 2).

According to the Ghose criteria for drug-likeness, total number of atoms should range from 20-70, molecular weight ranges from 160 to 480, molar refractivity ranges from 40 to 130 and The computed log P ranges from −0.4 to 5.6.[26] In the present study, only 16 out of 29 compounds tested passed Ghose criteria for drug-likeness (*Table* 2). Veber rule defines drug-likeness constraints as rotatable bond count ≤ 10 and topological polar surface area (TPSA) ≤ 140.[20] All compounds except isobiflorin, 8-Shogaol, 10-Shogaol and capsanthin validated veber rule for drug likeliness (*Table* 2). The Egan computational model for human passive intestinal absorption of small molecules accounts for active transport and efflux mechanisms and is therefore robust in predicting the absorption of drugs.[26] As depicted in *Table* 2 only capsanthin did not follow Egan criteria for drug likeliness. Muegge criteria for drug likeliness include molecular weight between 200-600 Da, carbon atoms > 4, Hydrogen bond donor ≤ 5, Hydrogen bond acceptor ≤ 10, heteroatoms > 1, XLOGP range -2 to 5, TPSA ≤ 150, and the number of rings ≤ 7.[26] Out of 29 compounds tested, only 11 compounds were able to meet Muegge criteria for drug likeliness. These 11 compounds (caryophyllenyl acetate, gingerol, 6-shogaol, gingerdiol, fraxidin, piperine, coumaperine, sesamolin, diferuloylmethane, curcumin and curcumin D6) validated all parameters of drug likeliness (*Table* 2).

Our study revealed that piperidine was predicted as the lowest CYPs promiscuity as it did not interact with all 5 available CYPs on virtual screening by acting as CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, and CYP3A4 inhibitor while sesamolin and piperine interacted with 4 and 3 CYPs respectively. The capsanthin did not interact with any of CYPs (*Table* 3). The sesamolin, and piperine exhibited high or favourable penetration through the blood–brain barrier. High GI absorption was observed in all screened compounds except four compounds i.e, isobiflorin, piperidine zingiberene and capsanthin (*Table* 3).

Thus, the piperine and sesamolin, among all phytochemicals investigated demonstrated best drug-like prop-

Table 2. List of the results of the phytochemical molecules drug likeness properties

S. No.	Compounds	Molecular weight (g/mol)	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity	Lipinski' RO5	Ghose	Veber	Egan	Muegge	Bio-availability score	TPSA (Å^2)	No. of rotatable bonds	Solubility (mol/L)
1	4-isopropylbenzyl alcohol	150.22	1	1	47.15	Yes	No	Yes	Yes	No	0.55	20.23	2	3.04E-03
2	4-isopropyl phenyl acetaldehyde	162.23	1	0	50.99	Yes	Yes	Yes	Yes	No	0.55	17.07	3	2.75E-03
3	4-isopropyl benzaldehyde	148.2	1	0	46.41	Yes	No	Yes	Yes	No	0.55	17.07	2	3.00E-03
4	2-methyl-3-(2-furyl) propenal	136.15	2	0	38.61	Yes	No	Yes	Yes	No	0.55	30.21	2	1.19E-02
5	Isobiflorin	354.31	9	6	84.12	Yes	No	No	No	No	0.55	160.82	2	1.74E-02
6	Caryophyllenyl acetate	262.39	2	0	79.68	Yes	Yes	Yes	Yes	Yes	0.55	26.3	3	3.12E-04
7	2-aminoisobutyric acid	103.12	3	2	25.86	Yes	No	Yes	Yes	No	0.55	63.32	1	2.27E+01
8	Furaneol	128.13	3	1	31.22	Yes	No	Yes	Yes	No	0.85	46.53	0	8.65E-02
9	Hydroxyisoleucine	147.17	4	3	36.3	Yes	No	Yes	Yes	No	0.55	83.55	3	1.67E+01
10	Diallyl sulphide	114.21	0	0	37.6	Yes	No	Yes	Yes	No	0.55	25.3	4	2.27E-02
11	Diallyl trisulfide	178.34	0	0	52.78	Yes	No	Yes	Yes	No	0.55	75.9	6	6.12E-03
12	Allicin	162.27	1	0	45.88	Yes	No	Yes	Yes	No	0.55	61.58	5	4.56E-02
13	Gingerol	294.39	4	2	84.55	Yes	Yes	Yes	Yes	Yes	0.55	66.76	10	1.11E-03
14	Zingiberene	204.35	0	0	70.68	Yes	Yes	Yes	Yes	No	0.55	0	4	7.94E-05
15	6-Shogaol	276.37	3	1	82.91	Yes	Yes	Yes	Yes	Yes	0.55	46.53	9	2.02E-04
16	8-Shogaol	304.42	3	1	92.53	Yes	Yes	No	Yes	No	0.55	46.53	11	3.95E-05
17	10-Shogaol	332.48	3	1	102.14	Yes	Yes	No	Yes	No	0.55	46.53	13	7.78E-06
18	Gingerdiol	296.4	4	3	85.51	Yes	Yes	Yes	Yes	Yes	0.55	69.92	10	5.14E-04
19	2-chloroacetophenone	154.59	1	0	41.65	Yes	No	Yes	Yes	No	0.55	17.07	1	3.21E-03
20	Fraxidine	222.19	5	1	57.49	Yes	Yes	Yes	Yes	Yes	0.55	68.90	1	3.21E-03
21	Piperin	285.34	3	0	85.47	Yes	Yes	Yes	Yes	Yes	0.55	38.77	4	1.84E-04
22	Piperidine	85.15	1	1	30.75	Yes	No	Yes	Yes	No	0.55	12.03	0	1.27E-01
23	Coumaperine	257.33	2	1	81.43	Yes	Yes	Yes	Yes	Yes	0.55	40.54	4	4.85E-04
24	Capsanthin	584.87	3	2	187.17	No	No	No	No	No	0.55	57.53	11	3.82E-10
25	Dronabinol	314.46	2	1	97.91	Yes	No	Yes	Yes	No	0.55	29.46	4	7.77E-07
26	Sesamolin	370.35	7	0	91.52	Yes	Yes	Yes	Yes	Yes	0.55	64.61	3	7.17E-05
27	Diferuloylmethane	368.38	6	2	102.8	Yes	Yes	Yes	Yes	Yes	0.55	93.06	8	1.15E-04
28	Curcumin	368.38	6	2	102.8	Yes	Yes	Yes	Yes	Yes	0.55	93.06	8	1.15E-04
29	Curcumin D6	368.38	6	2	102.8	Yes	Yes	Yes	Yes	Yes	0.55	93.06	8	1.15E-04

***Table* 3.** The pharmacokinetic parameters of the identified compounds

S. No.	Compounds	GI Absorption	BBB permeant	P-GP substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 Inhibitor	Log Kp (cm/s)
1	4-isopropylbenzyl alcohol	High	Yes	No	Yes	No	No	No	No	-5.55
2	4-isopropyl phenyl acetaldehyde	High	Yes	No	No	No	No	No	No	-5.55
3	4-isopropylbenzaldehyde	High	Yes	No	Yes	No	No	No	No	-5.52
4	2-methyl-3-(2-furyl) propenal	High	Yes	No	Yes	No	No	No	No	-6
5	Isobiflorin	Low	No	No	No	No	No	No	No	-8.96
6	Caryophyllenyl acetate	High	Yes	No	No	Yes	Yes	No	No	-5.3
7	2-aminoisobutyric acid	High	No	No	No	No	No	No	No	-8.92
8	Furaneol	High	Yes	No	No	No	No	No	No	-6.6
9	Hydroxyisoleucine	High	No	No	No	No	No	No	No	-9.2
10	Diallyl sulphide	High	Yes	No	No	No	No	No	No	-5.46
11	Diallyl trisulfide	High	Yes	No	No	No	No	No	No	-5.51
12	Allicin	High	Yes	No	No	No	No	No	No	-6.36
13	Gingerol	High	Yes	No	Yes	No	No	Yes	No	6.14
14	Zingiberene	Low	No	No	No	Yes	Yes	No	No	-3.88
15	6-Shogaol	High	Yes	No	Yes	Yes	No	Yes	No	-5.15
16	8-Shogaol	High	Yes	No	Yes	No	No	Yes	Yes	-4.55
17	10-Shogaol	High	Yes	No	Yes	No	No	Yes	Yes	-3.95
18	Gingerdiol	High	Yes	Yes	Yes	No	No	Yes	No	-5.79
19	2-chloroacetophenone	High	Yes	No	Yes	No	No	No	No	-5.76
20	Fraxidin	High	Yes	No	Yes	No	No	No	No	-6.59
21	Piperine	High	Yes	No	Yes	Yes	Yes	No	No	-5.58
22	Piperidine	Low	No	No	No	No	No	No	No	-6.22
23	Coumaperine	High	Yes	No	Yes	Yes	No	No	No	-5.72
24	Capsanthin	Low	No	Yes	No	No	No	No	No	-2.34
25	Dronabinol	High	Yes	No	No	Yes	Yes	Yes	No	-3.27
26	Sesamolin	High	Yes	No	Yes	No	Yes	Yes	Yes	-6.44
27	Diferuloylmethane	High	No	No	No	No	Yes	No	Yes	-6.28
28	Curcumin	High	No	No	No	No	Yes	No	Yes	-6.28
29	Curcumin D6	High	No	No	No	No	Yes	No	Yes	-6.28

erties (better ADME scores and RO5 obeying; *Table* 2, 3) and higher binding affinities (*Table* 1 and Supplementary Table S1).

Despite extensive research and significant success in treatment of primary tumors, we have not been able to fully translate our success in case of metastatic disease with metastatic BCa remaining largely incurable and a fatal disease. The severity of invasive/ metastatic BCa can be gauged by the fact that about 25% of the patients suffering from triple-negative breast cancer (TNBC) succumb to recurrence within 5 years of their diagnosis. On average reoccurrence rate of invasive BCa in women lies between 20-30%.

The chemokine interferon-γ inducible protein 10 kDa (CXCL10), a member of the CXC chemokine family, is associated with several conditions, including infectious diseases, immune dysfunction, chronic inflammation, tumor development, metastasis and dissemination.[35] BCL3 has been reported to promote the proliferation of the TNBC cell line, promoting erbb2-positive tumor metastasis, and regulating TGFβ-signalling in BCa metastasis.[10,36–39]

The potential of herbs and their metabolites as an effective strategy for anti-metastatic therapy has been successfully demonstrated in a study conducted on *Alisma canaliculatum*. In this study, the ethanolic extract of A. canaliculatum demonstrated inhibition of TNFα-induced migration of MDA-MB-231 metastatic breast cancer cells. Additionally, it prevented TNFα-induced CXCR3 and CXCL10 expression by inhibiting the IκB kinase (IKK)-mediated NF-κB pathway. The findings suggest the potential of ethanolic extract of A. *canaliculatum* as a supplement to inhibit invasion and metastasis of breast cancer cells.[40] Flavonoid Apigenin (4,5,7,-trihydroxyflavone) mediated suppression of the CXCL10 secretion significantly reduced the aggressive phenotype of human breast cancer cells.[41] Several *in vitro* and *in vivo* studies have highlighted the anticancer potential of both piperine and piperidine. These phytochemicals exhibit the capability to activate signaling pathways like NF-κB, PI3k/Aκt that are involved in cancer progression including caspase-dependent pathways to induce apoptosis.[42–45] Treatment with piperine in breast cancer cell lines resulted in inhibition of the Akt signaling pathway via decreasing Ser473 residue phosphorylation on Akt. Inhibition of Akt signaling pathway upon treatment with piperine was responsible for inducing BCa cell apoptosis.[44] Inhibition of MMP-2 and MMP-9 gene expression associated with the induction of metastasis in breast cancer was reported in TNBC cells upon treatment with piperine.[44,46]

The piperidine derivative 1-(2-(4 (Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl)piperidine (DTPEP) was able to inhibit cell proliferation of two different cell lines i.e, MDA-MB-231 and MCF-7 by restricting the cell cycle in the G0/G1 phase.[47] Treatment with capsanthin resulted in a significant decline in growth of MCF-7 cell line post 24 hrs of treatment. The decrease in viability of cells due to capsanthin was attributed to oxidative stress and DNA damage along with increased mitochondrial apoptotic mechanism-mediated cell death after p53 and Bax protein activations.[48] Sesamolin treatment was reported to significantly reduce the viability and proliferation of melanoma cells.[49] In an *in vitro* study on colorectal cancer, sesamolin prevented invasion and proliferation of cancer cells in HCT116 cell line by inducing apoptosis via inhibition of the JAK2/STAT3 signaling pathway.[50]

Since all herbal medicine are administered orally and oral absorption is a major determining process for the bioavailability of active biomolecules from the plant remedies, it becomes crucial to have a better understanding of the absorption rate, mechanism, and influencing factors.[51] The bioavailability of herbal components is associated with many presystemic processes, including the solubility in the gastrointestinal fluid, membrane permeability, degradation in the gastrointestinal tract, transporter [*e.g.*, P-glycoprotein (P-gp/MDR1/ABCB1)]-mediated intestinal efflux, presystemic gut wall metabolism, and presystemic hepatic metabolism. Nowadays, gastrointestinal absorption models are crucial tools for investigating transport mechanisms, determining the intestinal effective permeability, and predicting the plasma pharmacokinetic profile throughout the drug discovery/development process.[10,52]

Among different GI absorption models, the in silico model based on stimulating the absorption process from the GI tract has recently gained prominence for its utility in optimizing the active pharmaceutical ingredient (API) release rate, dose, and dose distribution from the various release fractions in modified-release dosage forms.[10] The high expression level of P-glycoprotein (P-gp) or multidrug resistance protein is adenosine triphosphate binding cassette transporter in the intestine apical membrane resulting in limiting the absorption of many drugs in the intestine making it a major obstacle in successful pharmacotherapy of cancers.[51,53–55] Cytochrome P450 (CYPs) metabolic enzymes are crucial enzymes involved in drug metabolism that account for approximately 75% of the total metabolic activity taking place in the organism. Deactivation of most drugs by CYPs is done either directly or by facilitated excretion from the body. CYPs are also capable of transforming certain substances into their active compounds.[56,57]

CONCLUSION

In this maiden in silico molecular docking study, we explored the potential of twenty-nine active phytochemicals from common Indian spices in combating breast cancer invasion and bone metastasis by targeting crucial proteins BCL3 and CXCL10. Sesamolin from sesame and piperine from pepper exhibited strong affinities towards both proteins, suggesting their promise as therapeutic candidates. The drug-likeness of these phytochemicals further supports their potential. However, experimental validation is essential to confirm their efficacy and safety *in vitro*. While the study offers rapid and cost-effective screening methods, the lack of experimental validation remains a limitation. Future directions include rigorous *in vitro* and *in vivo* studies to confirm efficacy and safety, determine optimal dosages and formulations, exploring modes of delivery, assessing synergistic effects with existing therapies, and ensuring comprehensive safety assessments for human consumption. Translation of these findings into clinical applications requires further preclinical and clinical studies to establish safety and efficacy profiles.

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