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Research Article

# Molecular Docking-Based Virtual Screening of Harmaline, Ephedrine, and Artemisinin Against 4 Major Breast Cancer Proteins EGFR, MTOR, PR and ER

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## ABSTRACT

Breast cancer is the second deadliest disease around the globe. It affects 1.38 million women annually and responsible for approximately 14% of cancer deaths. The inquisition for superlative solution is crucial demand for researchers and community. Synthetic drugs with more side effects are nowadays dominated by natural products mainly plants-based medicine. The present review cum research summarizes the plants used against breast cancer, invitro, invivo studies and docking of three important medicinal plants against four major breast cancer proteins. About 87 plants (belonging to 34 families) were found to be used by tribal communities of the world to beat breast cancer. Fabaceae, Zingiberaceae and Asteraceae were represented by highest number of plants. While *Abrus precatorius* L., *Curcuma longa* L., *Andrographis paniculata* (Burm.f.) Nees, *Strobilanthes crispus, Brassica oleraceae, Mangifera pajang* Kosterm., *Labisia pumila* Blume. and *Artocarpus altilis* Parkinson were highly cited plants. Many of these plants are under examination for invitro, invivo as well as bioinformatics studies to design a better drug. The plants-based chemicals harmaline, ephedrine and artemisin were first time docked against mammalian target of rapamycin (mTOR), Epidermal Growth Factor Receptor (EGFR), Estrogen receptor (ER) alpha and Progestrone receptor (PR). PyRx and discovery studio visualiser were used for docking studies and analysis. All three studied natural compounds exhibited drug likeness properties according to Lipniski's Rule and expressed very good binding affinities with active sites of studied breast cancer proteins. They may be studied under multitargeted drugs investigation against different breast cancer proteins. Currently only few valuable natural drugs like taxnes, paclitaxel, tamoxifen and rapamycin are available in the market but still not enough effective to combat breast cancer, so this area of research is highly demanding for its impact on the precious lives. Further these types of natural compound need to be optimized for producing potential drugs against breast cancer as they have minimum or no side effects in contrast to synthetic compounds.

**Keywords:** Breast Cancer, Medicinal plants, Drug design, Docking, Artemisin, Harmaline, Ephedrine

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## INTRODUCTION

Cancer is one of the highly dreaded complaints. It is likely that by means of 2030, 26.4 million human beings in single year can be detected with cancer and 17 million human beings will die from it (World Health Organization, 2010). The peculiar division observed in cancer cells results from harm in those cell's DNA (genetic codes that determines cell traits and functioning). There are several reasons e.g environmental elements (including exposure to tobacco smoke), radiations, free radicals in various sources etc, can provoke a chain of activities that consequences in DNA defects, causing cancers. Inheritance of defective DNA also contributed to

cancer percentage. Tumor is resulted via division of cancerous cells forming a clump (Kadioglu et al., 2017). Surrounding healthy non-cancerous cells are destroyed by tumors as abnormal division of cancer cells pressurize them (ACS, A. 2010). Immune system of human plays key role in combating malignant tumor cells (Cheng et al., 2021).

Breast cancer is the second prominent motive of death across the board. About one in 9 girls will broaden this disease at some stage in her lifetime (Anim et al., 2005). In statistics of 2020, lung cancer surpassed by breast cancer with 11.7% among all cancer types representing 2.3 million new cases (Yang et., 2021). In drug discovery unique molecules with potential effects against BC studied from natural products. Raw material has been provided for in depth research via availability of accumulated cancer genomic data. Network pharmacology and data analysis are effective tools in molecular drug design based on natural products (Li et al., 2021). In the framework of this study herbal drugs for treatment of breast cancer were reviewed along with docking of three novel potential anti-breast cancer plants based natural compounds.

## MATERIALS AND METHODS

### *In-Vivo* and *In-Vitro* Studies of Medicinal Plants Used Against Breast Cancer

Annually 458000 deaths of women caused by breast cancer accounting 14% of total deaths resulted from cancer. It effects 1.38 million per annum around the globe (Jemal et al., 2011). Chemical compounds from natural sources exhibit anticancer properties, they induce apoptotic effect on cancer cells thus controlling cancer (Portt et al., 2011). These natural compounds change biochemical as well as morphological features of cancer effected cells. Membrane blebbing, DNA destruction and cell volume reduction results from treatment with natural compounds (Golshan et al., 2016). Isolated compounds from *Fagonia cretica* display a different extent of scope of biological activities including anti-hemorrhagic, anti-tumor, anti-inflammatory, and neuro-protective effects (Razi et al., 2011). Leaf extract of *P. lanceolata* possessed remarkable apoptotic impact on cancer cell lines (triple negative CAL51 (Alsaraf et al., 2017).

Organosulfur compounds of *Allium sativum* control cancer cells through generation of reactive oxygen species causing cell cycle arrest. Additionally elevated amount of these compounds reduced the proliferation of lymphocyte. Cell arrest also carried at G2-M phase leading to apoptosis. The premier component of organosulfur compounds is γ-glutamylS-methylselenocysteine (Islam et al., 2011).

The key component of garlic termed as ajoene, reported for having cytotoxic properties against cancer cells via apoptotic mechanism (Karmakar et al., 2011). The flowcytometry analysis of *Lasiosiphon glaucus* expressed that it has cytotoxic by controlling proliferation of breast cancer cells (MCF-7) (Abhilash et al., 2022). *Tribulus terrestris* (TT) was assessed for cytotoxic effects against MCF-7 cells. Its extracts did not harm the non-cancerous cells. TT caused prominent destruction in DNA of MCF-7 human breast cancer cells via apoptotic induction (Patel et al.,2019).

*Baila litchi* L. extract repress the development and growth of BrDU breast cancer cells, causing remarkable inhibition of tumor (Wang, x., et al., 2006). *Rabdosia rubescens* expressed toxicity against Akt/EGFR BC proteins (Kadioglu et al., 2017). *Salvia miltiorrhiza* derived diterpenoid Cryptotanshinone (CPT) suggested to be a potential drug that inhibit the migration and proliferation of BC cells positive to Estrogen Receptor (ER) in cell line and high-throughput data analysis (Li et al., 2021).

### Chemical Compounds

The plants reviewed in this study have compounds such as alcaloids, flavonoids, isothiocynate, phenolic acids, terpenoids, stilbenes, lignans etc. These compounds have active molecules through which these plants can cure breast cancer cells. The active molecules within these compounds are berberine, caffine, anthocyanins, apigenin, curcumin, epigallocatechin-3-gallete, garcinol, plumbagin, xanthohumol, sesamin, oleanolicacid, resveratrol, ursolicacid, thymoquinone and polyphyllin D.

All these bioactive molecules reduce tumor growth in different cell line like BC MDA-MB-231, MCF-7 by stimulating apoptosis and autophagy. Anthocyanins inhibited BC cell growth and cyclooxygenase enzyme activity (Zhang et al., 2005). Apigenin reduced cell proliferation and endogenous 26S proteasome, downregulating cyclin A and B leading to arrest cell line (Noolu et al., 2016). Curcumin decreased tumor multiplication (Carroll et al., 2010).

Polyphyllin D induced the disruption of DNA and dissipation of mitochondrial membrane potential, leading to dysfunction of mitochondria in MCF-7 and MB-231 breast cancer cell lines (Lee et al., 2005). Xanthohumol decreased the inflammatory cell number and focal proliferation areas through modulation of apoptotic cells (Monteiro et al., 2008) and tumor suppressor prohibition interaction (Yoshimaru et al., 2014). Treatment by sesamin reduced MCF-7 xenograft tumor growth at high level of circulating estrogen (Truan et al., 2012). Garcinol reduced the growth on different cell lines by promoting caspase -3 mediated cell death sand lower signaling pathway (Ahmad et al., 2010). Thymoquinon

inhibited the expression of breast cancer cell by targeting several pathways (Rajput et al., 2013). Detail of plants used in breast cancer treatment in different ways are given in Table 1.

Table 1. Plants used for breast cancer treatment

| Plant name | Family | Reference |
|---|---|---|
| *Abrus precatorius* L. | *Fabaceae* | Manoharan, S., & Kaur, J. (2013), Bhutia,et al., (2016), . Ibahim M et al., (2011). |
| *Bauhinia purpurea* L. | | Balijepalli M, et al., (2010), Singh et al., (2006) |
| *Glycine max* L. | | Elgadir et al., (2015) |
| *Pueraia mirifica* | | Elgadir et al., (2015) |
| *Pueraria lobate* ohwi | | Elgadir et al., (2015) |
| *Trigonella foerum - graecum* L. | | Narrima et al., (2014), Ayob Z et al., (2013) |
| *Cristia vespertilionis* (L.f.) Bakh.f | | Lee et al., (2020) |
| *Erythrina corallodendron* L. | | Xing et al., (2019) |
| *Albizia zygia* (D.C) J.F.Macbr | *Leguminosae* | Kokila K et al., (2003) Ong H and Norzalina J,(1999) |
| *Alpinia conchigera* Griff. | *Zingiberaceae* | Zakaria et al., (2011) |
| *Alpinia officinarum* Hance. | | Wong et al.,(2013), Sathishkumar P et al.,(2014) |
| *Curcuma longa* L. | | Elgadir et al., (2015), Nataru et al (2014), Yue et al., (2010). |
| *Curcuma amada* Roxb. | | Manoharan S and Kaur J. (2013) |
| *Curcuma zanthorrhiza* Roxb. | | Cheah YH et al., (2006) |
| *Curcuma zedoaria* | | Dashora N et al., (2011) |
| *Etlingera elatior* (Jack) RMSm | | Zan CH et al., (2011) Ghasemzadeh A et al.,( 2015) |
| *Zingiber officinale* Var. | | Rahman S et al., (2011), Raghavendra L and PrashithKekuda R (2018). |
| *Alternanthera tenella* Var. | *Amaranthaceae* | Ozsoy N et al., (2009) |
| *Amaranthus lividus* L. | | Lee and Moon; (2016), Jadhao and Thorat; 2014) |
| *Amaranthus gangeticus* L. | | Sani HA et al., (2004) |
| *Chenopodium ambrosioides* L. | | Jia liang et al., (2014), Ya nan et al., (2015) |
| *Withania somnifera* L.(Dunal) | *Solanaceae* | Manoharan and Kaur (2013) Ghasemzadeh and Jaafar, (2011) |
| *Physalis minima* L. | | Ooi et al., (2010), Nassar et al.,(2010) |
| *Capsicum annuum* L. | | Jayakumar et al., (2014), Maisarah et al.,(2014) |
| *Thelesperma megapotamicum* | *Asteraceae* | Ling AL et al., (2015), Baharum Z et al., (2014). |
| *Vernonia amygdalina* Delile. | | Awang et al., (2010) |
| *Silybum marianum* L. | | Elgadir et al., (2015) |
| *Eupatorium odoratum* L. | | Rosna and Khandaker, (2015) |
| *Elephantopus scaber* Var. | | Harun et al., (2012) |
| *Echinacea angustifolia* Var. | | Lumpur K; (2008) |
| *Centratherum anthelminticum* L. | | Abu Bakar et al., (2010) |
| *Artemisia maritima* Pourr ex Willk. & Lange | | Teimouri, M., and Odoumizadeh, M. (2021). |
| *Allium sepa* | *Liliaceae* | Elgadir et al., (2015) |
| *Allium sativum* L | | Elgadir et al., (2015),32,33,60 |
| *Alstonia scholaris* (L.) R.Br | *Apocynaceae* | Jagetia and Baliga (2003), Jayanthi and Smitha (2012) |
| *Calotropis gigantean* | | Thoennissen N, et al., (2010) |
| *Catharanthus roseus* ( L.) G.Don | | Ahmad et al., (2010), Hasanzadeh et al., (2011) |

| | | |
|---|---|---|
| *Picralima nitida* (Stapf) T.Durand & H.Durand | | Engel et al., 2014 |
| *Andrographis paniculata* (Burm.f.) Nees | *Acanthaceae* | Suhail et al., (2011), Hossain et al., (2014), Roslida et al., (2011) |
| *Sanchezia speciose* | | Ho KL et al.,(2015) |
| *Strobilanthes crispus* | | Zan et al.,(2011), Aslam et al.,(2010), Ibahim et al.,(2011) |
| *Thunbergia laurifolia* Lindl. | | Khan et al., (2022) |
| *Justicia gendarussa* Macrae ex Nees. | | Khan et al., (2022) |
| *Ardisia crispa* Thunb. | *Myrsinacea* | Hamsin et al., (2014), Veerakumar et al., ( 2016) |
| *Ardisia brevicaulis* Diels. | | Aisha et al.,(2012) |
| *Labisia pumila* Blume. | | Rasool (2015), Al-Mekhlafi et al., (2012), Singh and Luqman (2014) |
| *Clausena excavata* Burm.f. | *Rutaceae* | Wong SK et al.,(2011) |
| *Murraya koenigii* L. | | Sayar K et al., (2014) |
| *Murraya paniculata* L. | | Fang and Ng (2015) |
| *Vaticadios pyroides* | *Dipterocarpaceae* | Biswal et al.,(2013) |
| *Annona muricata* L. | *Annonaceae* | Endrini et al., (2015), Syed Najmuddin et al., (2016) |
| *Annona squamosal* | | Mariod et al., (2012), Nguyen MT et al., (2015) |
| *Artocarpus altilis* Parkinson. | *Moraceae* | Sikarwar et al., (2014). Zakaria et al., (2011), Arung et al., (2009) |
| *Artocarpus obtusus* F.M.Jarrett | | Hashim et al., (2012) |
| *Ficus deltoidea* Var | | Alabsi et al., (2013) |
| *Typhonium flagelliforme* Roxb. ex G. Lodd | *Araceae* | Mohan et al., (2010), Srisawat T et al.,(2018) |
| *Theobroma cacao* L. | *Malvaceae* | Nurrochmad et al., (2011) |
| *Argemone Mexicana* L. | *Papveraceae* | Elgadir et al., (2015) |
| *Tinospora crispa* (L.) Hook.f.and Thomson | *Menispermaceae* | Bhagat and Chaturvedi, (2016) |
| *Stephania venosa* Spreng | | Khan et al., (2022) |
| *Azadirachta indica* A.Juss | *Meliaceae* | Andrade S (2012) |
| *Dysoxylum cauliforum* | | Chicca et al., (2010) |
| *Sandoricum koetjape* Burm f. Merr | | Kuppusamy et al., (2015) |
| *Brassica oleraceae* | *Brassicaceae* | Arisanty (2013), Tang et al.,(2013), Arbab et al., (2013) |
| *Raphanus sativus* L. | | Hu X et al., (2010) |
| *Buxus sempervirens* L. | *Buxaceae* | Ait Mohamed et al.,(2011) |
| *Origanum acutidens* Hand-Mazz | *Lamiaceae* | Tuncer et al., (2013) |
| *Phyllanthus amarus* Schumach and Thonn | *Phyllanthaceae* | Moongkarndi et al., (2004) |
| *Phyllantus pulcher* | | Aslam et al.,(2010) |
| *Scrophularia oxysepala* Boiss | *Scrophulariaceae* | Valiyari et al., 2012 |
| *Schinus molle* L. | *Anacardiaceac* | Khan et al., (2022) |
| *Schinus terebinthifolius* Var. | | Khan et al., (2022) |
| *Mangifera indica* L. | | Abdullah et al.,(2014) Roslen et al., (2014) |
| *Mangifera pajang* Kosterm. | | Banerjee et al.,(2016), Ghil S. (2013) Wong FC etal.,(2013) |
| *Dendrophthoe pentandra* L. | *Loranthaceae* | Wong FC etal.,(2013),136,92 |
| *Scurrula ferruginea* Roxb ex. Jack | | Looi et al., (2013); Rahmat et al., (2006) |
| *Cinnamomum zeylanicum* Var. | *Lauraceae* | Abdul wahab and adzmi (2017) |
| *Heliotropium indicum* L. | *Boraginaceae* | Moongkarndi et al., (2004) |

| | | |
|---|---|---|
| *Juniperus oxycedrus* L. | *Cupressaceae* | El abid et al., (2019) |
| *Juniperus phoenicea* L. | | El sawi et al.,(2008) |
| *Juniperus excels* | | Khanavi et al., (2019) |
| *Peganum harmala* L. | *Nitrariaceae* | Zhang et al., (2022) |
| *Piper nigrum* L. | *Piperaceae* | Poompavai and Gowri Sree (2021) |
| *Linum usitatissimum* L. | *Linaceae* | Szewczyk et al., (2014) |
| *Fagonia indica* Burm.f | *Zygophyllaceae* | Waheed, et al.,(2012. |
| *Fagonia cretica* L. | | Matt Lam et al., (2014). |
| *Ephedra alata* Decne. | *Ephedraceae* | Danciu et al., (2018) |

Members of family like Zingiberaceae, Fabaceae, Liliaceae, Anacardiaceae and Moraceae has reported for anticancer potential especially for breast cancer. In this review about 87 plants (belonging to 34 families) were found to be used by tribal communities of the world to overcome breast cancer. Fabaceae, Zingiberaceae and Asteraceae were represented by highest number of plants. While *Abrus precatorius* L., *Curcuma longa* L., *Andrographis paniculata* (Burm.f.) Nees, *Strobilanthes crispus, Brassica oleraceae, Mangifera pajang* Kosterm., *Labisia `pumila* Blume and *Artocarpus altilis* Parkinson were highly cited plants.

**Natural Drugs for the Treatment of Breast Cancer**

Drug design is the procedure or steps to words creating or designing the novel drug from substances using experimental, clinical, computational and translational modes for diseases (Zhou and Wei-Zhu, 2017). Designing the drug from plants constituents is becoming popular across the globe by driving the ethnobotanical knowledge and then testing them through pharmaceuticals, bioinformatics and other different protocols, leading to the discovery of novel drug. More than 60% crucial medicines consist of compounds obtained from plants. Several drugs acquired from plants, bacteria, fungi, marine organism etc., used for the treatment of various types of cancer i.e breast, ovarian, lungs, stomach, brain, pancreatic cancer (Cragg and Pezzuto, 2016).

Novel drugs available in market synthesized from various plant species containing chemicals having potential against breast cancer (BC), e.g taxnes is one of the most important classes of cancer chemotherapeutic drugs in clinical use. The paclitaxel and its analogue docetaxel, used against cancer extract from *Taxus brevifolia* (Suffness, 1995; Gordaliza, 2007). Few drugs which are highly effective against breast cancer derived from bacteria and fungi and marine organisms. Rapamycin is one of the major drugs used against different types of cancer, highly effective in treating BC. Trabectedin from marine organism used to treat BC, undergoes in trails (Cragg and Pezzuto, 2016). Tamoxifen is one of the major drugs against BC commercially accessible in market, binds with estrogen receptor and slabs its function thus preventing BC (Ahmed et al., 2014). To treat BC, there is search for drug that is plant based, effective on all stages of BC, as plant derived medicine are generally nontoxic having minimum or no side effects.

**Insilico Drug Design for Breast Cancer Treatment**

Genes that control growth of cell, if over expressed cause tumors in breast (Idris et al., 2021). Major proteins that have been studied insilico in breast cancer are estrogen receptor alpha (ER), progesterone receptor (PR), epidermal growth factor receptor (EGFR), mammalian target of rapamycin (mTOR) (Firdous et al., 2014; Acharya et al., 2019), BRAC1 (Saravanan et al., 2021), Beta tubulin receptor protein (Yadav et al. 2017), co-chaperone HSP90, HER2/neu receptor (Pick et al., 2007), Topo2$\alpha$receptor (Idris et al., 2021). Natural products possessing potential to prevent cancer will provide a mechanism that will be more effective in terms of non-toxicity and other positive aspects. Researchers dock these chemicals against cancerous proteins. Molecular docking is the strategy which utilizes the binding affinity of chemicals with cancerous proteins to inhibit those (Yadav et al., 2017).

Data bases that have been used frequently in molecular drug design are PyRx, SwissADME, PubChem (3D structure of compound), PDB database (for target protein), Uniport database, Discovery studio visualizer, BioSlove IT FlexX, CASTp program/server (Computed Atlas of Surface topography of protein), Argus lab docking software, Sybyl-X (Surfex-dock module), UCSF Chimera, Dock Prep (for energy minimization and geometry optimization of proteins), Dunbrack rotamer library (to repair side chain) (Yadav et al., 2017; Acharya et al., 2019; Saravanan et al., 2022), Server BSP-SLIM (for protein docking) (Supramaniam and Asita, 2020).

These bioinformatics tools play important role in studying the molecular reactions in drug discovery. Drug bank is remarkable resource that combines both detailed drug data with inclusive target molecule. PDB (Protein Data Bank) (Acharya et al., 2021) and PubChem are the global archives to acquire structural data of biological macromolecule and ligands respectively. These resources prove best structures in different formats, along with very important information

about the molecules. Softwares like PyRx and Discovery studio visualizer are the best and easily used sources to find the binding affinity of potential drug (ligand) against BC protein (target). ACD/chemsketch Hex, mainly used for the chemical drawing and graphics. Hex is for calculating and showing feasible docking forms of pairs of protein and DNA molecules. Molecular graphics program, Rasmol used for the structural results of nucleic acid, protein and other micro molecules (Mathew, 2009). The SWISS-Model is used for the construction of protein. The ExPASy's ProtParam Proteomics server was used to assess the physiochemical nature of ligand and the Procheck, Errat, which confirmed the 3D structure of protein. Server BSP-SLIM for protein docking (Supramaniam and Asita, 2020). Molinspiration server provides calculation for Lipniski's rule. The application of Lipinski's rule of five checks the drug likeness property through calculating the molecular weight, number of H-bond donors and acceptors, LogP values and molar refractivity (Acharya et al., 2021).

Based upon the information of use of plants against breast cancer, different molecules of these plants have been docked against major proteins of breast cancer. Phytocompunds of *Solanum torvum* (Saravanan et al., 2022), furanocoumarins (Acharya et al., 2019), different flavonoids (Suganya etal.,2014), withanolides analogs from *Withania somnifera* (Yadav et al., 2017) were studied against BC proteins. Docking of plant constituents provided new insights in research on BC treatment. The binding affinity of one of the compound from the unripe fruits of *Solanum torvum*, named ergost-25-ene-3,6-dione,5,12-dihydroxy-, (5.alpha., 12.beta.) with BRCA1 was higher than synthetic drug doxorubicin. These plants based natural compounds have almost no side effects as compared to synthetic drugs (Saravanan et al., 2021). Similarly, xanthotaxol (type of furanocoumarins) best worked with ER alpha, PR, EGFR and mTOR, recommended for BC treatment (Acharya et al., 2021). Chrysin and Equol (types of flavonoids) exhibited best binding affinity with ER. While the binding affinities of some compounds like analogs of withanolides with beta tubulin was suggested to be optimized for designing the inhibitors of protein beta tubulin. Plant products with potential anticancer activities have become a vital source of novel agents in treating cancer. *Adiantum capillus* and *Pteris Quadriureta* are such traditional herbs with potential pharmacological properties for BC (Rautray et al., 2018).

**Docking of Hermaline, Ephedrine and Artemisin Against Four Major BC Proteins**

Three important medicinal plants of Pakistan Peganum, Ephedra and Artemisia that have been reported for having potential against breast cancer yet not analysed for drug design, were docked against four major breast cancer proteins naming, Estrogen receptor (ER) alpha, Progestrone receptor (PR), Epidermal Growth Factor Receptor (EGFR) and mammalian target of rapamycin (mTOR). The plants-based chemicals selected were harmaline ephedrine and artemisin.

**Protein Retrieval and Preparation**

BC proteins were retrieved from PDB online available database RCSB PDB: Homepage. Structures of these BC proteins were prepared for docking using discovery studio visualizer. All hetero atoms including water and ligands were removed from protein molecule. To the prepared proteins, added hydrogen atoms for polarity and then saved in PDB format. While further they are converted in pdbqt format. Protein data base is one of the best sources to obtain the protein structure with all details. Crystal structures of proteins with bound ligands and other hetero molecules provide important information of protein characteristics. Active sites of BC proteins were highlighted using discovery studio visualizer. Constructed the Hydrophobicity and Ramachandran charts/plots for BC proteins (Figure 1).

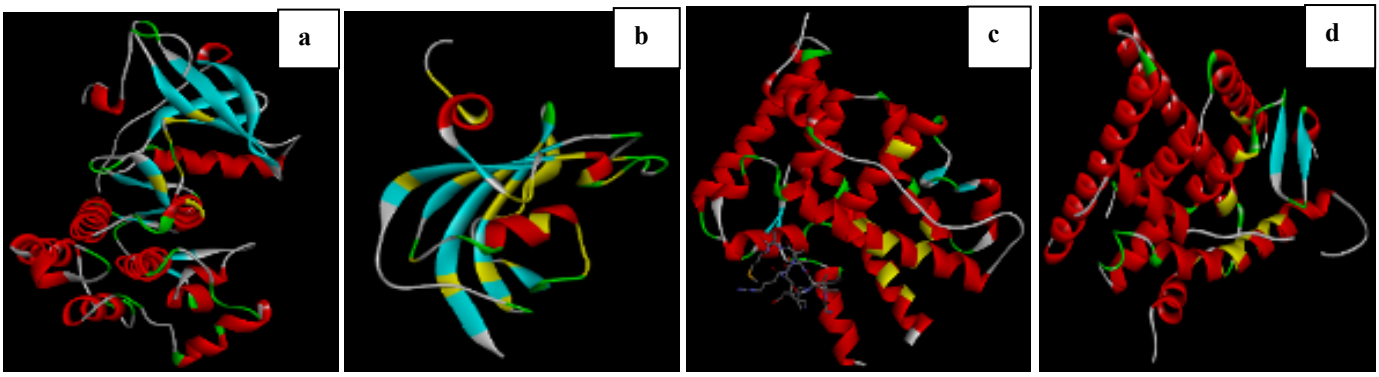



Figure 1. Proteins prepared structures (active site highlighted in yellow) for docking (a) 2j6m (b) 4drh (c) 4oar (d) 5w9c

**Ligand Extraction and Optimization**

3D and 2D structures of harmaline, ephedrine and artemisin retrieved from PubChem database available at PubChem (nih.gov). These ligands were optimized for energy in PyRx and then saved in autodock pdbqt format.

**Drug Likeness of Ligands**

Drug likeness assessment is the first step of possibility of a molecule to become a drug, related to the oral bioavailability. The ligands retrieved from PubChem were then analysed for different parameters using Lipniski's Rule of Five. Selected only those compounds fulfilling the criteria of Lipniski's rule of five regarding mass, molar refractivity, LOGP value, number of hydrogen bond donors and acceptors (Acharya et al., 2021).

**Docking**

PyRx software was employed for docking analysis. The loaded proteins and ligands were converted to same format (pdbqt). Run the Vina Wizard to dock ligands against BC proteins. The best docked models having minimum lowest energy and RMSD values were saved. Also, the best docked models were saved in PDB format for further analysis (Figure 2).

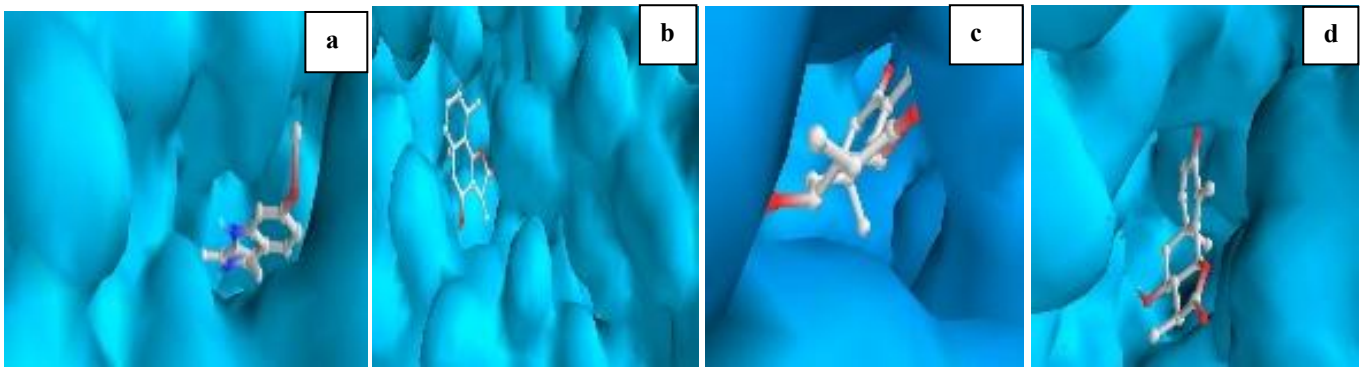



Figure 2. 4oar docked with (a) harmaline (b) artemisin (c) 5w9c and (d) 2j6m with artemisin (surface structures).

**Visualization of Docking Results**

Discovery studio visualizer was used to probe interactions of ligand with active site and the results were saved in 2D (Figure 3) and 3D (Figure 4). The interactions of H-bond donor and acceptor in the attachment site were visualized. The ionizability around and in the attachment site of ligand with active site was noted.

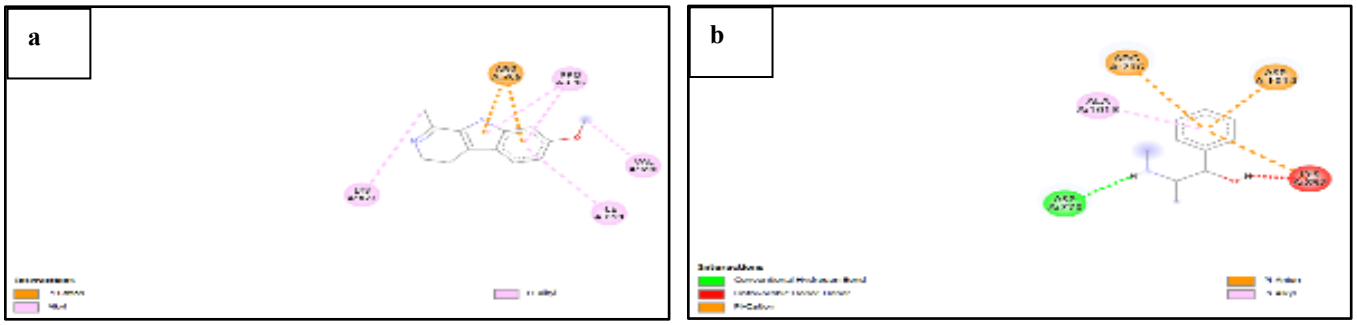



Figure 3. 2D structural interaction of (a) 4aor with harmaline and (b) 2j6m with ephedrine.

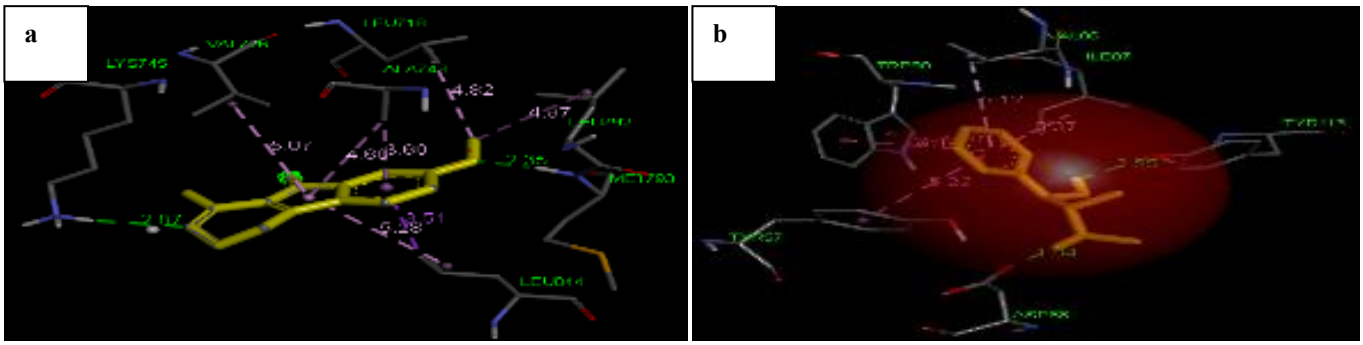



Figure 4. 3D structural interaction of (a) 2j6m with harmaline (b) 4drh with ephedrine.

## RESULTS AND DISCUSSION

Insilco drug design is powerful tool in drug discovery exploiting the interaction of receptor biomolecule and small ligand molecule finding their affinity (Liu et al., 2019a). Harmaline, artemisin and ephedrine are plants-based compounds having important medicinal properties. These are all cyclic organic compounds. These compounds have been reported in cell line, invitro, invivo and ethnobotanical studies for having potential against BC proteins (Lai and Singh, 2006; Shabani et al., 2015; Chen et al., 2016; Tang et al., 2020; Xu et al., 2020). Artemisin in clinical trials proved to be effective in metastatic breast cancer and solid tumor malignancies required to be tested for potential harms and drug likeness properties (Xu et al., 2020). Ephedrine have substantial antiproliferation activity against BC cell lines through induction of cell cycle arrest (Chen et al., 2016). Plant extract of *Peganum harmala* induced the apoptotic mechanism thus decreasing the growth of cancer cell lines (Shabani et al., 2015).

Drug likeness through Lipinski rule provides the very first information about the potential of molecule to be used as drug. This rule is highly considered in evaluation of drug absorption, distribution, metabolism and excretion (ADME) (Kadioglu et al., 2017). Artemisin and Harmaline have one and Ephedrine possessed two H-bond donors (Hydrogen bond donors e.g OH and NH groups must not exceed five), Ephedrine and Harmaline have two while Artemisin have four H-bond acceptors (no more than ten H-bond acceptors commonly N, O), molecular weight of Ephedrine calculated as 165 g/mol, Harmaline 214 g/mol and Artemisin 262 g/mol (molecular weight of molecule must be under 500 g/mol), log-p value of Ephedrine was 1.32, Artemisin 1.39 and Harmaline 2.54 (log-p i.e partition coefficient must be less than five). Artemisin, Harmaline and Ephedrine all were fulfilling the criteria of Lipniski's rule thus possessing the drug likeness property (Table 2). Molecular docking highly utilizes the use of Lipniski's rule in drug design for BC treatment (Suganya etal., 2014; Kadioglu et al., 2017; Yi et al., 2017; Abdullahi et al., 2022).

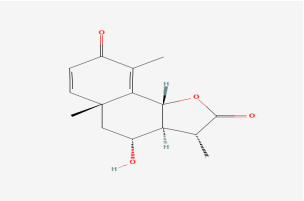
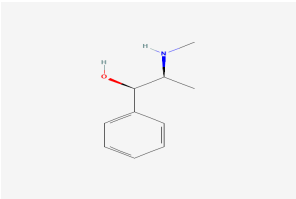
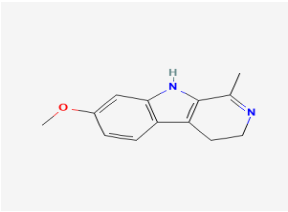
Table 2. Drug-likeness property using Lipinski rule

| Compound | LogP value | H-bond donor | H-bond acceptor | Mass (g/mol) | Molar Refractivity ($m^3$/mol) | Drug-likeness |
|---|---|---|---|---|---|---|
| Artemisin | 1.3904 | 1 | 4 | 262 | 68.111786 | Yes |
| Ephedrine | 1.3279 | 2 | 2 | 165 | 49.923489 | Yes |
| Harmaline | 2.5416 | 1 | 2 | 214 | 65.63269 | Yes |

Ramachandran plots of BC proteins gives the detail about protein confirmation and polypeptide description using torsion angles as in structural biology it is the main concept. Its use is becoming widespread. For protein structure Ramachandran also named as ϕ, ψ-plot became necessary part. Similarly, hydrophobicity plots of BC proteins helped to evaluate the protein structures as water mediates the protein-protein interactions. This provides large area protein-protein interfaces (temporarily stable and close), by compacting individual proteins (Bhadra, 2020). Ramachandran plots results for ER, EGFR and mTOR revealed that in favoured region maximum number of amino acids were present while only few fallen in not allowed area representing them as good model for molecular docking (Figure 8). Ravnik et al. (2021) created these plots for TGRS BC protein, then used optimized structure for docking with bacterial secondary metabolites. Extracellular pH for healthy breast tissues reported to be 6, while pH of acidic values provides the suitable microenvironment for tumors, thus hydrophobicity calculation charts comfort in BC proteins research (Ravnik et al., 2021).

Executed the molecular docking of harmaline, artemisin and ephedrine against four major BC proteins along with review of potential anti-BC plants in this study (Figure 5, 6, 9). Optimized energies for compounds artemisin (340.45 kcal/mol), ephedrine (150.09 kcal/mol), and harmaline (409.78 kcal/mol) before docking employing PyRx. Ligands bound to the active sites of the BC proteins and expressed promising results in terms of drug likeness, binding affinities, bonding interactions etc. In the current study artemisin (-7.8 kcal/mol) and harmaline (-7.3 kcal/mol) (Detail table 3) possessed higher binding affinities comparatively, as -6.93 kcal/mol binding energy scored for human Bcl-2 with paclitaxel (Ansari et al.,2021), oridonin from *Rabdosia rubescens* against Akt/EGFR BC proteins (Kadioglu et al., 2017) obtained binding energy -6.633 kcal/mol proving them strong candidates in anti-BC agents. Sarvanan et al. (2021) calculated -7.3 kcal/mol for ergost-25-ene-3,6-dione, with amino acid residues of active site TYR, MET, LEU.

**Table 3.** Docking results of selected natural compounds against major BC proteins

| Protein | | Ligand | | | | Binding Affinity (kcal/mol) |
|---|---|---|---|---|---|---|
| Name | PDB ID | Name | PubChem ID | Structure | Minimized energy E (kcal/mol) | |
| EGFR | 2j6m | Artemisin | 65030 |  | 340.45 | -7.7 |
| MTOR | 4drh | | | | 340.45 | -6.8 |
| PR | 4oar | | | | 340.45 | -7.8 |
| ER | 5w9c | | | | 340.45 | -7.2 |
| EGFR | 2j6m | Ephedrine | 9294 |  | 150.09 | -5.6 |
| mTOR | 4drh | | | | 150.09 | -6.3 |
| PR | 4oar | | | | 150.09 | -6.2 |
| ER | 5w9c | | | | 150.09 | -6.1 |
| EGFR | 2j6m | Harmaline | 3564 |  | 409.78 | -6.6 |
| mTOR | 4drh | | | | 409.78 | -5.9 |
| PR | 4oar | | | | 409.78 | -7.3 |
| ER | 5w9c | | | | 409.78 | -6.9 |

Binding of ligands with BC proteins involved bonding types of Alkyl, Pi-Alkyl, Pi-Anion, Hydrogen bond (Figure 7), Van der Waals interaction, Pi-Sigma, Pi-Pi-Stacked, Unfavourable donor-donor and acceptor-acceptor (Table 4). Ionizability if almost all docked molecules towards neutral. LEU718 was bound by H-bond ligand with bond distance of 2.91Aº. Similarly, Artemisin docked with 2j6m specifically on active site previously described on crystal structure of EGFR. Artemisin bound by H-bond with MET793 and unfavourable acceptor with ASP855. The bond distances were 2.15 Aº and 2.9 Aº. The region was lying in coil structure of 2j6m. Docking of artemisin with ER also expressed high binding affinity. Artemisin docked with 17 amino acids of active site through vender Waals interactions. Amino acids LEU and MET were highly interacted. Harmaline docked with PR also expressed positive results it docked with active site through Pi-cation, Alkyl, Pi-Alkyl bonds in coil and helix structures of proteins. The active amino acids were PRO696, VAL698, ILE699, ARG766, LYS822. The minimum distance was 3.66 Aº and largest bond was 5.3 Aº. Overall results are summarized in (Tables 2 and 3). Decidedly used amino acids were LEU (14), MET, LYS (6), ASP, VAL (5), ALA, PHE, TYR, ILE (4), ARG, TRP (3), THR, PRO, GLU, GLY (2), SER, HIS (1).

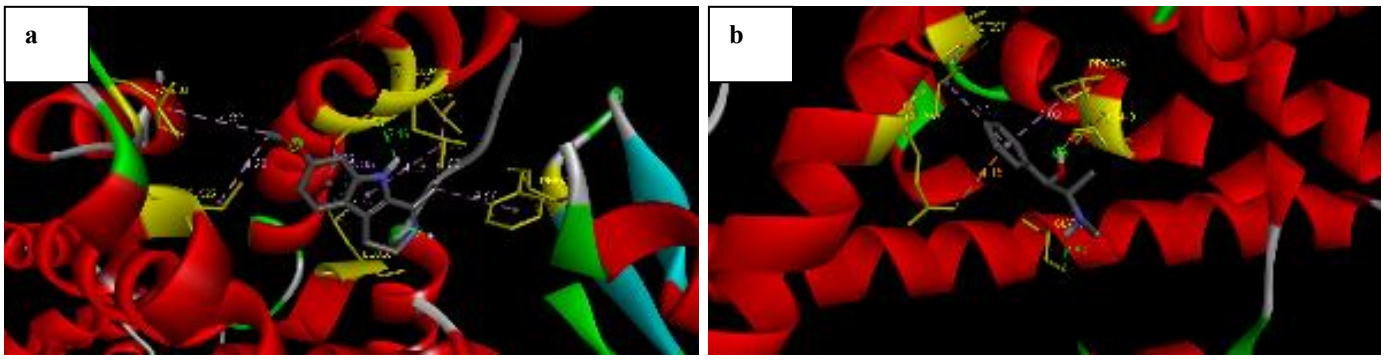



Figure 5. Protein interaction of (a) 5w9c with harmaline (b) ephedrine.

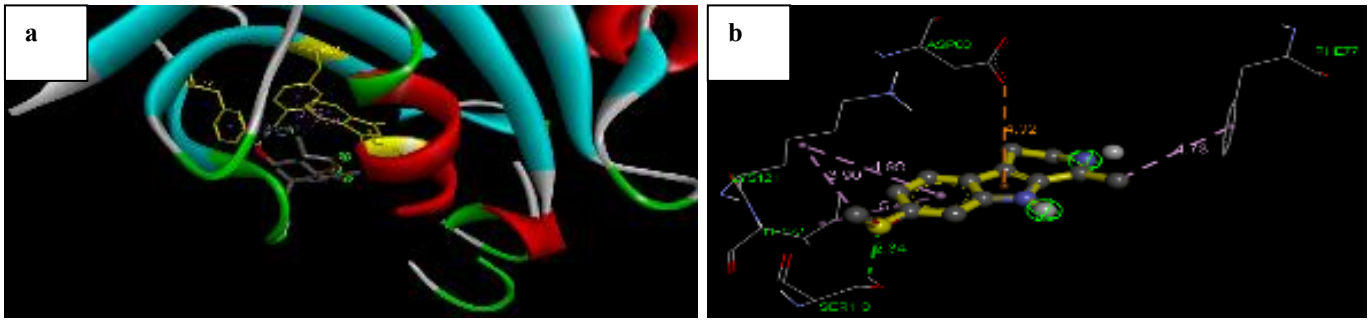



Figure 6. Interaction of (a) 4drh with artemisin and (b) harmaline

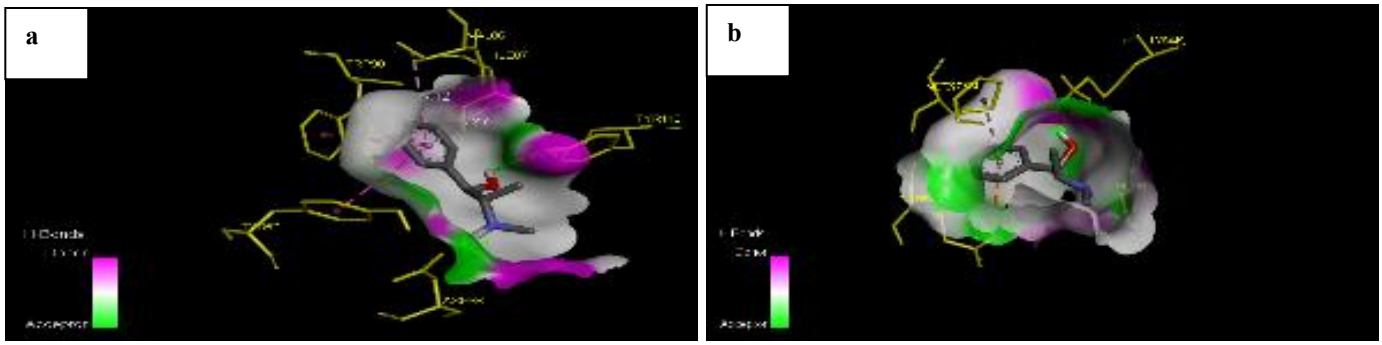



Figure7. Hydrogen bond donor and acceptor in (a) 4drh with ephedrine (b) 5w9c with ephedrine.

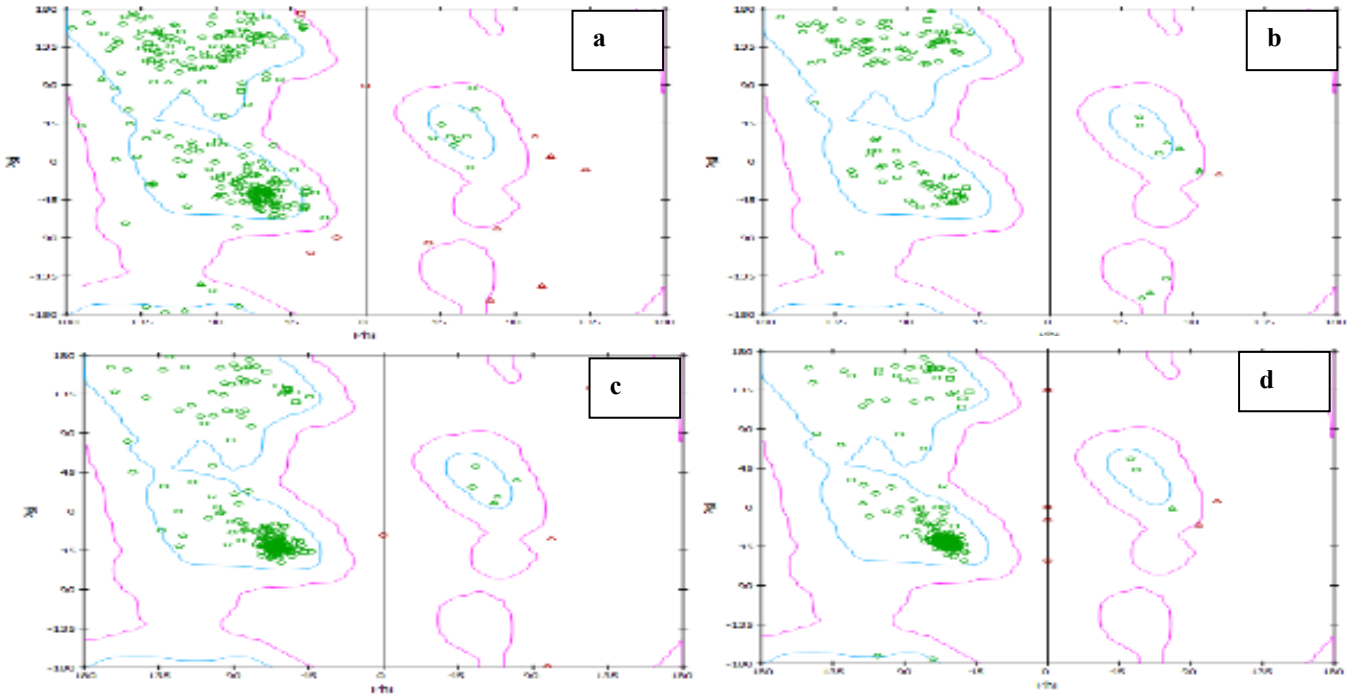



Figure 8. Ramachandran plots of proteins structures showing phi (ϕ), psi (ψ) torsional angles for (a) 2j6m (b) 4drh (c) 4oar (d) 5w9c Inside Outside Hard-Sphere Overlap

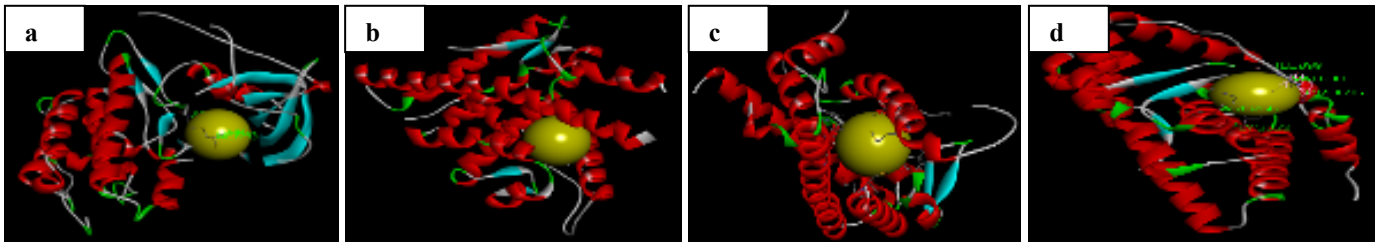



Figure 9. Artemisin docked with (a) 2j6m (b) 4oar (c) 5w9c and harmaline with (d) 4oar.

Table 4. Interactions of ligand with targeted BC proteins

| Biomolecule | PDB ID | Interacting compound | Nature of interaction | Structures Involved in interaction | Aminoacids on active site | Bond distances Angstrom (A°) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lowest | Highest |
| EGFR | 2j6m | Artemisin | Conventional H-bond, Unfavourable acceptor-acceptor | Coil, | MET793, ASP855 | 2.15 | 2.9 |
| | | Ephedrine | Conventional H-bond, Unfavourable donor-donor, Pi-Catio, Pi-Anion, Pi-Alkyl | Coil, Sheet, Helix, | ASP770, ARG776, LYS852, ALA1013, ASP1014 | 1.33 | 4.64 |
| | | Harmaline | Conventional H-bond, Pi-Sigma, Alkyl, Pi-Alkyl | Sheet, Coil | LEU718, VAL726, ALA743, LYS745, LEU792, MET793, LEU844 | 2.35 | 5.28 |
| mTOR | 4drh | Artemisin | Conventional H-bond, Pi-Sigma | Sheet, Helix | TYR57, PHE77, TRP90 | 2.09 | 3.99 |
| | | Ephedrine | Conventional H-bond, Pi-Pi stacked, Pi-Pi T-shaped, Pi-Alkyl | Sheet, Coil, Helix, Turn | TYR57, ASP68, VAL86, ILE87, TRP90, TYR113 | 1.94 | 5.37 |
| | | Harmaline | Conventional H-bond, Pi-Anion, Alkyl, Pi-Alkyl | Sheet, Coil, Turn | ASP68, PHE77, SER118, LYS121, ILE122 | 2.34 | 5.49 |
| PR | 4oar | Artemisin | Conventional H-bond | Helix | LEU718 | 2.91 | |
| | | Ephedrine | Conventional H-bond, Pi-Pi stacked, Pi-Alkyl | Turn, Sheet, Helix, Coil | TYR753, THR829, HIS881, LYS919, VAL925 | 1.85 | 5.13 |
| | | Harmaline | Pi-Cation, Alkyl, Pi-Alkyl | Coil, Helix | PRO696, VAL698, ILE699, ARG766, LYS822 | 3.66 | 5.3 |
| ER | 5w9c | Artemisin | Van der Waals | Sheet, Coil, Helix, Turn | LEU525, 384, 387, 391, 428, 346, THR347, MET343, 388, 421, TRP383, ALA350, GLU353, ARG394, | -- | -- |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | PHE404, GLY521, ILE424 | | |
| | | Ephedrine | Conventional H-bond, Unfavorable donor-donor, Pi-Anion, Pi-Alkyl | Coil, Helix | PRO324, GLU353, MET357, GLY390, LYS449 | 1.45 | 5.5 |
| | | Harmaline | Conventional H-bond, Pi-Sigma, Alkyl, Pi-Alkyl | Helix, Coil | LEU346, LEU349, ALA350, LEU387, PHE404, LEU525, VAL533 | 2.49 | 5.29 |

Conventional H-bonding and Pi-bonding were immensely displayed (Table 4) in contrast to other bonding types like Van der Waals interactions. Polar H-bonding and hydrophobic interactions were extensive between BC proteins and furanocoumarins (Achrya et al., 2019). Active amino acids were members of helix, turn, coil, sheet. Minimum bond distance was 1.33 A$^{o}$ for ephedrine with 2j6m and 5.5 A$^{o}$ measured for ephedrine with 5w9c.

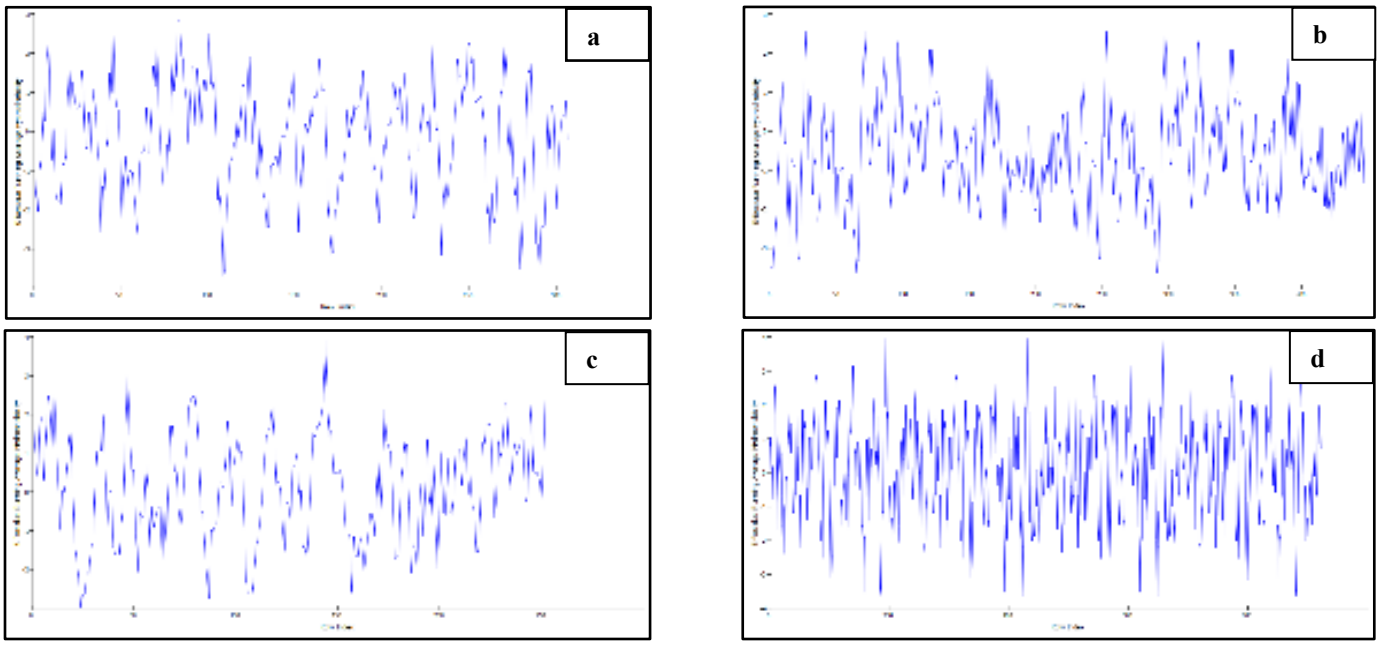



Figure 10. Hydrophobicity plots for (a) 2j6m (b) 4drh (c) 4oar (d) 5w9c.

Docking of artemisin with 5w9c calculated 17 (highest number) amino acids residues interacted with ligand in active site expressing efficient bonding in terms of amino acid residues (Ansari et al., 2021) whereas only single amino acid was involved in bonding of artemisin with 4oar with highest binding affinity of -7.8 kcal/mol. Resibufogenin from *Bufo bufonis* suppresses BC angiogenesis via blocking specific signalling pathway interacting through hydrophobic interaction with seven amino acid residues (Leu840, Leu1035, Leu889, Val899, Val848, Val914 and Ala866) (Figure 10). Another interaction was H-pi between Lys868 residues and resibufogenin (Yang et al., 2021).

In drug discovery multitargeted ligands are of high importance, because one drug targeting many BC proteins will reduce the requirement of different drug for each type. The studied molecules docked with all four BC proteins. These compounds have been reported for having potential against BC proteins. From Artemisia species, artemisinin and its derivatives have been proven having potential against cancer cell lines, tested on the ethnobotanical knowledge of using Artemisia as infusion. It expressed cytotoxic effect against MCF7 BC cell lines, which produce estrogen

dependent ductal carcinoma (Suberu et al., 2014). In clinical trials artemisinin derivatives expressed broad spectrum antitumor activities (Xu et al., 2020). Ephedrine inhibited the growth of SKBR3 tumor cell lines at Go/G1 phase, even up to 500 µg/ml ephedrine resulted in death of SKBR3 cells in human BC cell lines (Chen et al., 2016). Harmaline a major alkaloid of Peganum was found to have inhibitory effect against MDA_MB_231, Mcf-7. Depending on the dose, harmaline decrease the cell viability resulting in the death of cancer cell lines (Tehrani et al., 2014). Utilizing high-throughput and network pharmacology techniques CPT also found to be multitargeted drug targeting ER-positive BRCA by regulating different pathways (Li et al., 2021). .

## CONCLUSIONS

It can be concluded that all three studied natural compounds possess potential properties against BC proteins. These compounds are used by different tribal communities to treat BC, so they can be optimized for producing highly effective drugs treating BC. Plants based natural drugs against cancer are the topmost and urgent demand in treating cancer. Artemisin, harmaline and ephedrine possessed commending drug likeness profile having high binding affinities and are multitargeted compounds. Only few natural drugs like taxnes, paclitaxel, tamoxifen and rapamycin are available in the market. In the first step the ethnobotanical knowledge of treating breast cancer need to be analysed and then tested clinically on cell lines. Then bioinformatics studies in second step to find the best natural compound of suggested plants. Finally, the application of best docked molecules invivo on lab animals.

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## AUTHOR CONTRIBUTIONS

Mushtaq Ahmad and Muhammad Zafar conceived idea, Bibi Sadia and Wajia Noor molecular docking and writeup, Aroosa Habib and Iqra Qayyum, Ayesha reviewed the plants against BC.

## COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

## REFERENCES

Kadioglu, O., Saeed, M., Kuete, V., et al. Oridonin targets multiple drug-resistant tumor cells as determined by in silico and in vitro analyses. *Front. Pharmacol.* 9, 355

Cokkinides, V., Albano, J., Samuels, A., et al. American cancer society: Cancer facts and figures. *Am. Cancer Soc.* Atlanta

Cheng, Z., Li, M., Dey, R., et al. Nanomaterials for cancer therapy: current progress and perspectives. *J. Hematol. Oncol.* 14(1), 85

Zhao, W., Li, X., Wang, W., et al. Association of Preoperative Serum Levels of CEA and CA15-3 With Molecular Subtypes of Breast Cancer. *Dis. Markers.* 2021(1), 5529106

Lin, L., Li, Z., Yan, L., et al. Global, regional, and national cancer incidence and death for 29 cancer groups in 2019 and trends analysis of the global cancer burden, 1990–2019. *J. Hematol. Oncol.* 14(1), 197

Jemal, A., Bray, F., Center, M. M., et al. Global cancer statistics. *CA Cancer J. Clin.* 61(2), 69–90

Portt, L., Norman, G., Clapp, C., et al. Anti-apoptosis and cell survival: a review. *Biochim. Biophys. Acta Mol. Cell Res.* 1813(1), 238–259

Golshan, A., Amini, E., Emami, S. A., et al. Cytotoxic evaluation of different fractions of Salvia chorassanica Bunge on MCF-7 and DU 145 cell lines. *Res. Pharm. Sci.* 11(1), 73–80

Razi, M. T., Saadullah, M., Murtaza, G., et al. Determination of the anti-diabetic effect of methanolic extract of Sphaeranthus indicus L. on alloxan induced diabetic rabbits. *Lat. Am. J. Pharm.* 30

Alsaraf, K. M., Mohamed, M. H., Al-Shammari, A. M., et al. Broad-spectrum cytotoxic effect of calendula officinalis L against breast cancer cells. *Indian J. Forensic Med. Toxicol.* 14(1), 803–809

Islam, M. S., Kusumoto, Y., Al-Mamun, M. A. Cytotoxicity and cancer (HeLa) cell killing efficacy of aqueous garlic (Allium sativum) extract. *J. Sci. Res.* 3(2), 375–382

Karmakar, S., Roy Choudhury, S., Banik, N. L., et al. Molecular mechanisms of anti-cancer action of garlic compounds in neuroblastoma. *Anti-Cancer Agents Med. Chem.* 11(4), 398–407

Abhilash, N., Ajay, S. K., Ramesh, K. V., et al. Lasiosiphon glaucus a potent ethnobotanical medicinal plant against breast cancer targeting multiple pathways: an invitro study. *Adv. Tradit. Med.* 23(2), 557–564

Patel, A., Soni, A., Siddiqi, N. J., et al. An insight into the anticancer mechanism of Tribulus terrestris extracts on human breast cancer cells. *3 Biotech.* 9(2), 58
Wang, X., Yuan, S., Wang, J., et al. Anticancer activity of litchi fruit pericarp extract against human breast cancer in vitro and in vivo. *Toxicol. Appl. Pharmacol.* 215(2), 168–178
Zhang, S., Won, Y. K., Ong, C. N., et al. Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. *Curr. Med. Chem. Anti-Cancer Agents.* 5(3), 239–249
Noolu, B., Gogulothu, R., Bhat, M., et al. In vivo inhibition of proteasome activity and tumour growth by Murraya koenigii leaf extract in breast cancer xenografts and by its active flavonoids in breast cancer cells. *Anti-Cancer Agents Med. Chem.* 16(12), 1605–1614
Herrero de la Parte, B., Rodeño-Casado, M., Iturrizaga Correcher, S., et al. Curcumin reduces colorectal cancer cell proliferation and migration and slows in vivo growth of liver metastases in rats. *Biomedicines.* 9(9), 1183
Lee, J., Cho, K. Flaxseed sprouts induce apoptosis and inhibit growth in MCF-7 and MDA-MB-231 human breast cancer cells. *In Vitro Cell. Dev. Biol. Anim.* 48(4), 244–250
Monteiro, R., Calhau, C., Silva, A. O. E., et al. Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J. Cell. Biochem.* 104(5), 1699–1707
Yoshimaru, T., Komatsu, M., Tashiro, E., et al. Xanthohumol suppresses oestrogen-signalling in breast cancer through the inhibition of BIG3-PHB2 interactions. *Sci. Rep.* 4(1), 7355
Truan, J. S., Chen, J. M., Thompson, L. U. Comparative effects of sesame seed lignan and flaxseed lignan in reducing the growth of human breast tumors (MCF-7) at high levels of circulating estrogen in athymic mice. *Nutr. Cancer.* 64(1), 65–71
Ahmad, P., Jaleel, C. A., Salem, M. A., et al. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotechnol.* 30(3), 161–175
Rajput, S., Kumar, B. P., Dey, K. K., et al. Molecular targeting of Akt by thymoquinone promotes G1 arrest through translation inhibition of cyclin D1 and induces apoptosis in breast cancer cells. *Life Sci.* 93(21), 783–790
Zhou, S. F., Zhong, W. Z. Drug design and discovery: principles and applications. *Molecules.* 22(2), 279
Cragg, G. M., Pezzuto, J. M. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Med. Princ. Pract.* 25(Suppl. 2), 41–59
Suffness, M. Taxol: science and applications. *CRC Press.*
Gordaliza, M. Natural products as leads to anticancer drugs. *Clin. Transl. Oncol.* 9(12), 767–776
Almomen, A. Working towards an optimized therapy for abnormal endometrial proliferation: Treatment approaches and disease modeling. *The University of Utah.* 2016
Idris, M. O., Adeniji, S. E., Habib, K., et al. Molecular docking of some novel quinoline derivatives as potent inhibitors of human breast cancer cell line. *Lab-in-Silico.* 2(1), 30–37
Firdous, A. B., Sharmila, G., Balakrishnan, S., et al. Quercetin, a natural dietary flavonoid, acts as a chemopreventive agent against prostate cancer in an in vivo model by inhibiting the EGFR signaling pathway. *Food Funct.* 5(10), 2632–2645
Acharya, R., Chacko, S., Bose, P., et al. Structure based multitargeted molecular docking analysis of selected furanocoumarins against breast cancer. *Sci. Rep.* 9(1), 15743
Saravanan, R., Raja, K., Shanthi, D. GC–MS Analysis, molecular docking and pharmacokinetic properties of phytocompounds from Solanum torvum unripe fruits and its effect on breast cancer target protein. *Appl. Biochem. Biotechnol.* 194(1), 529–555
Yadav, D. K., Kumar, S., Saloni, H. S., et al. Molecular docking, QSAR and ADMET studies of withanolide analogs against breast cancer. *Drug Des. Dev. Ther.* 1859–1870
Pick, E., Kluger, Y., Giltnane, J. M., et al. High HSP90 expression is associated with decreased survival in breast cancer. *Cancer Res.* 67(7), 2932–2937
Saravanan, R., Balasubramanian, V., Balamurugan, S. S., et al. Zinc transporter LIV1: A promising cell surface target for triple negative breast cancer. *J. Cell. Physiol.* 237(11), 4132–4156
Supramaniam, G., Elengoe, A. In Silico Molecular Docking of Glycyrrhizin and Breast Cancer Cell Line Proteins. In *Plant-derived Bioactives*, 575–589. Springer, Singapore, 2020
Acharya, P., Ramana, M. M., Korgavkar, N., et al. Synthesis, Docking Studies, and Biological Evaluation of Betti Bases as Promising Anti-tubercular Agents. *Lett. Drug Des. Discov.* 20(6), 724–737
Mathew, A. Insilico Docking Studies on Anticancer Drugs for Breast Cancer. In *2009 Int. Assoc. Comput. Sci. Inf. Technol.-Spring Conf.*, 567–570. IEEE, 2009
Suganya, J., Radha, M., Naorem, D. L., et al. In silico docking studies of selected flavonoids-natural healing agents against breast cancer. *Asian Pac. J. Cancer Prev.* 15(19), 8155–8159
Rautray, S., Panikar, S., Amutha, T., et al. Anticancer activity of Adiantum capillus veneris and Pteris quadriureta L. in human breast cancer cell lines. *Mol. Biol. Rep.* 45(6), 1897–1911
Liu, Z., Li, Y., Han, L., et al. PDB-wide collection of binding data: current status of the PDBbind database. *Bioinformatics.* 31(3), 405–412

Lai, H., Singh, N. P. Oral artemisinin prevents and delays the development of 7,12-dimethylbenz [a] anthracene (DMBA)-induced breast cancer in the rat. *Cancer Lett.* 231(1), 43–48

Shabani, S. H. S., Tehrani, S. S. H., Rabiei, Z., et al. Peganum harmala L.'s anti-growth effect on a breast cancer cell line. *Biotechnol. Rep.* 8, 138

Chen, D., Ma, F., Liu, X. H., et al. Anti-tumor effects of ephedrine and Anisodamine on Skbr3 human breast Cancer cell line. *Afr. J. Tradit. Complement. Altern. Med.* 13(1), 25–32

Tang, C., Zhao, C. C., Yi, H., et al. Traditional Tibetan medicine in cancer therapy by targeting apoptosis pathways. *Front. Pharmacol.* 11, 976

Xu, C., Zhang, H., Mu, L., et al. Artemisinins as anticancer drugs: Novel therapeutic approaches, molecular mechanisms, and clinical trials. *Front. Pharmacol.* 11, 529881

Kadioglu, O., Law, B. Y., Mok, S. W., et al. Mode of action analyses of neferine, a bisbenzylisoquinoline alkaloid of lotus (Nelumbo nucifera) against multidrug-resistant tumor cells. *Front. Pharmacol.* 8, 238

Yi, F., Sun, L., Xu, L. J., et al. In silico approach for anti-thrombosis drug discovery: P2Y1R structure-based TCMs screening. *Front. Pharmacol.* 7, 531

Abdullahi, S. H., Uzairu, A., Shallangwa, G. A., et al. In-silico activity prediction, structure-based drug design, molecular docking and pharmacokinetic studies of selected quinazoline derivatives for their antiproliferative activity against triple negative breast cancer (MDA-MB231) cell line. *Bull. Natl. Res. Cent.* 46(1), 1–2

Bhadra, P. Green Chiretta (Andrographispaniculata): In silico Analysis of Therapy for Breast Cancer. *Indian J. Nat. Sci.* 10(60), 20645–20652

Ravnik, Z., Muthiah, I., Dhanaraj, P. Computational studies on bacterial secondary metabolites against breast cancer. *J. Biomol. Struct. Dyn.* 39(18), 7056–7064

Ansari, M. N., Saeedan, A. S., Kaithwas, G., et al. Archana Bharti Sonkar1, Abhishek Verma1, Sneha Yadav1, Rohit Kumar1, Jyoti Singh1, Amit K. Keshari2, Soniya Rani1, Anurag Kumar1, Dharmendra Kumar1, Neeraj Kumar Shrivastava1, Shubham Rastogi1, Mariam K. Alamoudi3. 2024

Saravanan, D., Rafi, S. M., Mohan, M. Identification of novel Bioactivities from Bee venom to target TNF‑α for cancer therapy. *Arch. Clin. Toxicol.* 5(1), 22–24

Suberu, J. O., Romero-Canelón, I., Sullivan, N., et al. Comparative cytotoxicity of artemisinin and cisplatin and their interactions with chlorogenic acids in MCF7 breast cancer cells. *ChemMedChem.* 9(12), 2791–2797

Seyed Hassan Tehrani, S., Hashemi Sheikh Shabani, S., Tahmasebi Enferadi, S., et al. Growth inhibitory impact of Peganum harmala L. on two breast cancer cell lines. *Iran. J. Biotechnol.* 12(1), 8–14