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In Silico And Molecular Docking Prediction Studies Elucidate Anti-Breast Cancer Activity Of Lycopene And Gallic Acid

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Abstract

Breast cancer holds for the maximum mortality rate in women. Though the current treatment and chemotherapies are killing cancer cells and also causing severe side effects in normal cells. Overcome the current chemotherapeutic challenges, plants derived nutraceuticals contain strong anticancer activity and less toxicity. Gallic acid and lycopene were one of the chemopreventive compounds with anticancer activity in breast cancer cells. This study analyzes the molecular docking of lycopene and gallic acid complex with breast cancer target protein and finds their binding score. The three-dimensional structure of proteins is retrieved from protein data bank and construction of complex structure with the use of molecular modeling ChemSketch. The molecular docking studies were done with the help of Autodock vina. The target protein PARP shown higher binding energy (-7.4 Kcal/mol) and the least binding energy found in BRCA1 (-4.1 Kcal/mol). Alone and in a combination of lycopene with gallic acid complex are potential to target breast cancer proteins through molecular docking approach. Molecular docking studied shows the PARP was maximum binding energy for the combination of lycopene and gallic acid complex.

Keywords: Breast cancer, Lycopene, Gallic acid, ChemSketch, Molecular docking

1. Introduction

Breast cancer is one of the foremost common female cancers in India. Early detection of breast cancer is often treated and chances of survival are high in such cases. The aggressive nature of the primary stage in breast cancer may cause a severe problem in further progression. Nowadays, the available breast cancer drugs are being targeted based on their receptor of the cell surface, angiogenesis blockade, histone deacetylase inhibitors, and other inhibitor's survival pathway [1]. Furthermore, the present therapy of carcinoma includes surgery, chemotherapy, radiotherapy, hormone therapy, and targeted therapy; these present therapies have harmful side effects like liver, kidney, heart failure, and mutation to normal healthy cells [2]. Therefore, there is a need for developing a new drug that overcomes the above side effects. Similarly, the huge number of available active agents remains a serious concern. This raises concern to identify the importance of novel cytotoxic agents which act as a modulator for breast cancer.

So, plant-derived compounds can be a new candidate for developing effective anticancer agents [3]. Lycopene is the red-hued pigmented plant supplement that acts as a solid cancer prevention agent and has been put in the spotlight by overall population and clinical examination [4]. The majority of the functional compounds in lycopene have been shown to disrupt the cell cycle in human breast cancer cell lines at various stages during their progression and also induce apoptosis through the inherent pathways [5].

Gallic acid is the major component of natural products plays an important role in the prevention of breast cancer development via apoptosis. The logical examination shows the molecular mechanisms of signaling pathways that have been explored in gallic acid were broadly critical in the current medical applications. Gallic acid induces apoptosis through mitochondria membrane permeabilization and caspase activation [6, 7].

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Cancer-targeted therapies play a major role in medicinal compound-based drugs which inhibit the growth of cancer cells by changing the fictional characterization. Breast cancer cells can overexpress specific receptors which, induce the expression of a gene in different cell cycle pathways [8]. The combination of gallic acid and lycopene complex for breast cancer therapy has not yet been studied and this is the first attempt to analyze their interaction in some breast cancer association proteins. In computational biology, tools are used to design ligand-based study and their interaction between the medicinal compounds in breast cancer proteins. These computer-based strategies provide a rapid efficient investigation of therapeutic drugs and breast cancer target proteins. The present study aims to developed three-dimensional (3D)structures of breast cancer proteins, physicochemical characterization, and disulfide bonds analysis, i.e. BRAC1, AKT, PARP, and PDK. Then evaluate their binding affinities with Lycopene, gallic acid, and gallic acid lycopene complex through molecular docking.

2. Materials and Experimental Methodology

2.1Retrieve the structure and sequence

The complete amino acid sequences were obtained from the National Centre of Biotechnology Information (NCBI). The database of protein data bank (PDB) is used to retrieve the 3D structure of the proteins and is also helpful to study three-dimensional structural data of large biological molecules.

2.2 Physiochemical characterization of proteins

The physical and chemical parameters of the protein were analyzed by using the ProtParam tool which allows computing various physicochemical properties of the given protein sequence. The protein sequences were uploaded in FASTA format in ProtParam and the results were obtained. The amino acid length, molecular weight, pI, negative and positive charged residues, extinction coefficient, instability and aliphatic index, and grand average of hydropathicity (GRAVY) of the proteins have been identified.

2.3 Analysis of disulfide bonds in proteins

Disulfide bonds in the target proteins are analyzed through the Cys_Rec program since the tridimensional structure of the protein is stabilized by cysteine.

2.4 Retrieval of Lycopene and Gallic acid structure

The structure of Lycopene and Gallic acid was retrieved from PubChem which was an interlinked database for chemical compounds and their mechanisms against biological discoveries. It is an open chemistry database containing nucleotides, carbohydrates, lipids, peptides, and chemically modified macromolecules.

2.5 Lycopene - Gallic acid complex

A complex structure of gallic acid and lycopene was developed in ChemSketch. ACD/ChemSketch is a molecular modeling program used to create and modify images of chemical structures. The structure of gallic acid, lycopene, and complex was demonstrated in Fig 1.1

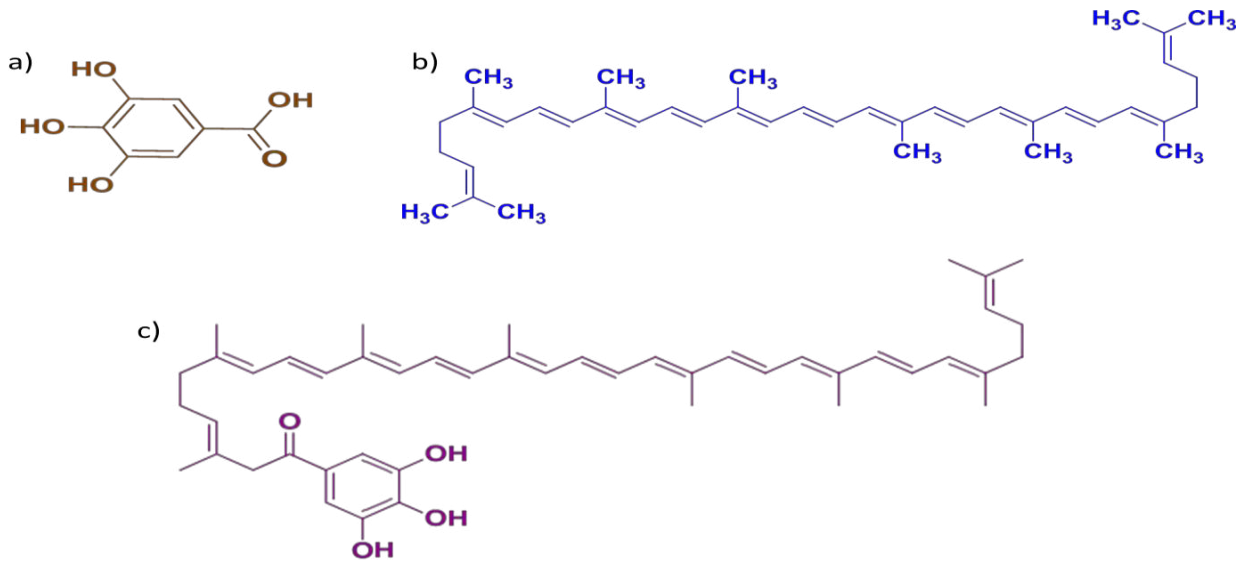

Figure 1.1 Structure of gallic acid (a), lycopene (b), and complex structure of gallic acid lycopene (c)

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2.6 Molecular docking and Visualization

Docking is a method that forms the stable complex with the preferred orientation of one molecule with another molecule. Docking was done for breast cancer target proteins with lycopene, gallic acid, and complex of lycopene with gallic acid in AutoDock vina. Stimulation of small and macromolecule systems and docking results were visualized in Discovery studio.

3. Results and discussion

3.1 Physiochemical characterization of proteins

The ProtParam tool of Ex-Pasywas used to analyze the physicochemical properties of the breast cancer target proteins. The FASTA format sequence used to analyze the number of amino acids, molecular weight, theoretical pI, negatively charged residues, positively charged residues, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of the proteins has been identified and the results are given in Table 1.1.

Protein	Length	Molecular Weight	pI	-R	+R	Extinction Coefficient	Instability Index	Aliphatic Index	GRAVY
BRCA1	1863	207720.85	5.29	283	213	101.190	54.68	69.01	-0.785
AKT	480	55716.44	5.75	77	66	65.320	36.04	71.69	-0.576
PDK	413	46626.23	6.67	46	44	42.290	45.72	84.53	-0.289
PARP	1014	113083.79	8.99	137	159	119.180	37.22	79.13	-0.536

Table 1.1 Physiochemical parameters of proteins in ProtParam

The isoelectric point (pI) value of BRAC1, AKT, and PDK was less than 7 which strongly indicates the acidic characteristics of the proteins, while the pI value of PARP is near 9 because of the phosphorylation states. In addition, the particular wavelength of the light extension was calculated using the extinction coefficient. The wavelength ranges are 101.190, 65.320, 42.290, and 119.180 M/cm for BRCA1, AKT, PDK, and PARP respectively. Furthermore, the negative (ASP+GLU) and positive (ARG+LYS) charged residues numbers are denoted as –R and +R. There was a total of 283 negatively charged and 213 positively charged residues for BRCA1, then 77 negatively charged and 66 positively charged residues for AKT. There are 46 negatively charged and 44 positively charged residues for PDK, while, 137 negatively charged and 159 positively charged residues in the protein sequence of PARP. Moreover, the negative charge of the very low grand average of hydropathicity (GRAVY) index confirms the hydrophilic nature of proteins. Therefore, all proteins have the same stability [9]

3.2 Analysis of disulfide bonds in proteins

The TheCys_Rec program was used to analyze the disulfide bonds in the cysteine stabilized tridimensional structure of target cancer proteins. Cys_Rec program is used for predicting S-S bonding states of cysteines and for locating disulfide bridges. The Cys_Rec analysis data of target proteins are demonstrated in table 1.2

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Protein	Cys_Rec	Score	Protein	Cys_Rec	Score
AKT	60	18.5	PDK	22	18.8
	77	31.6		28	0.3
	224	15.6		60	26.1
	296	19.1		163	7.6
	310	34.8		603	2
	344	22.5		686	18.3
	460	14.3		688	18.1
				695	3.9
Protein	**Cys_Rec**	**Score**	**Protein**	**Cys_Rec**	**Score**
PARP	21	5.2	BRCA1	39	44.2
	24	7.1		44	41.6
	56	23.8		61	23.5
	125	9.4		91	12.5
	128	12.9		197	19.7
	162	14.9		328	11.6
	256	22.9		903	2.7
	295	23.2		953	4.3
	298	13.8		1291	8.7
	311	3.8		1372	3.4
	321	13.2		1382	2.6
	429	24		1511	3.7
	845	19.9		1697	4.5
	908	34.4		1847	6.3

Table 1.2 Disulphide bonds in the target proteins by using Cys_Rec

The programing tool CYS_REC was used to investigate the positions of the cysteine and analyze the S-S bond pattern target protein sequence. The tool CYS_REC helps to recognize the total number of residues in the target proteins of AKT, BRCA1, PDK, and PARP. Relatively, the CYS-REC tool performing the prediction of S-S bonding states of cysteines was also reported [10].

3.3 Molecular Docking of gallic acid, lycopene, and gallic acid lycopene complex with target proteins

Further, the gallic acid, lycopene, and gallic acid lycopene complex have been analyzed as the proteins involved in breast cancer. The overexpression of gene BRAC1 is involved in the huge risk of causing breast cancer and many risk factors modify the regulation of BRCA mutation. This attention in BRCA1 genes indicates the specific function of breast tissues and is also helpful to examine the detailed functions. So, the role of BRCA1 role in the breast cancer cell can inhibit the cell cycle and DNA repair mechanisms are hugely correspond with their anti-oncogene activities [11]

The protein kinase B (PKB), plays a key role in signaling downstream of growth factors, regulates the cellular functions including proliferation and survival. Akt isoforms in cellular phenotypes commonly deregulated in malignancies, especially cell migration and metastatic dissemination were identified in cancer [12]. In the breast cancer therapies and inhibitors pathways, the phosphorylation of Akt play a major role in cell growth, proliferation, motility, and survival in both normal and tumor cells which were involved in the enhancement of epithelial-mesenchymal transitions and angiogenesis during tumorigenesis [13, 14].

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Phosphoinositide subordinate protein kinase 1 (PDK1) might be a vital modifier of ribociclib affectability in estrogen receptor-positive MCF-7 carcinoma cells. Pharmacologic restraint of PDK1 synergistically hindered multiplication and expanded apoptosis during a board of ER-positive carcinoma cell lines. PDK1 has been embroiled in significant cell measures including endurance, digestion, and tumorigenesis. PDK1 has highly expressed in many human neoplastic cell lines and breast tumors, suggesting a task for PDK1 in cancer progression [15]. In breast cancer, the kinases signaling pathways activation contributes to varied malignant which suggest that activation of those kinase pathways by phosphorylation may partially account for the molecular pathogenesis of human breast carcinoma, particularly moderate to the highlevelofPDK-1phosphorylationwasfoundin86%ofhigh-grade metastasized breast tumors [16].

The computational tool AutoDock vina helps to analyze the molecular docking breast cancer target protein with gallic acid and lycopene. The binding affinity of the breast cancer target proteins was obtained and the protein-ligand binding interactions were visualized in Discovery studio.Among the4 proteins,BRAC1hasa maximum binding affinity with -6.3Kcal/Mol andstronglybindswithgallicacidandAKT hashigherbinding scores with -7.9Kcal/Moland effectivelybindswithlycopene.Thedockingresultsaregiven in Fig 1.1 and table5.3.

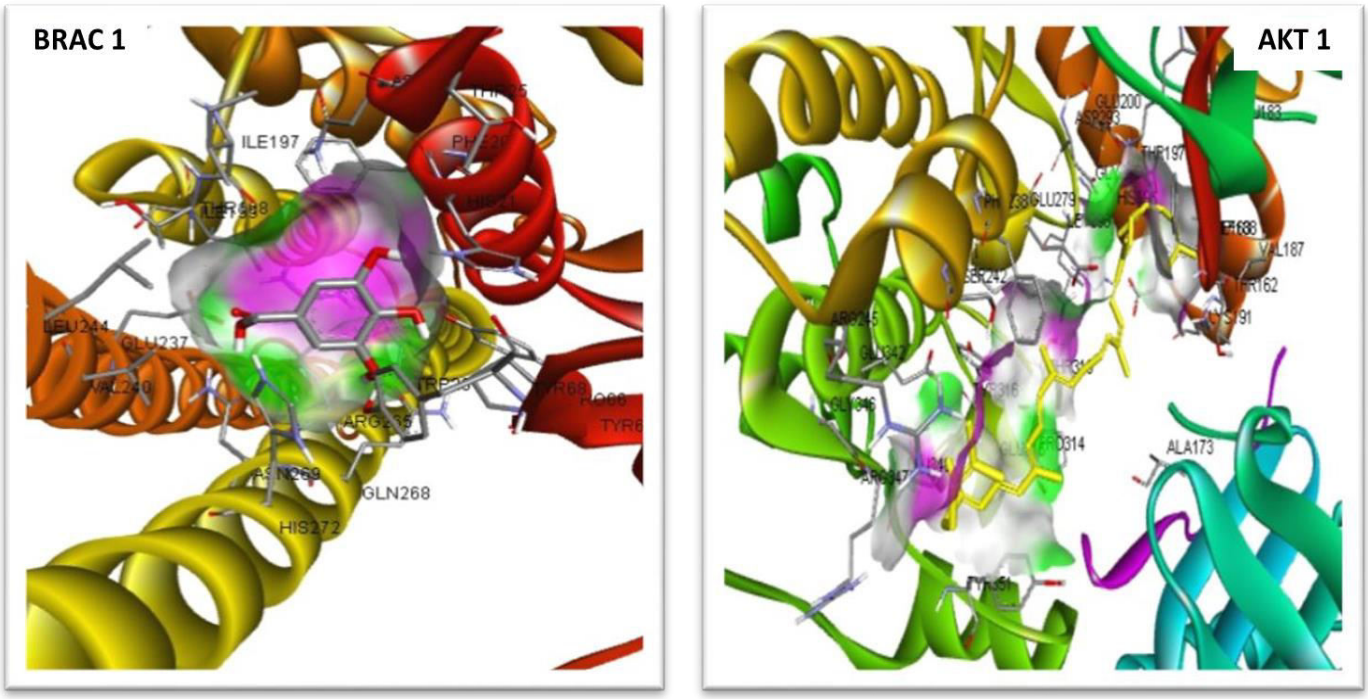

Figure 1.2 Binding of gallic acid inBRCA1 and lycopene in AKT

The binding energy of breast cancer target proteins for lycopene and gallic acid		
Ligand	**Protein**	**Binding energy (Kcal/mol)**
Gallic Acid	BRCA1	-6.3
	AKT	-5.7
	PARP	-5.9
	PDK	-5.6
Lycopene	BRCA1	-3.7
	AKT	-7.9
	PARP	-6.3
	PDK	-7.0

Table 1.3 Binding affinity of target proteins for gallic acid and with lycopene

PARP-1 and PARP-2 are DNA damage sensors that are generally dynamic throughout the S-period of the phonecycle and have a wider range of roles in DNA repair than previously thought. Active PARP monotherapy phase I clinical trial and II trials hint at a willingness to explore their use in the germline of the mutations to tumors with defects in homologous recombination repair [17]. PARP inhibitors are the primary cancer therapeutics designed to take advantage of synthetic lethality. PARP suppression can promote genomic

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instability, which can lead to reversion mutations, whichcanrestoreBRCA1/BRCA2function and PARP resistance [18].

In the gallic acid lycopene complex, among the 4 proteins, PARP shows maximum binding affinity with -7.3 Kcal/Mol. The results are given in Fig 1.3 and table1.4.

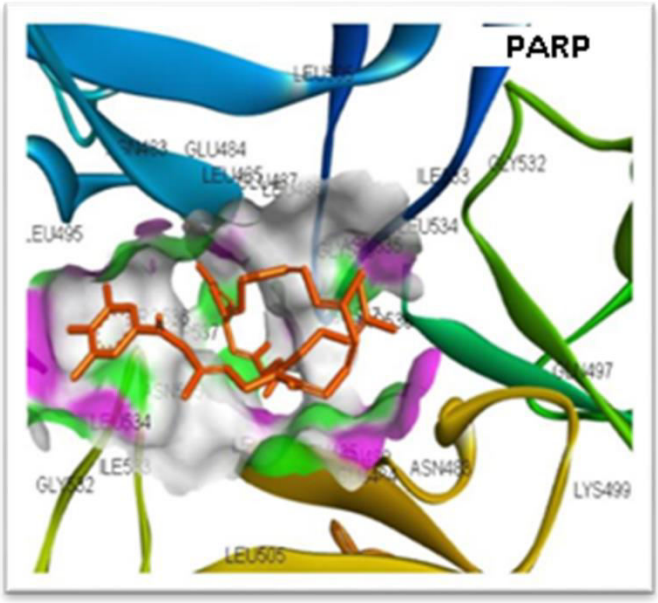

Figure 1.3 Binding of gallic acid lycopene complex inPARP

The binding energy of breast cancer target proteins for lycopene and gallic acid		
Ligand	**Protein**	**Binding energy (Kcal/mol)**
Gallic Acid + Lycopene complex	BRCA1	-4.1
	AKT	-7.3
	PARP	-7.4
	PDK	-6.4

Table 1.4 Binding affinity of target proteins for gallic acid lycopene complex

4. Conclusion

Breast cancer target proteins' properties are identified by using the protparam of Expasy's server. Disulfide bonds in the target proteins are analyzed with help of the Cys_Rec program since cysteine stabilizes the tridimensional structure of the protein. Molecular docking was done for 4 breast cancer target proteins with lycopene and gallic acid. Among them, BRCA1 has a higher binding energy of -6.3 Kcal/mol for gallic acid and AKT for lycopene with -7.9Kcal/Mol. Moreover, the docking was carried out with a complex ligand of lycopene and gallic acid on target proteins and among them, PARP shows higher binding energy of -7.4 Kcal/mol. Finally, PARP is the significant target for lycopene–gallic acid complex. Therefore, this complex will be explored in further research.

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