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Research Article

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Screening of plant-derived natural compounds as potent chemotherapeutic agents against breast cancer: An *in silico* approach

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ABSTRACT

In present study, we aimed to investigate the chemotherapeutic potential of 27 dietary phytochemicals with the motive of developing in silico protocol against transcription factor- NF-κB; growth factor-EGF; antiapoptotic proteins-Bcl-2 and survivin; protein kinase-HER-2; cell cycle protein-cyclin D1 and metastasis proteins-5-LOX, COX2 and VEGF. 2-D structures of all phytochemicals were retrieved from PubChem Compound database and their subsequent conversion into 3-D structures was performed by using online software system CORINA. The X-ray crystallographic structure of proposed target proteins was extracted from RCSB Protein Data Bank. Molecular docking simulation study was carried out by using AutoDock Tools 4.0. The docking results revealed that quercetin (BE: -7.75 Kcal/mol; Ki: 385.26 nM) exhibited better binding interaction to NF-κB than its known inhibitors. Resveratrol (-7.11 Kcal/mole; 6.12 µM) was found to bind to EGF with tighter interaction than several reported EGF inhibitors. Quercetin (-7.86 Kcal/mole; 1.72 µM) and guggulsterone (-7.90 Kcal/mole; 1.62 µM) were best bound to Bcl-2 and Survivin respectively. Emodin (-7.60 Kcal/mole; 2.69 µM) was best docked with HER-2. Guggulsterone (-9.84 Kcal/mole; 60.76 nM) was further best bound to Cyclin D1. Moreover, dibenzoylmethane (-8.05 Kcal/mole; 1.25 µM), guggulsterone (-11.15 Kcal/mole; 0.0067 µM) and Quercetin (-8.75 Kcal/mole; 0.3852 µM) showed very good binding interaction with 5-LOX, COX2 and NF-κB respectively. Our in silico findings have explored the chemopreventive potential of phytochemicals and further, being natural, they have minimal or null side effects on human body as compared to the synthesized anti-breast cancer agents and thus could be their promising alternatives.

Keywords: Phytochemicals, Mammary cancer, Docking, Quercetin, Guggulsterone

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INTRODUCTION

The worldwide burden of cancer increased to an estimated 14 million new cases per year and statistics expected to rise to 22 million annually within the next two decades [1]. Over the same period, cancer deaths are predicted to rise from an estimated 8.2 million annually to 13 million per year [2]. Across the world, in 2012 the most common cancers diagnosed were those of the lung (1.8 million cases, 13.0% of the total), mammary (1.7 million, 11.9%), and large intestine (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%) [3]. As a result of growing and ageing

populations, economically developing countries are strangely affected by the increasing numbers of cancers. More than 60% of the world's total cases occur in Africa, Asia, and Central and South America, and these regions account for about 70% of the world's cancer deaths, a situation that is made worse by the lack of early detection and access to treatment [4]. For instance the incidence of breast cancer in India is on the rise and is rapidly becoming the number one cancer in females pushing the cervical cancer to the second spot. The number of women estimated to be dying of breast cancer every year has also been steadily raising. As against an estimated 48,170 women who died of breast cancer in 2007, the number breached the 50,000 mark in 2010. Uttar Pradesh recorded the highest number of breast cancer deaths among states in 2010, 8,882 followed by Maharashtra (5,064), Bihar (4,518), West Bengal (4,095), Andhra Pradesh (3,863), Madhya Pradesh (3,179) and Rajasthan (3,097) [5].

It was revealed by published data so for that unhealthy life style is the major culprit for most of the cancer and other inflammatory diseases along with some other factors [6]. Epidemiological studies have indicated that populations that consume food rich in fruits and vegetables have a lower incidence of cancers [7–9]. Review of results from 206 human epidemiologic studies and 22 animal studies has suggested indicated that the incidence of cancer, cardiovascular diseases and other aging-related pathologies can be reduced significantly by increased consumption of fruit and vegetables [10, 11].

There are several compounds of plant origin, generally known as phytochemicals, which includes different heterogeneous class of molecules including carotenoids and several food polyphenols, such as flavonoids, phytoalexins, phenolic acids indoles etc interfering with known pathways of cancer induction [12-15]. Since last few years, phytochemicals has been attracting a great interest of researchers because of the reports that shows biological targets of these phytochemicals in mammalian cells were also involved oncogenic alterations, such transformation of cell cycle control, apoptosis evasion, angiogenesis and metastases. In addition, it has been suggested by a large number of epidemiological studies that a regular intake of phytochemicals is capable of reducing the incidence of several types of cancers [13, 14, 16, 17]. Being natural these dietary compounds have seldom side effects and most of them are already present in regular diet of humans, hence, if they are proved to be effective anti-breast cancer agents, only the change in food habit may reduce the risk of mammary cancer and other diseases in human. The present study was carried out to explore the chemotherapeutic potential of proposed dietary phytochemicals against pathway-specific molecular targets together with transcription factor- NF-κB; growth factor-EGF; antiapoptotic proteins-Bcl-2 and survivin; protein kinase-HER-2; cell cycle protein-cyclin D1 and metastasis proteins-5-LOX, COX2 and VEGF.

EXPERIMENTAL SECTION

Preparation of target proteins

The crystal structure of all the target enzymes Bcl2, Survivin, Cyclin D1, EGF, 5-LOX, COX-2, VEGF, HER-2 and NF-κB (pdb ids 2O2F, 1E31, 2W96, 2FDB, 2ABV, 1V0X, 1MKK, 1MFG and 1SVC respectively) involved in various cancer pathways were extracted from Protein Data Bank [18]. All the water molecules and hetatms were removed and further hydrogen atoms were added to the protein. CharMM force field [19] was applied to all the structures followed by energy minimization of each structure for 1000 steps using steepest descent method.

Preparation of library of dietary phytochemicals and docking simulation

All the dietary phytochemicals taken in this study were searched from different literatures. The chemical structures of the selected compounds were available with PubChem compound database and were downloaded from this database. A series of docking experiments were carried out with all the selected dietary phytochemicals against the above selected cancer targets using AutoDock Tools 4.0 [20] for search of compounds with possible anti-carcinogenic activities. The compounds were screened on the basis of their binding free energies and those reflecting good binding affinity were further analyzed on *in silico* platform. Lamarckian genetic algorithm, which is a combination of the genetic algorithm and the local search Pseudo-Solis and Wets algorithm, was employed as a parameter for the molecular docking. The grid box was set to 60*60*60 Å, generated around active site of all the target enzymes, making sure all the selected compounds can freely rotate inside the grid. The total docking runs was set to 10. Each molecular docking experiment was repeated twice to check the precision of results. The finally obtained docked complexes were subsequently analyzed using ligplot [21] and the best complexes were visualized using PyMol [22].

RESULTS AND DISCUSSION

Molecular docking has been an efficient method for discovery and development of new drug candidates [23-26]. In this study we have used molecular docking approach in order to find out natural dietary compounds with better inhibition potential against various biological target enzymes involved in causing mammary cancer [27-32].

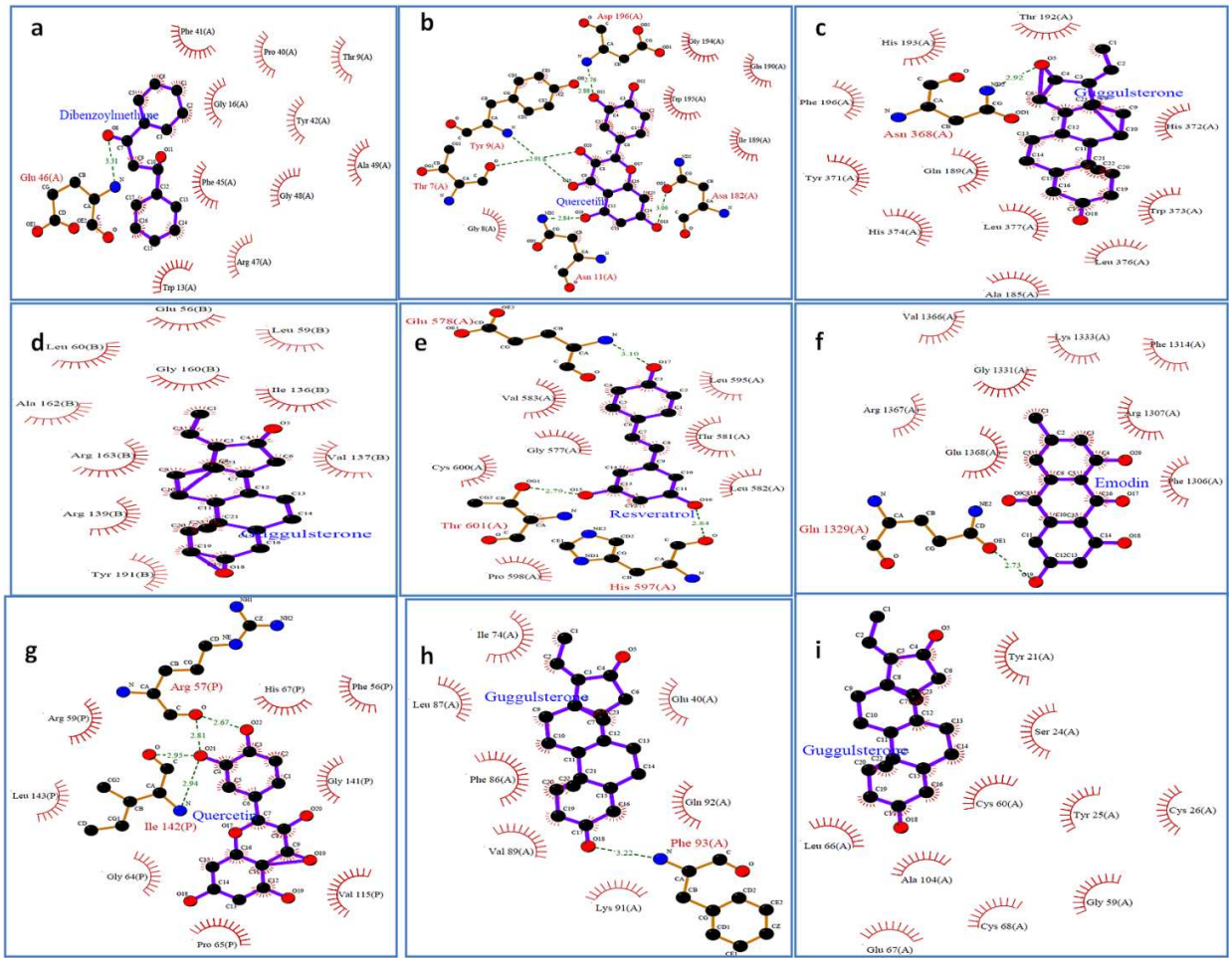
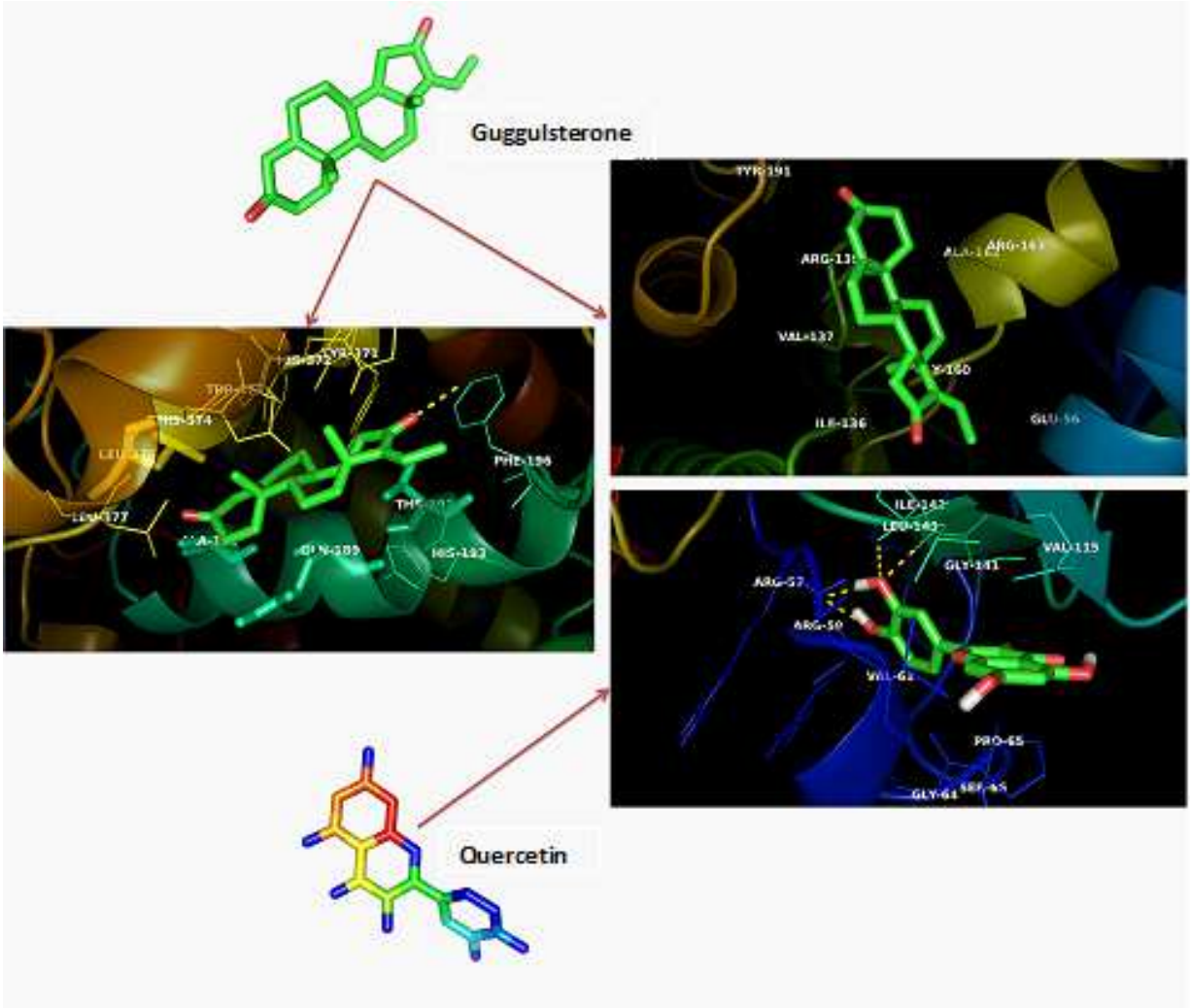


Fig 1 Interaction of best screened out dietary phytochemicals with active site residues of their respective targets

Guggulsterone has earlier been reported to inhibit NF-κB and IκBα kinase activation in human non-small cell lung carcinoma (H1299) and human lung epithelial cell carcinoma (A549) cells [33]. In terms of free binding energy our *in silico* findings revealed that, guggulsterone was showing a very strong and effective binding against most of the selected target enzymes. Furthermore, guggulsterone is reported to be the most notable phytochemicals which down-regulate the expression of apoptosis suppressor proteins in several cancer cell lines [33] that was supported by our dry-lab findings as guggulsterone showing strong inhibition against Survivin, Cyclin D1, COX-2 and VEGF having binding free energies of -7.9, -9.84, -11.15 and -7.24 Kcal/mol respectively (Figure 1 and 2, Table 1).

It was further, investigated that A185, Q189, T192, H193, F196, Y371, H372, W373, H374, L376, and L377 residues were engaged in guggulsterone-COX-2 complex formation. The interaction of guggulsterone within the active site of COX-2 seems to be mostly driven by hydrophobic contacts, wherein, N368 was involved in hydrogen bonding stabilizing the complex (Table 1). Moreover, was also showing plausible binding with both VEGF and Cyclin D1. The higher inhibition potential of guggulsterone might be prove itself as prominent leads against the selected protein markers and forecast their major role in inducing apoptosis in mammary cancer cells.

Fig 2 Visual representation of the interaction of Guggulsterone with different amino acids within the active site of cox-2 and cyclin d1 and the interaction of quercetin within the active site of NF-κB



In continuation, our docking results depicted that quercetin was another potent inhibitor against most of the studied enzymes and it had much better efficacy against NF-κB as compare to other phytochemicals. Various reports suggested that the overexpression/activation of NF-κB in breast cancer cells, where it affects cell proliferation, suppresses apoptosis as well as promotes tumor growth [34–36] and increased binding activity of NF-κB-DNA has been displayed in a variety of mammary cancer cell lines [37]. Quercetin displayed high binding efficacy against NF-κB and Bcl-2 with binding free energies of -7.86 and -8.75 kcal/mol (Table 1) respectively that might be reason to induce apoptosis in quercetin treated cancer cell lines. Our study also epitomized that quercetin interacts by making both hydrophobic contacts as well as hydrogen bonds with the active site residues of all the targets enzymes. Our results strongly favored the previous findings which suggest quercetin to be a very effective inhibitor against NFκB [38].

Dibenzoylmethane [39], an active component of Licorice (*Glycyrrhiza echinata*) showed high potency against 5-LOX as compare to other studied dietary phytochemicals. 5-LOX was responsible for generating leukotrienes (LT) from arachidonic acid, and was supposed one of the most studied cancer targets [32, 40, 41]. Our study elucidated that dibenzoylmethane was found to be the most active inhibiting agent against 5-LOX (ΔG, -8.05 Kcal/mol) (Table 1) in which T9, W13, G16, P41, Y42, F45, and R47 (Table 1) residues were involved in hydrophobic interaction and hydrogen bond was formed by E46 residue providing stability to the dibenzoylmethane-5-LOX complex.

Likewise, emodin depicted strong inhibition against Human Epidermal growth factor Receptor-2 (HER-2), an enzyme responsible for modulation in signal transduction pathways, thereby helping in cell growth and differentiation reported in certain types of cancers [42]. This receptor protein was effectively inhibited by emodin as reflected by its free binding energy (ΔG, -7.6 Kcal/mol) (Table 1) supporting the previous investigations [43].

Table 1: Binding affinity of dietary phytochemicals against their respective target enzymes and the amino acid residues involved in their interactions

Protein	PDB ID	Most effective Phytochemicals (CID)	Binding Free Energy, ΔG (Kcal/mol)	Inhibition Constant (μM)	Residues Involved	
					H-Bonds	Hydrophobic Interaction
Bcl2	2O2F	Quercetin (5280343)	-7.86	1.72	T7, Y9, N11, N182, D196	G8, Y9, N11, I189, Q190, G194, W195
Survivin	1E31	Guggulsterone (6450278)	-7.9	1.62	F93	E40, I74, F86, L87, V89, K91, Q92
Cyclin D1	2W96	Guggulsterone (6450278)	-9.84	0.06076	NA	E56, L59, L60, I136, V137, R139, G160, A162, R163, Y191
EGF	2FDB	Resveratrol (445154)	-7.11	6.12	E578, H597, T601	G577, T581, L582, V583, L595, P598, C600, T601
5-LOX	2ABV	Dibenzoylmethane (8433)	-8.05	1.25	E46	T9, W13, G16, P41, Y42, F45, R47
COX-2	1V0X	Guggulsterone (6450278)	-11.15	0.0067	N368	A185, Q189, T192, H193, F196, Y371, H372, W373, H374, L376, L377
VEGF	1MKK	Guggulsterone (6450278)	-7.24	4.97	NA	Y21, S24, Y25, C26, G59, C60, L66, E67, C68, A104
HER-2	1MFG	Emodin (3220)	-7.6	2.69	Q1329	F1306, R1307, F1314, G1331, K1333, V1366, R1367, E1368
NF-κB	1SVC	Quercetin (5280343)	-8.75	0.3852	R57, I142	F56, R59, G64, P65, H67, V115, G141, L143

Resveratrol (trans-3,4′,5 trihydroxystilbene) is a polyphenolic natural product, present in grape is well known to have chemopreventive and antitumor activities [44,45]. The antioxidant, proapoptotic, and antigrowth properties of resveratrol make it an anti-cancerous agent [44, 46, 47]. This phytochemical has been reported to bind and activates ERs (α and β) to exert both estrogenic and antiestrogenic effects [48,49]. Resveratrol is also been reported to be important for breast cancer prevention because it inhibits breast cancer cell growth [48, 50, 51]. Our docking results exhibited that resveratrol showing plausible binding potential (ΔG of -7.1 Kcal/mol) against EGF, a tyrosine kinase-type integral membrane receptors which regulates the signals that are relevant to proliferation and survival as well as migration and invasion [52, 53]. Furthermore, resveratrol also demonstrated a strong binding within the active site of EGF, where both hydrogen bonds and hydrophobic interactions contribute equally for the positioning of this compound within the active site. E578, H597 and T601 residues of EGF were making hydrogen bonds and G577, T581, L582, V583, L595, P598, C600, T601 residues involved in hydrophobic interactions for the proper lodging of resveratrol within the binding site of EGF. Interestingly, overexpression of EGF is reported in breast cancer contributing to tumor malignancy and poor prognosis [54]. As compare to other compounds, resveratrol was found to be most active inhibitor against EGF. These *in silico* findings are in great agreement with the previous reports [55]. In continuation our docking experiments also suggested emodin (ΔG, -7.6 Kcal/mol) as one of the best inhibitor of HER-2, which is a transmembrane tyrosine kinase act as a coreceptor for other epidermal growth factor receptors associated with a poor prognosis in cancer [56, 57].

The current findings based on the pathway specific targets, dietary phytochemicals certainly authenticate them as excellent anti-mammary cancer drug leads of natural origin [58]. Thus our *in silico* data has elucidated the *in vitro* data with possible underlying mechanism(s) of action, hence it could be used in the mechanism-based screening of new compounds against mammary cancer and other diseases by using disease-specific molecular and cellular targets.

Validation of docking methodology: To ensure that the ligand orientations and positions obtained from the docking studies were likely to represent valid and reasonable potential binding modes of the inhibitors, the docking methods and parameters used were validated by redocking experiments. The ligand was docked into the native protein to determine the ability of AutoDock program to reproduce the orientation and position of the ligand observed in the crystal structure. The top ranking conformational clusters from this dock were evaluated in terms of

root mean square deviation between docked position and experimentally determined position for the ligand. The low RMS (1.20 Å) between the experimental and docked co-ordinates of ligand indicated same binding orientation that favored the validation of docking method (Figure 3).

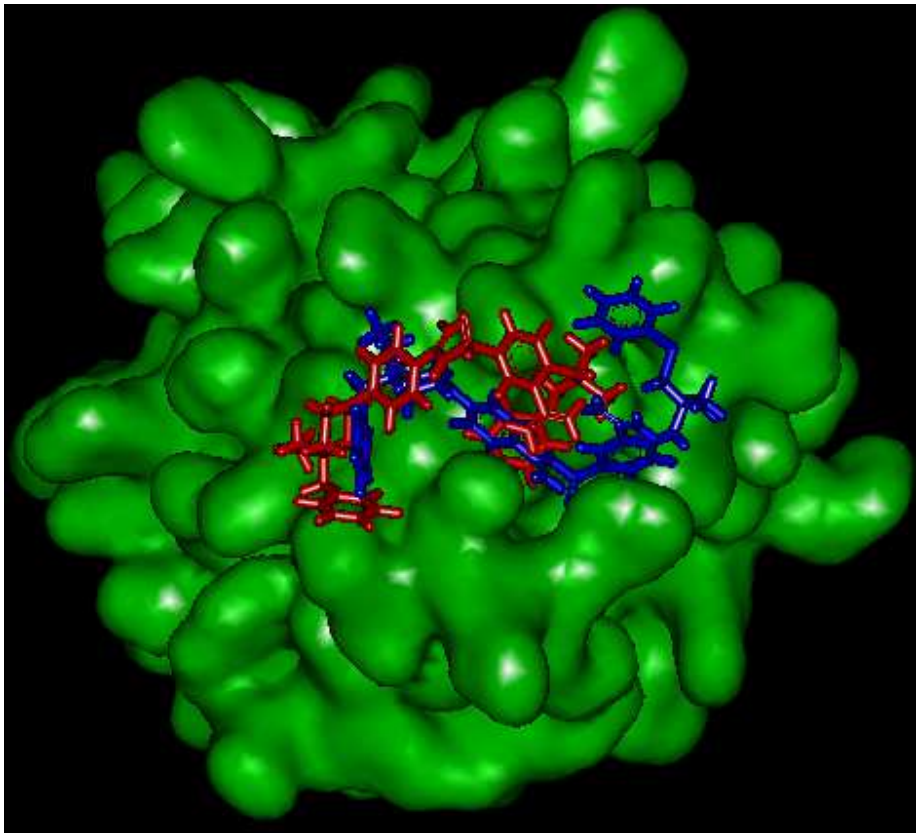


Fig 3 Validation of docking method by superimposing the inhibitor present in the crystal structure of Bcl2 (red) and that after redocking the same (blue) with AutoDock Tool 4.0

CONCLUSION

Breast cancer is one of the major health problems in adult women in developed and developing countries, and is the field of most active research. In continuous offer to explore new biocompatible agents, the current study was focused on natural products to find new safer, cost effective anti-breast cancer compounds. Some phytochemicals have been found to be acting against breast cancer by interfering with one or more carcinogenesis pathways. In present study, a total of 27 dietary phytochemicals were docked against different cancer markers for their binding efficiencies. Our results showed some compounds have good binding efficiencies against known cancer markers and thus can act as effective potential anti- cancer agents. Furthermore, guggulsterone exhibited better inhibition against Survivin, Cyclin D1, VEGF and COX-2, while quercetin showed better inhibition efficacy against Bcl-2 and NFκB, resveratrol against EGF, dibenzoylmethane and Emodin against HER-2. All of these compounds are usually present in common dietary plants. Further, being natural products, these compounds have minimal side effects on human body as compared to the synthesized anti-breast cancer agents and thus could be their promising alternatives.

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