

In-silico analysis of bioactive compounds gingerol-10 and epigallocatechin 3 gallate: in breast cancer therapy

Green tea and ginger extracts in breast cancer therapy

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Abstract—Green tea and Ginger are used as a cure for many illnesses in human. Literary works have suggested that these phytochemicals Epigallocatechin 3 gallate (EGCG) in green tea and 10- gingerol have a vital role in reducing free radicals in the body as well as physiological role. Previous studies have shown that 10-gingerol has pharmacological functions in controlling breast cancer cell growth and development. EGCG has shown to intervene with estrogen receptor activity, suppress estrogen-induced breast cancer cell proliferation, and make hormone-responsive tumours more sensitive to steroid receptor-targeting drugs in breast cancer. This research aimed to study the effectiveness of the phytochemicals in the therapy of breast cancer using In Silico analysis. In this study, phytochemicals from green tea leaves were extracted using Soxhlet extraction with different solvents. Phytochemical analysis was performed to study the bio-active compounds. Pharmacokinetic properties of drugs were studied using PreADMET tool. Molecular Docking was performed using AutoDock in Linux environment to study the protein-ligand interactions. The molecular dynamics results were examined using RMSD, RMSF graphs while the binding affinity of the phytochemicals were analyzed using MMPBSA calculations. Green tea leaves extract showed the presence of glycosides, tannins, saponin, terpenoids, flavonoids while Ginger extract showed the presence of alkaloids, glycosides, phenol, saponin, terpenoids, flavonoids. The interaction between the protein and ligands were analyzed using docking score. The protein-ligand complex was further subjected to optimization by MD simulations using WebGROW server. Also, MD simulation trajectories was adopted as inputs for MMPBSA calculations of ligand binding free energies, and analysis of their binding process. The study interprets that combined treatment of EGCG and 10-gingerol can lead to potential anti-tumour effect on breast cancer cell lines, with a focus on their ability to modulate multiple signaling transduction pathways involved in cancer.

Index terms: *In-Silico, Molecular docking, Molecular dynamics, Breast cancer.*

I. INTRODUCTION

Breast cancer prevalence continues to increase after generations of experimental, etiological, and clinical trials. Breast cancer is now the most common malignancy source of disease risk for women, impacting one out of every 20 women around the world and one out of every eight in strong nations. Recent studies have suggested that risk of breast cancer can be reduced by adapting new medications, but it is yet to be challenging when used widely. If we develop a better understanding of breast cancer initiation and continue designing and researching novel flea preventative, we must remember the obstacles to acceptance and adherence to existing medications [1]. Breast cancer risk gene (BRCA1) mutations are closely related to familial breast and ovarian cancers. Female who acquires these mutations have a 60% increased risk of developing the disorder. Important intervals of risk during typical female growth are linked to the early onset of breast cancer. As reactive oxygen species are produced during the metabolic production of estrogen, these events associate with the formation of DNA lesions in mammary tissue [2].

Breast invasive ductal carcinoma (IDC) is a widespread breast cancer that is responsible for many cancer-related deaths among women around the world. The second most frequent histologic form of breast cancer, invasive lobular carcinoma, accounts for 5% to 15% of all invasive breast cancers. In a fibrous stroma, it is made up of non-cohesive cells that are distributed or arranged in a single-file sequential order. Lobular carcinoma in situ is often associated with invasive lobular carcinoma. Early breast cancer has a favourable prognosis. Both stage 0 and stage I have a 5-year survival rate of 100%. Stage II and stage III breast cancer have 5-year survival rates of around 93 percent and 72 percent, respectively. The disease's prognosis deteriorates dramatically as it progresses across the body. Just 22% of stage IV breast cancer patients will live for another five years [3-5].

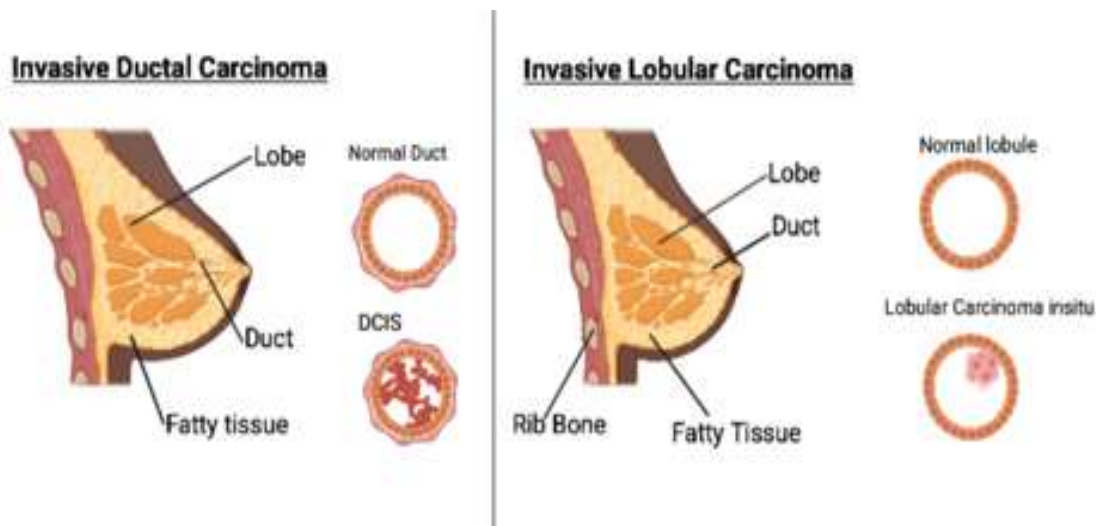


Figure 1: Diagrammatic representation of Invasive ductal and Invasive lobular carcinoma

II. RISK FACTORS, SIGNS, AND SYMPTOMS

Breast cancer is linked to several other well risk factors, the most well-known of which are age and female sex. A first-degree relative is (i.e., mother, sister, or daughter) family background is extremely important, particularly if the cancer is found before menopause. Including many second-degree relatives with breast cancer can raise the risk of breast cancer even more, although this risk has not been measured. It is worth noting that most breast cancer patients have no exposure to risk factors.

Genetically inherited breast cancer accounts for about 8% in all cases. Around half of these instances are linked to mutations in the BRCA1 and BRCA2 breast cancer susceptibility genes. Pre-menopausal women are most likely to have inherited breast cancer, which is more often bilateral than nonhereditary breast cancer. By impacting lifelong hormone levels, early menstruation and age at menopause can also raise the risk of breast cancer. Women who have a first pregnancy after the age of 30 or who have never been fertile are also at a higher risk. A rise in female sexual hormones seems to speed up cell division in breast tissue, increasing the chances of mutations [4].

One of most popular breast conditions about which people prefer health assistance is the presence of a breast mass. Benign lesions account for about 90% of all breast masses. In women, fibroadenoma is normally linked with smooth and spongy masses in the age between 20-30 years. One of the common symptoms is the bloody discharge, nipple pain and redness on the skin.

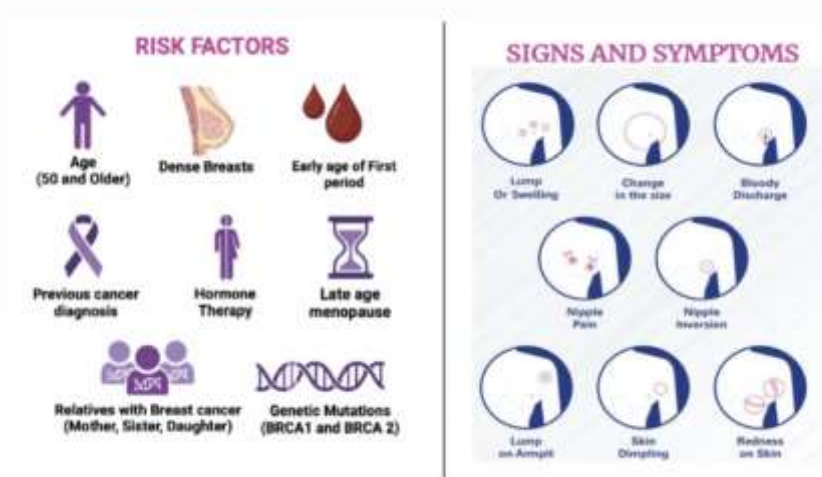


Figure 2: Representation of various risk factors, signs and symptoms involved in breast cancer

Green tea and Ginger are two well-known plant source having pathological and physiological benefits. The bioactive compounds present in these plants have role in the development of cancer therapeutics. In earlier research it has been suggested that green tea have anti-cancer properties and its consumption may decrease the risk of other chronic diseases also. Based on many clinical trials, it has been observed that these bioactive compounds have an efficacy towards the Breast cancer therapy [6,7]. There is a lot of evidence showing the health effects of green tea for several conditions, such as cancer, heart disease, liver disease, and so on.

Green tea can also help with diabetes, exercise, inflammatory bowel disease, skin conditions, hair loss, weight loss, and oxidative stress, among other things. It also protects Nervous System, Vascular System and even helps in oral disease treatment [8,9].

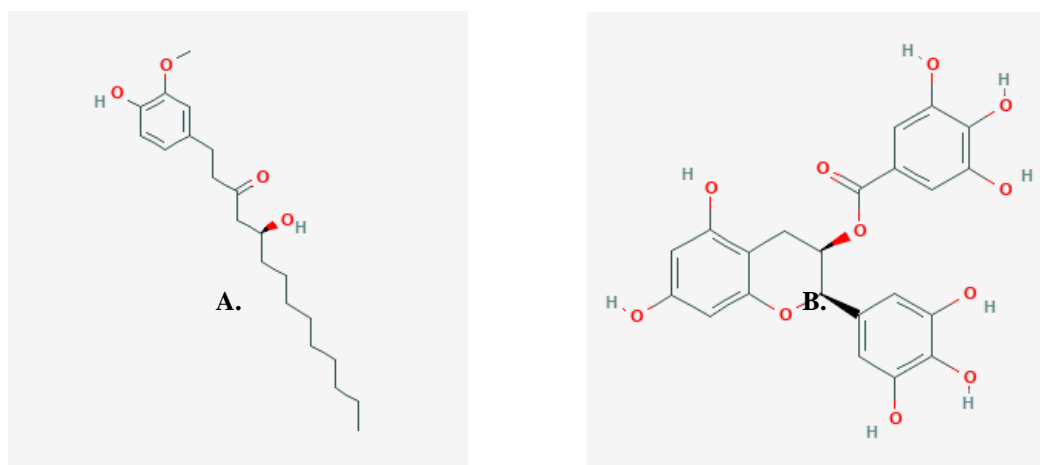


Figure 3: 2D structure of 10-gingerol and Epigallocatechin 3-gallate, A.10-gingerol, B. Epigallocatechin 3-gallate (EGCG).

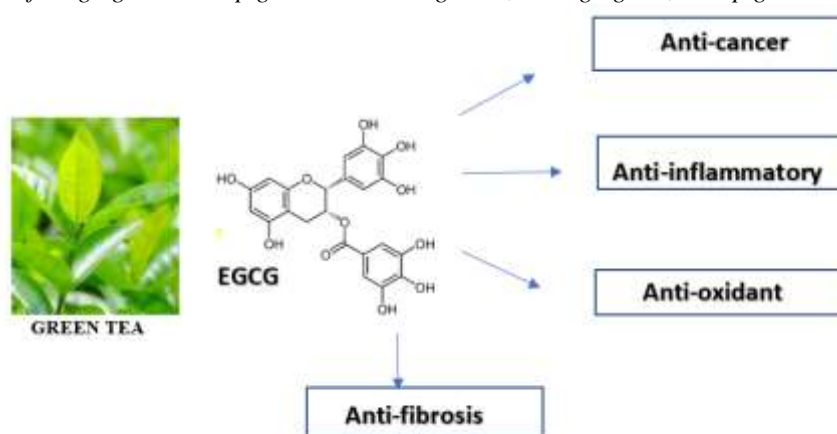


Figure 4: Benefits of Green tea

Ginger is also responsible for its pharmacological and health-promoting properties. There is various role of ginger in the improvement of diseases like degenerative diseases, digestive illness, cardiovascular disorders, vomiting, diabetes mellitus, and cancer. It also has anti-inflammatory and antioxidant effects that help to slow down the ageing process [10-14].

Fresh ginger comprises a variety of phytochemicals that have biological functions that are important to diseases caused by reactive oxygen species (ROS). Bio-active constituents like 6- shogaol, 10- gingerol, showed cytotoxic properties in cell lines [15].

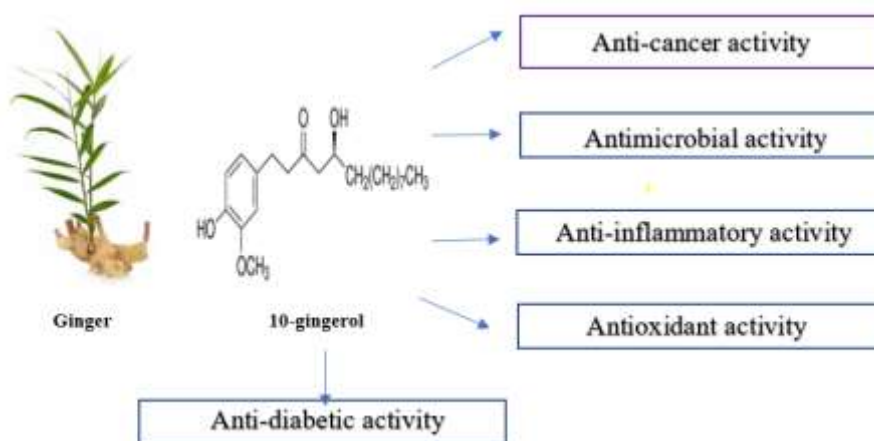


Figure 5: Benefits of Ginger

It has been found from epidemiological, animal, and in vitro studies that ginger and its bio-active compounds cause apoptosis and inhibit the proliferation in numerous types of cancer including breast cancer. Most of the in vivo studies have shown the efficacy of green tea and its bio-active compounds in causing apoptosis [5, 16-18]. When naturally available bio-active compounds are combined they can reduce the side effects as well as can give the better result than commercially available drugs. In this study, we had aimed the efficacy of phytochemical compounds present in ginger and green tea namely 10-gingerol and EGCG respectively as therapeutic bio-active compound in breast cancer using In Silico analysis.

An efficient strategy can be carried out by combining two naturally occurring bio-active constituents from the plant source. This can be useful for breast cancer as well as it will be effective against the side effects caused due to radiotherapy or chemotherapy. PreADMET is a web-based application which helps in predicting ADME data and produces predictions for Drug likeliness, Toxicity and ADME. This in silico analysis of compound is important to assess the behaviour of the compounds that can be used pharmaceutically.

BRCA 1 protein

Protein used for performing molecular docking and molecular dynamics is BRCA 1 protein RCSB PDB Id: 3K0K. The BRCA1 gene in humans produce a protein known as breast cancer type 1 susceptibility protein. BRCA1 is a human tumour suppressor gene that is involved in DNA repair. BRCA1 interacts with its binding partner, the BRCA1-associated Ring Domain protein (BARD1), in the nucleus to aid in the coordination of DNA modification repair. These functions are carried out by the BRCA1-BARD1 hetero-dimer interfering with other repair proteins, such as BRCA2, at affected DNA sites. BRCA1 functions as a tumour suppressor in this case, ensuring genome reliability. Inherited mutations in BRCA1 can result in functional defects in the protein, which can impair BRCA1's ability to suppress tumours. Although the molecular structure of the entire BRCA1 protein has yet to be ascertained, structural data for the N-terminal RING domain and the C-terminal (BRCT) region, which contain numerous clinical mutations, is accessible [2].

Molecular Docking and Molecular Dynamics

Molecular docking is a useful and capable technique. It is becoming increasingly relevant in the development of rational drugs. Docking is a computational technique for finding a suitable ligand that matches the protein's binding site energetically and geometrically. In other words, it is the analysis of how two or more molecules, such as a ligand and a protein, interact with one another. Docking is accomplished in two steps: first, sampling ligand conformations in the active site of the protein, and then rating these conformations using a scoring function. In an ideal world, sampling algorithms would be able to replicate the experimental binding mode, and the scoring function would give it the highest score out of all produced conformations [19]. Molecular dynamics simulation (MDs) is a valuable instrument for studying biomolecular processes in precise detail. MD and similar approaches are on the verge of becoming standard statistical instruments for drug development. Their primary benefit is that they directly address systemic stability and entropic consequences. As improved algorithms and hardware designs expand their use, this makes for a more precise approximation of the thermodynamics and kinetics involved with drug target identification and binding [20].

III. METHODS AND MATERIALS

Plant extract preparation

Soxhlet extraction: 30g of the green tea plant extract was weighed and placed in a Soxhlet apparatus. The extraction was carried out using 250 ml of the ethanol solvent. In case of ginger, the same amount of plant extract was weighed and placed into the apparatus. Here the process was carried out using solvent called acetone. After the process of 6 hours, the suspension was filtered and concentrated to dryness using rotary evaporator [21]. The dried extract was scrapped out using spatula and kept in an eppendorf's tube in the deep refrigerator for further experiments.



Figure 6: Ginger and Green tea extract obtained after Soxhlet extraction

Qualitative Analysis

Phytochemical Analysis: It is the process of screening and identification of bio-active constituents which are present in the plant source. Those bioactive constituents which can be derived from plant sources are alkaloids, carbohydrates, terpenoids, flavanoids, saponins, tannins, steroids and phenolic compounds.

Molecular Docking

Ligands: In this study the bio-active compounds present in a plant source, ie: 10-gingerol, Epigallocatechin 3-gallate (EGCG) were used as ligands and they were downloaded using PubChem. These ligands were prepared using Open Babel by adding hydrogen and converting the .sdf file into .pdbqt format.

PreADMET, a web-based technology that predicts the absorption, distribution, metabolism, and elimination/excretion (ADME) properties of compounds and builds a drug-like library using an in-silico method, was used to assess the toxicity of the bioactive compounds. This analysis was performed using sdf format of the ligands and after the submission process, results were obtained which has shown a good range of ADME, non-toxic and drug-likeness properties of the ligands.

Preparation of protein:

The protein BRCA 1 crystal structure was downloaded from RCSB Protein Data Bank (PDB ID: 3K0K). Protein structure was processed in Autodock Software by adding polar hydrogen and Kollman charges, correcting bond orders, replacing absent atoms and residues, deleting ions, and evaluating and correcting side chain protonation states [22]. A grid box of size 22 x 27 x 22 was created with default parameters and no restrictions, with an inner box (10 x 15 x 10 3) focused on X, Y, Z coordinates -23.75, 48.00, and 4.0. [23]. This obtained grid surrounds the region of active site of the protein. Molecular Docking of protein was performed with two different ligands namely ECGC and 10-gingerol in the linux environment using AutoDock Vina. It has shown a good docking score of the ligands when interacted with the protein. Therefore, this interaction between the protein-ligand complexes were analyzed using PyMOL and docking score is conserved for further references [5, 24].

Molecular Dynamics

Molecular Dynamics was performed using WebGro web-based simulation tool [25]. The forcefield used for simulation used for simulation is GROMOS96 43a1. The water model surrounding protein and ligand to mimic the environment is SPC water model. Energy Minimization Parameters helps in representing the minimum energy conformation for the structure. The algorithm used during simulation is Steepest Descent is used. NaCl is used neutralizing and for equilibration of the environment NVT is used that is where N represents Constant Number, V represents Volume and T represents Temperature.

For Molecular Dynamics, Leap-frog algorithm is used. The frames per simulation was 1000 and total time interval is 50ns [23]. Root Mean Standard Deviation (RMSD) is a quantitative measure of resemblance between two or more protein structures that is commonly used. RMSD graphs which revealed stability of the protein complexes throughout the simulation time interval. RMSF analysis helped in examining fluctuations occur in each residue during simulation, here we observed the fluctuations in the active site residues of the protein complex with ligands in the simulation time [26].

The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approach has been widely used to model molecular recognition, such as protein-ligand binding interactions, as an effective and accurate free energy simulation tool [27, 28]. Binding energy calculations of the ligand with the protein for last 20 nano seconds (30ns to 50ns) of the simulation time was carried out.

IV. RESULT AND DISCUSSION

Table 1: Summary of Plant extraction

Sample	Sample weight taken in grams	Solvent (250ml)	Yield in grams
Green tea	30g	Ethanol	1.543 g
Ginger	30g	Acetone	1.235 g

Phytochemical Analysis results:

A phytochemical screening test was done on green tea leaves and Ginger to evaluate for the presence of phytochemicals. Table 2: describes the presence and absence of phytochemicals in both the extracts. Green tea leaves shown positive results for glycosides, tannins, saponin, terpenoids, flavonoids and negative results for alkaloid, steroid and phenol and Ginger shown positive test results for alkaloids, glycosides, phenol, saponin, terpenoids, flavonoids and negative results for steroid and tannins.

Table 2: Phytochemical screening of ECGC and 10-gingerol extracts for bioactive compound

Tests	Green tea leaves	Ginger
Alkaloid	-	+
Glycosides	+	+
Steroid	-	-
Tannins	+	-
Phenol	-	+

Saponin	+	+
Terpenoids	+	+
Flavonoids	+	+

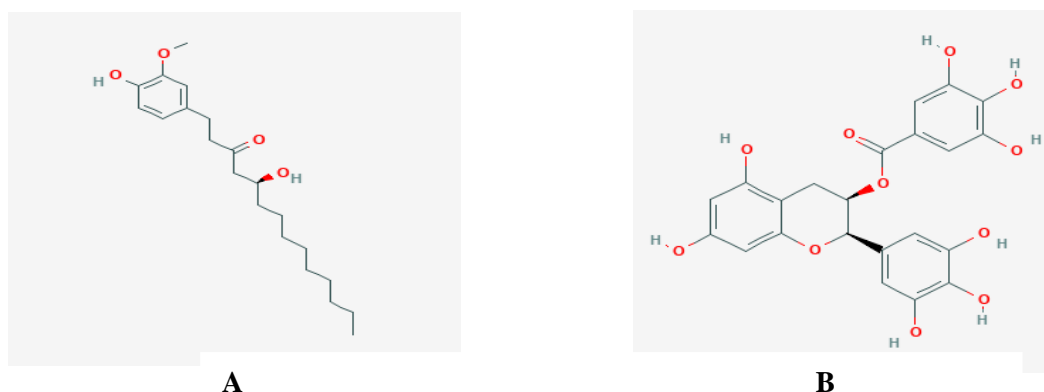


Figure 7: Structure of ligands A.10-gingerol, B. Epigallocatechin 3-gallate (EGCG).

Ligand	Blood Brain Barrier (BBB)	Plasma Protein Binding (PPB)	CYP_2D6 inhibition	Pure water solubility
10-gingerol	4.95718 (High absorption to CNS)	100.00 (Chemicals strongly bound)	Non	4.12244
Epigallocatechin 3-gallate (EGCG)	0.0875288 (CNS active compound)	100.00 (Chemicals strongly bound)	Non	552.794

Table 3: ADME Properties of Ligands

Pre-ADMET Analysis:

Epigallocatechin 3-gallate (EGCG) and 10-gingerol both have qualified in drug-likeness property as well as shown to be non-toxic according to the PreADMET. Thus, these two phytochemicals were considered for further studies. ADME properties also represents that these bioactive compounds are fulfilling the desired range to be classified as potential therapeutic compound available from plants.

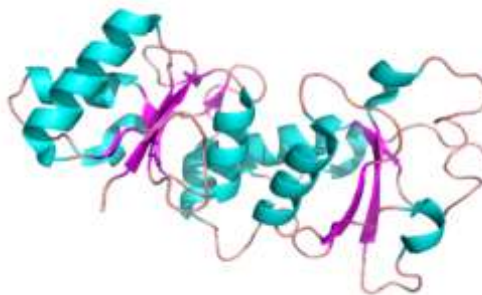


Figure 8: 3D view of 3K0K protein captured in PyMOL. Structural Analysis: BRCA1 BRCT crystal structure in complex with a free carboxy C-terminus limited recognition tetrapeptide

Molecular Docking

The proteins were prepared for docking by removing bound water molecules, added non-polar hydrogens and Kollman charges. Molecular Docking interactions of BRCA1 protein binds with the receptor EGCG and 10-gingerol were visualized using PyMOL software [22]. The Protein-Ligand complex having good docking score were screened and the bonded interactions were highlighted as yellow broken lines, the bonded and non-bonded residues of the protein were represented as green sticks and the Ligands were represented as ball and stick model having magenta color as shown in Fig. 9.

These 2D interaction plot of Protein and Ligands were prepared using LigPLOT software and 3D interaction images were prepared using the PyMOL software.

Table 4: Docking Table

Ligand name	Docking score (kcal/mol)	Number of H-bonds	Bonded interactions	Non-bonded interactions
Epigallocatechin 3-gallate (EGCG)	-6.6	0	-----	Thr1700, Asn1774, Leu 1701, Ile1680, Lys1702, Gln1779
10-Gingerol (GIN)	-5.2	3	Asn1678 (3.11), Ser1655(3.12), Gly1656 (2.85)	Val1654, Phe1662, Leu1657, Lys1702, Leu1701, Ile1680, Thr1700

The docking score of Epigallocatechin 3-gallate (EGCG) is -6.6 kcal/mol, and 10-Gingerol (GIN) is -5.2kcal/mol. In EGCG there are no bonded interactions as hydrogen bonds are not formed between the ligand and the protein and has good docking score in comparison with 10-Gingerol. There are 3 Bonded interactions between the Protein and 10-Gingerol with the docking score - 5.2kcal/mol.

The non-bonded interacting residues of protein upon docking with ECGC ligand are Thr1700, Asn1774, Leu 1701, Ile1680, Lys1702, Gln1779. The bonded and non-bonded interacting residues of the protein 3K0K upon docking with 10-Gingerol are Asn1678 (3.11), Ser1655(3.12), Gly1656 (2.85) and Val1654, Phe1662, Leu1657, Lys1702, Leu1701, Ile1680, Thr1700.

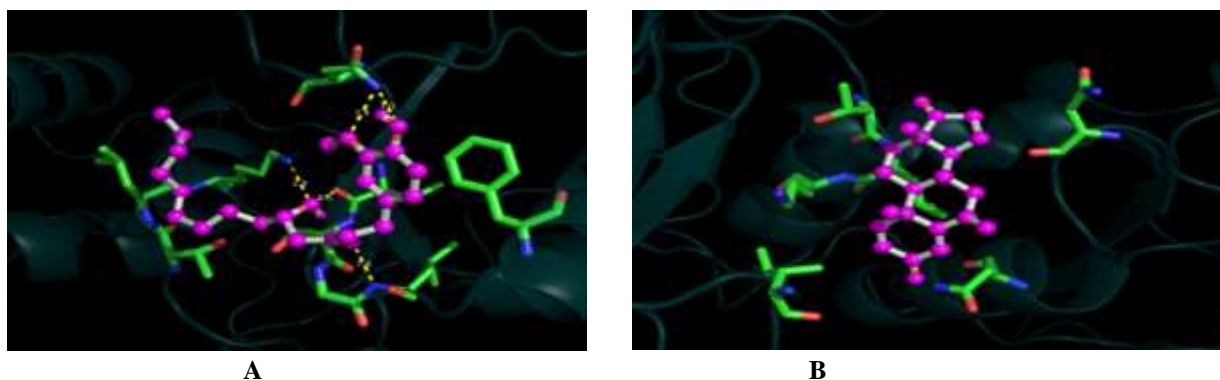


Figure 9: The 3D interaction images between the receptor and the ligands were taken using PyMOL software (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC). The Magenta color ball and stick model in the images represents the respective drug molecule and green color sticks are the interacting residues (bonded and non-bonded) and the bonded interactions are shown in yellow broken lines with the distance mentioned in Angstroms. A: 3D images of 3K0K_GIN complex, B: 3D images of 3K0K_ECG complex

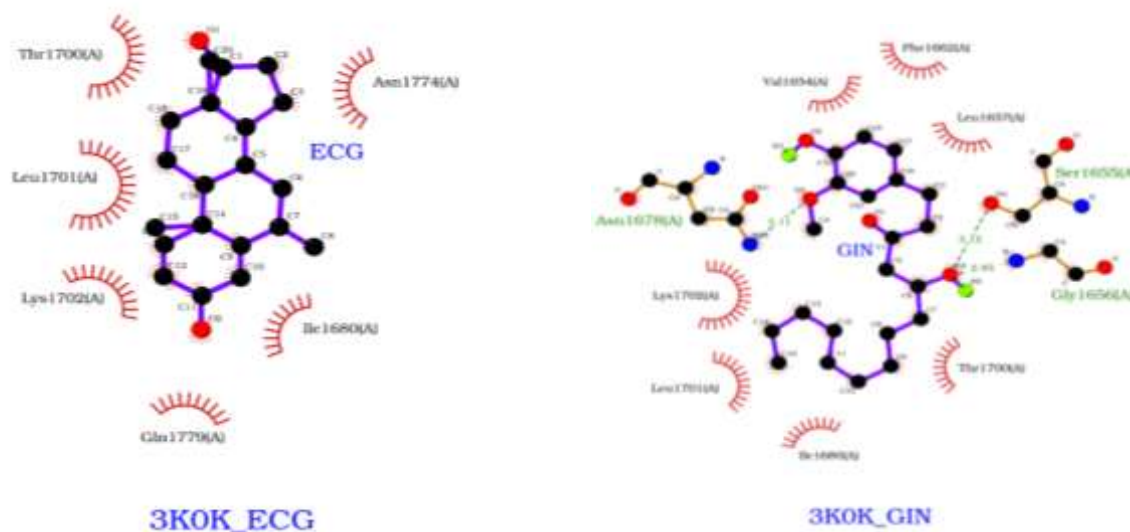


Figure 10: The above images are prepared using LigPLOT software. This image shows the bonded and non-bonded interactions between the ligands and protein 3K0K. 3K0K_ECG image shows the interaction between 3K0K protein receptor and Epigallocatechin 3-gallate (ECGC) ligand. 3K0K_GIN represents the interaction between the 3K0K protein receptor and 10-Gingerol.

Molecular Dynamics

The Root Mean Square Deviation (RMSD) of the model protein systems is calculated, which is a common metric for comparing two structures. The RMSD is determined by rotating and mapping the instantaneous structures' coordinates to superimpose them with the reference structure with the maximum overlap. In Fig.11, the RMSD shows the overall structural dynamics. Here, X-axis represents time interval in nano seconds and Y-axis represents deviation in nano meters.

Specific residue stability, or how frequently a single residue shifts (fluctuates) during a simulation, is calculated using Root Mean Square Fluctuation (RMSF), it shows the fluctuations of the residues throughout the simulation. In Fig.12, the RMSF per residue is usually plotted against the number of residues, and it will show the amino acids in a protein contribute the most to molecular motion structurally. Here, X-axis is the amino acid residues and Y-axis is the fluctuation in nano meters.

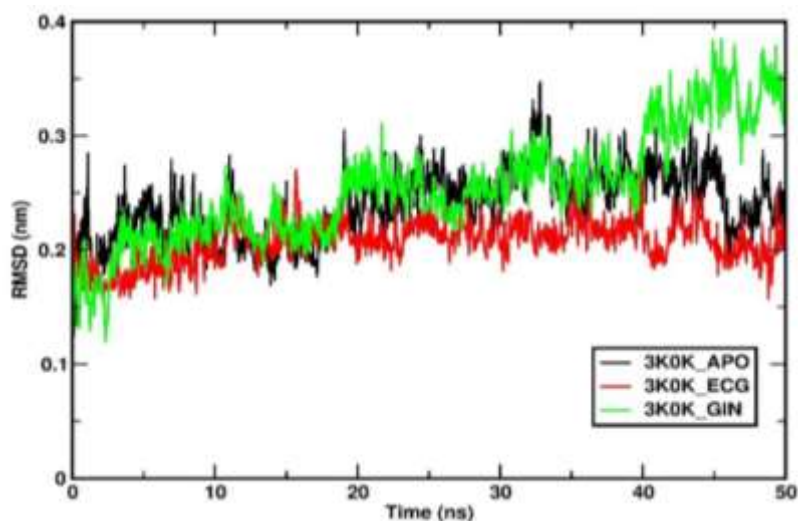


Figure 11: RMSD Graph showing overall structure

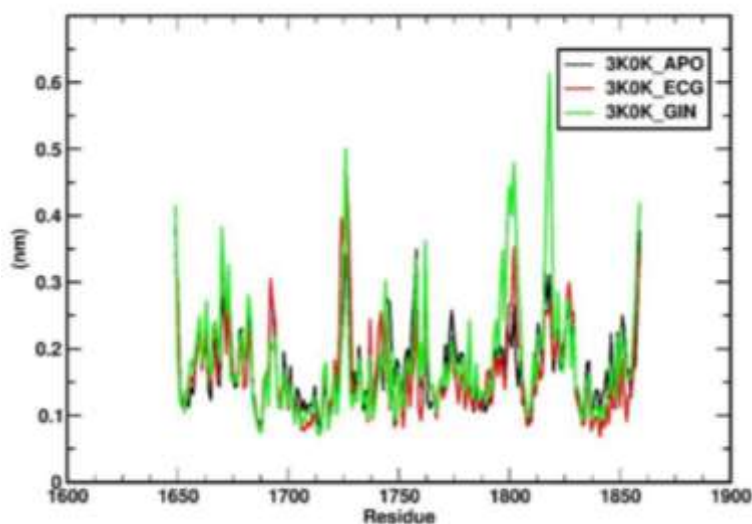


Figure 12: RMSF Graph showing fluctuation of residues throughout the simulation

The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculation tables:

The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) free energy simulation obtained for the 3K0K_GIN and 3K0K_ECG for the last 20 nanoseconds of the simulation (30ns-50ns). As it is free energy simulation method and used to understand the protein-ligand binding affinity. Binding energy of 10-gingerol is -137.234 kJ/mol and EGCG is -104.141 kJ/mol

This MMPBSA table represents that the bio-active compound 10-Gingerol which has the least binding energy and thus, it can be more suitable for binding to the active site of the protein complex 3K0K which makes it better as comparison to EGCG.

Table 5: MMPBSA calculation for 10-gingerol and EGCG

10-Gingerol	Binding energy = -137.234 +/- 0.000 kJ/mol
EGCG	Binding energy = -104.141 +/- 0.000 kJ/mol

V. SUMMARY AND CONCLUSION

These bioactive compounds EGCG and 10-gingerol can be used in many therapeutical aspects and can also reduce free radicals' production in the body. These bioactive constituents have anti-cancer property according to the earlier studies. It has been studied that 10-gingerol is having the ability to inhibit cell growth and it also induces S phase cell cycle arrest and apoptosis. Also, EGCG is found to be beneficial in biological as well as in anti-cancer properties. The estrogen-sensitive MCF-7 breast cancer cell line and EGCG has been shown to bind to ER to prevent proliferation. In Breast cancer genes BRCA1 and BRCA2 has an ability to induce breast cancer especially in the young women. In general, these genes are responsible for carrying out DNA repair mechanism to prevent cancer but when these genes get mutated it causes breast cancer as their DNA repair mechanism gets damaged. In phytochemical screening, polar solvent was used for EGCG i.e., ethanol as it extracted most of the phytochemicals based on the literary source and yield was obtained. It has shown positive results for secondary metabolites i.e., glycosides, tannins, saponin, terpenoids, flavonoids. In case of ginger based on earlier literature studies dipolar solvent was used i.e., acetone which has given positive results for alkaloids, glycosides, phenol, saponin, terpenoids, flavonoids.

This indicated that these two bioactive constituents are extracted from these two plant sources. In this study, In Silico analysis was carried out with BRCA 1 protein having the PDB ID: 3K0K. The ligands were downloaded from PubChem and processed it in Open BABEL. These bioactive compounds have qualified in drug likeliness properties using PreADMET tool. Using the same software toxicity was analyzed which has shown that the ligands are non-toxic in nature. ADME results shown good range for BBB, PPB, CYP_2D6, Pure water solubility. The interaction between the protein and ligand was determined using docking score of both ligands in which EGCG has good inhibition towards 3K0K protein than 10-gingerol in Docking studies. After docking, the 2D and 3D docking images are constructed using LigPLOT and PyMOL.

MMPBSA Calculation in Molecular Dynamics has shown that the Binding affinity of 10-gingerol and EGCG with the protein where 10-gingerol had minimum binding energy as compared to EGCG, this makes 10-gingerol as a good inhibitor of 3K0K protein in dynamic studies. Also, RMSD shown that the structure is stable and RMSF shown that the fluctuation in active site is less. By analyzing these data obtained by In Silico study, we can conclude that these bioactive compounds have efficiency to suppress the one of the mutations causing gene BRCA1 in breast cancer. If this BRCA1 gene gets mutated it is found to be fatal as it causes breast cancer. So, these bioactive compound acts as ligands and get binds to the protein which says that these bioactive compounds can be considered for inhibiting cancer cell proliferation.

Hence, we can conclude that these two phytochemicals have potential for suppressing the breast cell growth and these phytochemicals can also be combined for better efficacy and it would be helpful in further in vivo and in vitro studies.

VI. ACKNOWLEDGEMENT:

The authors would like to acknowledge the Management of JSS Academy of Higher Education & Research, Mysuru, Karnataka, for supporting the basic research ideas and for the resources provided.

REFERENCES:

- [1] K. L. Britt, J Cuzick and KA Philips, "Key steps for effective breast cancer prevention," Nat. Rev. Cancer, vol. 20, no. 8, pp. 417-436, 2020.
- [2] B. L. Gilmore, Y Lian, C Winton et al., "Molecular analysis of BRCA1 in human breast cancer cells under oxidative stress," Sci. Rep., vol. 7, p. 43435, 2017.
- [3] D. A. Mahvi et al., "Local cancer recurrence: The realities, challenges, and opportunities for new therapies," CA Cancer J. Clin., vol. 68, no. 6, pp. 488-505, 2018.
- [4] Z. Chen et al., "Invasive lobular carcinoma of the breast: A special histological type compared with invasive ductal carcinoma," PLOS ONE, vol. 12, no. 9, p. e0182397, 2017.
- [5] S. A. Narod, "Personalised medicine and population health: Breast and ovarian cancer," Hum. Genet., vol. 137, no. 10, pp. 769-778, 2018.
- [6] E. H. Cain et al., "Multivariate machine learning models for prediction of pathologic response to neoadjuvant therapy in breast cancer using MRI features: A study using an independent validation set," Breast Cancer Res. Treat., vol. 173, no. 2, pp. 455-463, 2019.
- [7] S. M. Chacko, P. T. Thambi, R. Kuttan, and I. Nishigaki, "Beneficial effects of green tea: A literature review," Chin. Med., vol. 5, no. 13, 2010.
- [8] P. Zivarpour et al., "Molecular and biological functions of gingerol as a natural effective therapeutic drug for cervical cancer," J. Ovarian Res., vol. 14, no. 1, p. 43, 2021.
- [9] V. R. Sinija and H. N. Mishra, "Green tea: Health benefits," J. Nutr. Environ. Med., vol. 17, no. 4, pp. 232-242, 2008.

- [10] C. Chu et al., "Green tea extracts epigallocatechin-3-gallate for different treatments," *BioMed Res. Int.*, vol. 2017, p. 5615647, 2017.
- [11] Y. Shukla and M. Singh, "Cancer preventive properties of ginger: A brief review," *Food Chem. Toxicol.*, vol. 45, no. 5, pp. 683-690, 2007.
- [12] S. D. Jolad et al., "Fresh organically grown ginger (*Zingiber officinale*): Composition and effects on LPS-induced PGE2 production," *Phytochemistry*, vol. 65, no. 13, pp. 1937-1954, 2004.
- [13] H. Jiang et al., "Metabolic profiling and phylogenetic analysis of medicinal *Zingiber* species: Tools for authentication of ginger (*Zingiber officinale* Rosc.)," *Phytochemistry*, vol. 67, no. 15, pp. 1673-1685, 2006.
- [14] B. H. Ali, G. Blunden, M. O. Tanira and A. Nemmar, "Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research," *Food Chem. Toxicol.*, vol. 46, no. 2, pp. 409-420, 2008.
- [15] R. Nicoll and M. Y. Henein, "Ginger (*Zingiber officinale* Roscoe): A hot remedy for cardiovascular disease?" *Int. J. Cardiol.*, vol. 131, no. 3, pp. 408-409, 2009.
- [16] F. Peng et al., "Cytotoxic, cytoprotective and antioxidant effects of isolated phenolic compounds from fresh ginger," *Fitoterapia*, vol. 83, no. 3, pp. 568-585, 2012.
- [17] Sahdeo Prasad, Amit K. Tyagi and quote; Ginger and Its Constituents: Role in Prevention and Treatment of gastrointestinal Cancer," *Gastroenterol. Res. Pract.*, vol. 2015, p. 11, 2015.
- [18] E. C. Stuart, M. J. Scandlyn and R. J. Rosengren, "Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer," *Life Sci.*, vol. 79, no. 25, pp. 2329-2336, 2006.
- [19] S. S. Azam and S. W. Abbasi, "Molecular docking studies for the identification of novel melatonergic inhibitors for acetyl serotonin O-methyltransferase using different docking routines," *Theor. Biol. Med. Model.*, vol. 10, pp. 63, 2013.
- [20] M. De Vivo, M. Masetti, G. Bottegoni and A. Cavalli, "Role of Molecular Dynamics and related methods in drug discovery," *J. Med. Chem.*, vol. 59, no. 9, pp. 4035-4061, 2016.
- [21] O. R. Alara, N. H. Abdurahman, C. I. Ukaegbu, N. A. Kabbashi, "Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques," *J. Taibah Univ. Sci.*, vol. 13, no. 1, pp. 414-422, 2019.
- [22] D. Prasanth et al., "In silico identification of potential inhibitors from Cinnamon against main protease and spike glycoprotein of SARS CoV-2," *J. Biomol. Struct. Dyn.*, pp. 1-15, Advance online publication (2020).
- [23] V. Kurdekar et al., "Structural features underlying the activity of benzimidazole derivatives that target phosphopeptide recognition by the tandem BRCT domain of the BRCA1 protein," *bioRxiv*, p. 555623, 2019.
- [24] (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC).
- [25] M. J. Abraham et al., "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," *SoftwareX*, vol. 1-2, no. 1-2, pp. 19-25, 2015.
- [26] J. Ramakrishnan et al., "Strong binding of leupeptin with TMPRSS2 protease may be an alternative to camostat and nafamostat for SARS-CoV-2 repurposed drug: Evaluation from molecular docking and Molecular Dynamics simulations," *Appl. Biochem. Biotechnol.*, vol. 193, no. 6, pp. 1909-1923, 2021.
- [27] O. Trott and A. J. Olson, "AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading," *J. Comp. Chem.*, vol. 31, no. 2, pp. 455-461, 2010.
- [28] C. Wang et al., "Recent developments and applications of the MMPBSA method," *Front. Mol. Biosci.*, vol. 4, p. 87, 2017.