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Research Article

Phytochemical Analysis And *In Silico* Pharmacological Profiling Of Methanolic Extract From *Annona Squamosa* L. Seeds For Breast Cancer

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ABSTRACT

Breast cancer remains a significant concern globally, with existing treatment options often associated with adverse effects. Consequently, there is a growing interest in exploring alternative treatments derived from natural sources. This study focuses on investigating the potential anti-cancer properties of plant extracts obtained from *Annona squamosa* L. seed. Extract were prepared using methanol via the maceration method. Analysis of the phytochemical composition using GC-MS and LC-MS techniques identified 43 compounds. Molecular docking against two proteins, 3ERT and 2J5F, revealed six compounds with lower binding affinity, including Piceatannol 3-O-glucoside, Cochlearine, Gerberinol, Ellagic acid, Dielsiquinone, and Annobraine. Among these, Dielsiquinone showed higher drug likeness properties. Further evaluation through MD simulation suggested Dielsiquinone as a potent anticancer agent *in silico*. This study highlights the potential of natural compounds, particularly Dielsiquinone, for developing effective anticancer drugs to combat breast cancer, offering a promising avenue for future research and drug development.

KEY WORDS: Breast cancer, *Annona squamosa* L., Analytical analysis, Molecular docking, Drug likeness, MD simulation

1. INTRODUCTION:

Cancer presents itself as one of the most formidable illnesses, marked by the unrestrained growth of cells within the body, leading to uncontrollable proliferation. Despite advancements in diagnosis, treatment, and prevention, it remains a significant cause of death globally, representing a severe metabolic disorder.[1] The impact of cancer is vast, as highlighted by the 2022 GLOBOCAN report, which compiles data from across the globe. Among both sexes, lung cancer leads with 12.4% incidence, closely followed by breast cancer at 11.6%. Colorectal cancer ranks third at 9.6%, trailed by prostate cancer at 7.3%. Stomach and liver cancers contribute 4.8% and 4.3%, respectively, to global cases. Notably, nearly half of all cancer instances, approximately 49.8%, fall into the "other" category, underscoring the wide array of cancers impacting populations worldwide.

Breast cancer, characterized by disrupted mammary epithelial cell function and high heterogeneity, stands as a formidable global health concern, ranking second in cancer diagnoses and a leading cause of female mortality worldwide. Its severity is compounded by escalating risk factors influenced by both internal and external factors, while conventional treatments such as chemotherapy and radiotherapy contribute to treatment burden and the development of multidrug resistance, resulting in disappointingly low survival rates. To address this critical situation, extensive research efforts are exploring alternative therapies, including medicinal plants and their bioactive compounds, as potential safer and effective options. Despite advancements in understanding its complex etiology and the use of biomarkers in diagnostics, breast cancer continues to challenge the healthcare community, necessitating ongoing innovation to improve patient outcomes and quality of life globally. [2, 3]

Medicinal plants have been used for centuries in traditional medicine systems due to their diverse pharmacological properties. Of particular interest are plant-derived phytochemicals, which exhibit various bioactivities, including potent anti-cancer effects. These phytochemicals represent a rich source of potential therapeutic agents for breast cancer treatment, offering the possibility of targeted therapies with fewer adverse effects compared to conventional treatments. The exploration of plant-derived compounds for breast cancer therapy aligns with the broader trend towards natural products and complementary medicine in oncology. By harnessing the power of nature's pharmacopeia, researchers aim to identify and develop novel drugs that specifically target cancer cells while sparing healthy tissues. In this context, the current study focuses on investigating specific plant phytochemicals for their anti-cancer properties in the context of breast cancer treatment. [4-6]

2. METHODOLOGY;

2.1. **Plant material collection, authentication and processing:** In October 2021, *Annona squamosa* L. seeds were collected from the Danta Taluka of Banaskantha district in Gujarat, India, and verified by Dr. Hitesh Solanki, a Professor at Gujarat University, Ahmedabad. The collected plant material was subjected to washing, followed by drying in shade for 7-15 days, grinding to ensure uniformity, and storage under low temperature conditions.[7,8]

2.2. Extract preparation: The extraction process utilized the maceration method, where 30 grams of seed powder was mixed with 300 ml of methanol. The mixture was agitated for one week to facilitate extraction. After agitation, the solvent was evaporated, resulting in the collection of the crude extract, which was subsequently stored at a lower temperature for further analysis.[9]

2.3. Phytochemical analysis: Phytochemical analysis of crude extract was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) techniques**.**

GC-MS:

The GC-MS analysis utilized a GCMS-TQ8040 NX system with an Auto Injector and Auto Sampler. A 30 m long analytical column with a diameter of 0.25 mm facilitated separation. Operating parameters included an inlet temperature of 250.00 °C, split injection mode with a ratio of 10.0, and helium as the carrier gas. The column oven temperature started at 50.0 °C, with a purge flow rate of 5.0 mL/min. Sample injection volume was 1 µL. The MS component featured an ion source temperature of 230.00 °C and an interface temperature of 250.00 °C. Calibration and operation were conducted using the GCMS-TQ8040 NX platform. Compound identification relied on peak retention time, area, height, and mass spectral fragmentation patterns correlated with the NIST library.[10,11]

LC-MS:

In this study, bioactive compound identification utilized liquid chromatography-mass spectrometry (LC-MS) with a Gemini C18 column (250mm x 4.6mm, 5µm). The mobile phase comprised Water (A) and Acetonitrile (B), with a gradient program: A(10.00%)/B(90.00%) at 30.00 mins, A(95.00%)/B(5.00%) at 35.00 mins, and A(95.00%)/B(5.00%) at 37.00 mins, maintaining a flow rate of 1.000 mL/min. Column oven temperature was 40±0.3°C, and autosampler temperature was 15±3°C. Injection volume was 10.0 µL, and detection used both PDA and Mass spectrometry, with a total run time of 45.0 minutes. These parameters enabled efficient chromatographic separation and accurate mass spectrometric analysis, facilitating successful compound identification by correlating peak retention time, area, height, and mass spectral fragmentation patterns with known compounds in the METLIN Metabolite PCDL. [12,13]

2.4. Molecular Docking

Molecular docking is a computational method used to predict how a ligand interacts with a receptor or target protein, aiming to determine binding affinity and favorable ligand conformations. In our investigation, we utilized Docking-based virtual screening (DBVS) with PyRx v0.8, integrating AutoDock, AutoDock Vina, and Open Babel.[14]

Target Protein:

In our breast cancer study, two pivotal proteins were selected: the Human Estrogen Alpha Receptor (PDB ID 3ERT) and the Epidermal Growth Factor Receptor (PDB ID 2J5F). These proteins are integral to breast cancer-related cellular processes and signaling pathways, rendering them crucial targets for investigation.[15]

Protein Preparation:

To prepare the target receptors, their 3D structures were obtained from the RCSB Protein Data Bank in formats like PDB. These structures were then imported into PyRx v0.8, supporting various file formats

including PDB, MOL2, and PDBQT. The receptor structures underwent refinement steps such as removing water molecules and adding hydrogen atoms, performed using Biovia Discovery Studio Visualizer or PyRx v0.8. The refined structures were converted to the PDBQT format, ensuring compatibility for subsequent molecular docking studies. This comprehensive preparation process is vital for accurate computational assessments of ligand-receptor interactions.[16]

Ligand Preparation:

In current study, phytochemicals were sourced from PubChem databases and prepared for molecular docking. Imported compounds underwent energy minimization to stabilize their structures. All ligands were converted to the PDBQT format using the Open Babel Tool, essential for chemical structure conversions in molecular docking simulations.[17]

Molecular Docking:

The molecular docking analysis utilized the AutoDock Vina tool within PyRx v0.8. Docking parameters were configured through the "Vina wizard" tab, and receptors and ligands were carefully selected. Active site coordinates were determined using the Active Site Prediction Server from SCFBio, IIT Delhi. Docking calculations involved posing and scoring ligand-receptor complexes, generating docking poses and scores. Results were analyzed via a .csv file to compare binding affinity scores. Emphasis was placed on ligand interactions with receptor amino acid residues and identification of bond types contributing to stable conformations. Visualization was accomplished using Biovia Discovery Studio Visualizer, facilitating insights into molecular interactions.

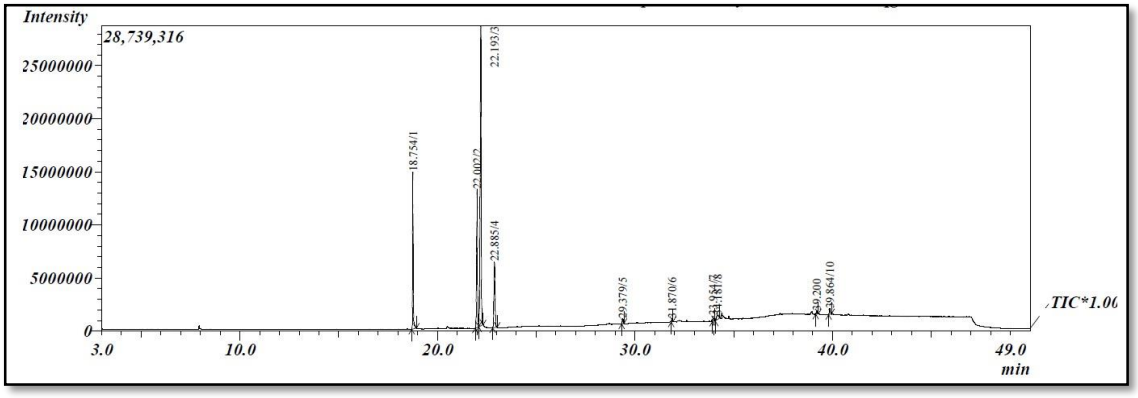
2.5. Drug likeness: In drug discovery, evaluating a compound's drug potential is essential. Drug likeness analysis assesses molecular properties and predicts biological behavior. The study utilized SwissADME, a popular computational tool from the Swiss Institute of Bioinformatics. SwissADME forecasts physicochemical properties, pharmacokinetic properties, and drug likeness property violations using various tools such as Lipinski, Ghose, etc.[18]

2.6. MD Simulation:

Dielsiquinone, a phytochemical with promising affinity and drug likeness, underwent 100 nanoseconds (ns) of molecular dynamics (MD) simulation using Desmond, a Schrödinger LLC package. Initial protein-ligand complexes were obtained from docking studies, providing a static prediction of ligand binding. Simulations aimed to predict ligand binding in physiological conditions, assessing conformational stability, intermolecular interactions, and binding site occupancy. Protein-ligand complexes were preprocessed and optimized using Protein Preparation Wizard or Maestro, then prepared with the System Builder tool. The OPLS_2005 force field and TIP3P solvent model were employed, with neutralization and addition of 0.15 M NaCl to mimic physiological conditions. Simulations were conducted in the NPT ensemble at 310 K temperature and 1 atm pressure. Trajectories were stored every 100 picoseconds (ps) for analysis, including Root Mean Square Deviation (RMSD), Root-mean-square fluctuation (RMSF), and protein-ligand interactions over time.[19,20]

3. RESULT:

3.1. GC-MS for CSM:



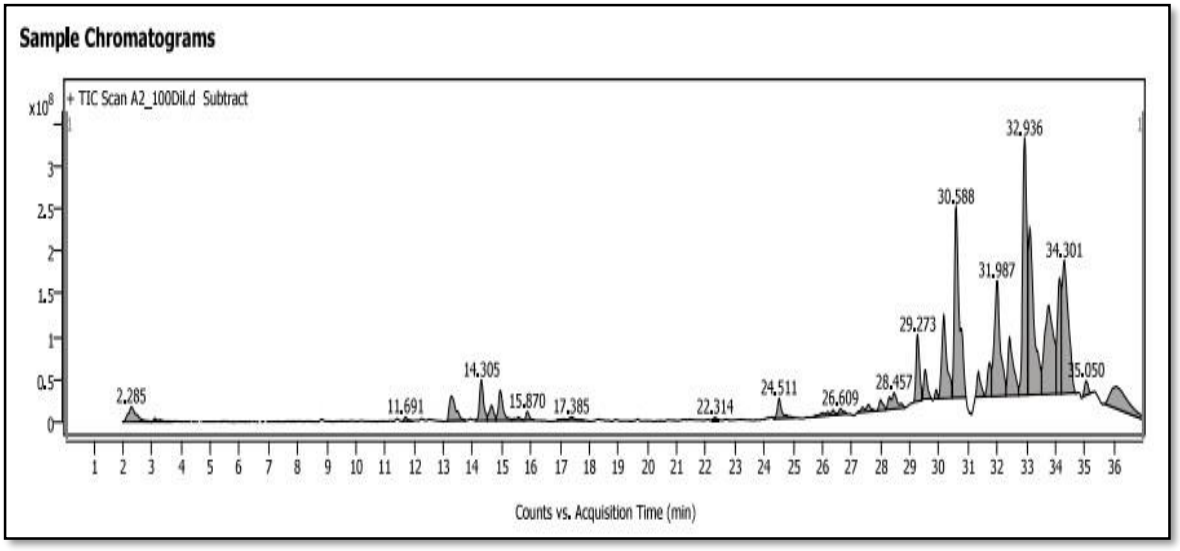
[Figure 1: GC-MS Chromatogram for CSM]

The GC-MS chromatogram for the sample labeled as CSM reveals a complex mixture of compounds. Figure 1 represents a hypothetical GC-MS chromatogram, and Table 1 represents the list of identified compounds for CSM, illustrating the separation of compounds over time (retention time), with peaks indicating the presence and abundance of different compounds.

[Table 1: Identified compound from CSM by GC-MS]

Sr.No	RT(min)	Name of the compound	Molecular formula	MW	Peak area%
1	18.754	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	15.67
2	22.002	Methyl linoleate	$C_{19}H_{34}O_2$	294.5	21.08
3	22.193	Methyl elaidate	$C_{19}H_{36}O_2$	296.5	47.61
4	22.885	Methyl stearate	$C_{19}H_{38}O_2$	298.5	11.18
5	29.379	Nonadecanoic acid	$C_{21}H_{42}O_2$	326.6	0.73
6	31.870	Glycidol oleate	$C_{21}H_{38}O_3$	338.5	0.32
7	33.954	Hydroxycitronellal, trimethylsilyl ether	$C_{13}H_{28}O_2Si$	244.45	0.54
8	34.181	Petroselinic acid, TMS derivative	$C_{21}H_{42}O_2Si$	354.6	1.43
9	39.200	Stigmasterol	$C_{29}H_{48}O$	412.7	0.50
10	39.864	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	0.94

3.2. LC-MS for CSM:



[Figure 2 : LC-MS Chromatogram for CSM]

The LC-MS chromatogram (Figure 2 and table 2) for the compound sample (CSM) provides valuable insights into the composition and abundance of various compounds present. Upon analysis of the data, it's evident that the sample contains a diverse array of compounds with differing molecular structures and weights.

[Table 2: Identified compound from CSM by LC-MS]

Sr. No	RT(min)	Name of the compound	Molecular formula	MW	Peak area%
1	2.285	Gerberinol (hydroxycoumarin)	$C_{21}H_{16}O_6$	364.3	11.29
2	3.101	pyrogallic acid	$C_6H_6O_3$	126.11	1.57
3	11.691	Lindenenone	$C_{15}H_{16}O_2$	228.29	1.57
4	13.273	1-benzopyran (3'-Deaminofusarochromanone)	$C_{15}H_{19}NO_4$	277.31	14.79
5	14.305	Piceatannol 3-O-glucoside	$C_{20}H_{22}O_9$	406.4	15.1
6	14.638	Cherimolacyclopeptide A	$C_{38}H_{63}N_9O_{10}S$	836	6.44
7	14.955	Cochlearine	$C_{15}H_{19}NO_3$	261.32	1.86
8	15.571	Cherimoline	$C_{37}H_{66}O_8$	638.9	1.41
9	15.870	Rutin	$C_{27}H_{30}O_{16}$	610.5	2.67
10	17.385	N-Acetyl-O-demethylpuromycin-5'-phosphate	$C_{23}H_{30}N_7O_9P$	579.5	2.63

11	22.314	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	368.3	0.70
12	24.511	Norisocorydine	$C_{19}H_{21}NO_4$	327.4	8.53
13	26.376	ellagic acid	$C_{14}H_6O_8$	302.19	4.35
14	26.609	Oxonantenine	$C_{19}H_{13}NO_5$	335.3	2.44
15	27.591	p-hydroxybenzoic acid	$C_7H_6O_3$	138.12	4.42
16	27.991	Coumarin	$C_9H_6O_2$	146.14	3.92
17	28.457	Butyl laurate	$C_{16}H_{32}O_2$	256.42	11.73
18	29.273	Squamolone	$C_5H_8N_2O_2$	128.13	21.91
19	29.522	Hydroxyhydroquinone	$C_6H_6O_3$	126.11	10.04
20	29.905	Methyl 2-furoate	$C_6H_6O_3$	126.11	1.95
21	30.172	3-formyl-4-hydroxy-2h-pyran	$C_6H_6O_3$	126.11	39.97
22	30.588	Annocherine A	$C_{17}H_{15}NO_4$	297.30	90.71
23	31.354	7-Methylinosine	$C_{11}H_{15}N_4O_5^+$	283.26	8.96
24	31.737	Phloroglucinol	$C_6H_6O_3$	126.11	11.35
25	31.987	gallic acid	$C_7H_6O_5$	170.12	64.43
26	32.419	7,8-Dihydroparasiloxanthin	$C_{40}H_{60}O_2$	572.9	30.31
27	32.936	Demethylspheroidene	$C_{40}H_{58}O$	554.9	100
28	33.119	Dihydroxylycopene/ OH-Rhodopin	$C_{40}H_{60}O_2$	572.9	91.18
29	33.768	Dielsiquinone	$C_{15}H_{11}NO_4$	269.25	73.96
30	34.134	(Z)-Tamarindienal	$C_6H_6O_3$	126.11	39.67
31	34.301	Annobraine	$C_{19}H_{11}NO_4$	317.3	80.18
32	35.050	benzoic acid	$C_7H_6O_2$	122.12	4.79
33	36.082	3,4,3',4'-Tetrahydrospirilloxanthin	$C_{42}H_{64}O_2$	601.0	36.32

3.3. Molecular docking of phytochemicals derived from CSM:

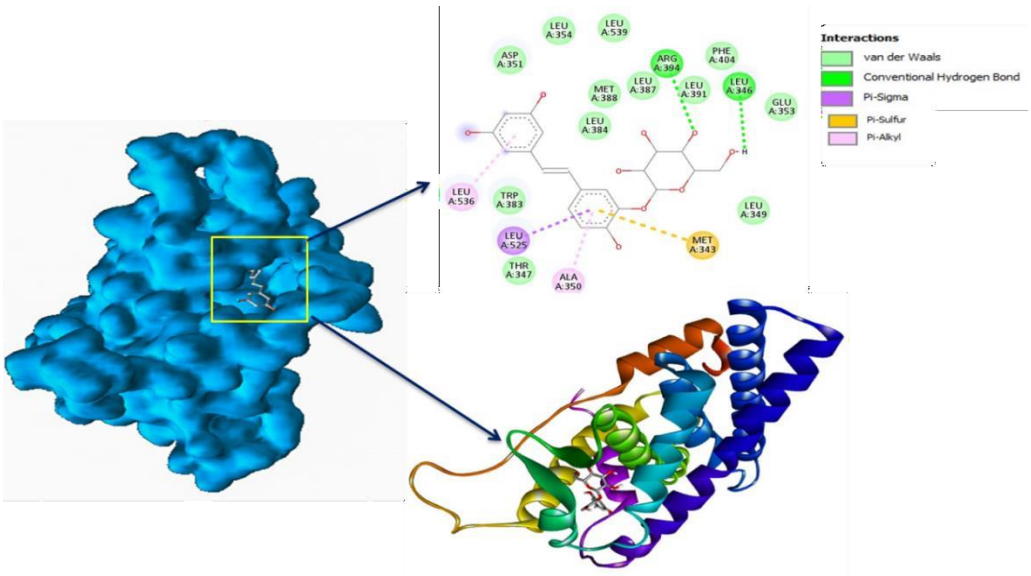
The molecular docking analysis encompassed the evaluation of 45 identified compounds sourced from the methanol extract of *Annona squamosa* L. seeds against both the Human estrogen Alpha receptor (PDB id: 3ERT) and the Epidermal growth factor receptor (PDB id: 2J5F). This comprehensive examination aimed to elucidate the binding affinities and potential interactions of these compounds with the respective receptors, providing valuable insights into their pharmacological activities and therapeutic potentials. By targeting multiple receptors, the study offers a broader perspective on the bioactivity of the compounds and their possible roles in modulating key signalling pathways implicated in estrogen regulation and epidermal growth factor signalling. The provided tables 3 present the binding affinity and amino acid interactions of various compounds identified in CSM extract, categorized by GC-MS and LC-MS analyses. Figures 4 and 5 depict the two-dimensional and three-dimensional interactions of molecular docking involving three potent phytochemicals with reduced binding affinity for the Human Estrogen Alpha receptor (PDB id: 3ERT) and the Epidermal Growth Factor receptor (PDB id: 2J5F), respectively.

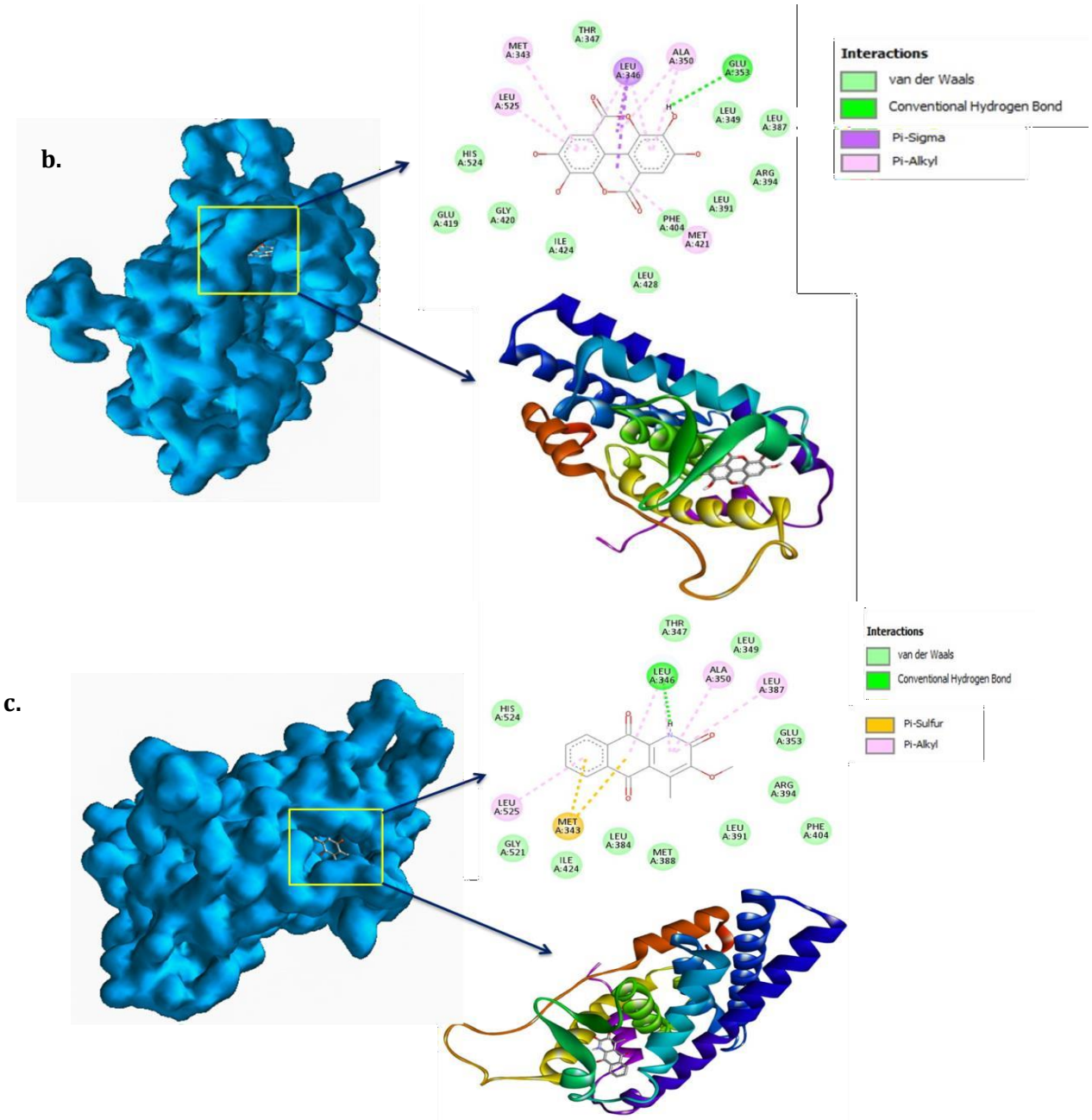
[Table 3: Binding Affinity of Compounds Identified in CSM Extract by GC-MS and LC-MS against 3ERT and 2J5F]

Sr.No	Identified Compound	Binding Affinity For 3ERT	Binding Affinity For 2J5F
1	Methyl palmitate	-5.3	-5.2
2	Methyl linoleate	-5.9	-5.3
3	Methyl elaidate	-5.6	-5.5
4	Methyl stearate	-5.5	-5.4
5	Nonadecanoic acid, methyl ester	-5.6	-5.3
6	Glycidol oleate	-5.8	-5.4
7	Farnesol	-6.3	-5.8
8	Rosmarinic acid	-6.5	-8
9	Stigmasterol	-5.1	-8.4
10	.gamma.-Sitosterol	-6.8	-7.6
11	N Gerberinol (hydroxycoumarin)	-6.5	-8.6
12	pyrogallic acid	-5.2	-4.8

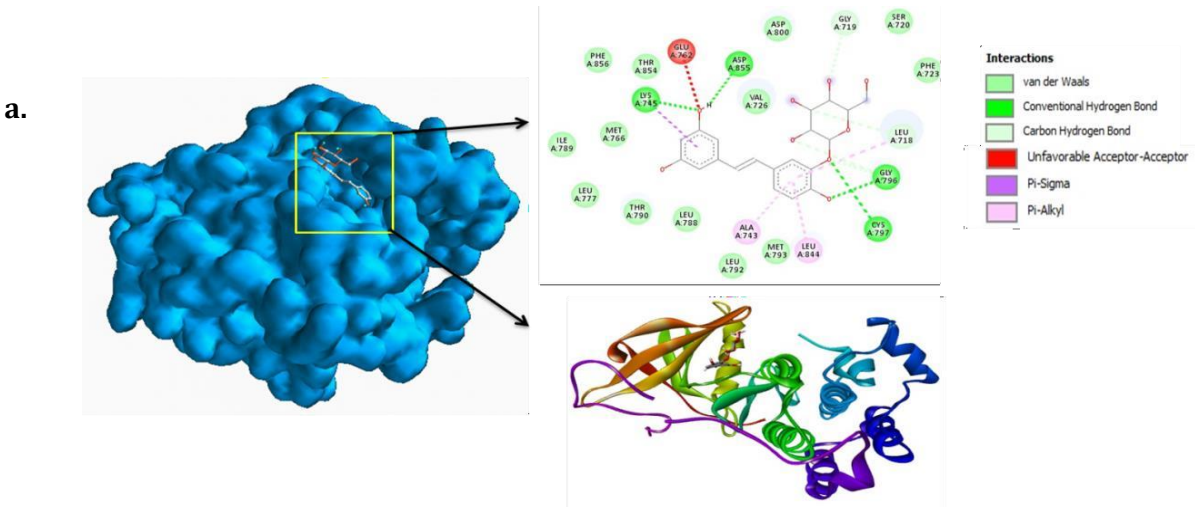
13	Lindenenone	-7.5	-7.6
14	1-benzopyran (3'-Deaminofusarochromanone)	-7.8	-7.4
15	Piceatannol 3-O-glucoside	-8	-8.1
16	Cherimolacyclopeptide A	-6.5	22.4
17	Cochlearine	-8.1	-7.6
18	Cherimoline	-7.3	-6.4
19	Rutin	-6.8	-7
20	N-Acetyl-O-demethylpuromycin-5'-phosphate	-6.9	-7.6
21	3-Feruloylquinic acid	-4.0	-4.8
22	Norisocorydine	-6.5	-7.7
23	ellagic acid	-8.2	-8.5
24	Oxonantenine	-6.5	-8.7
25	p-hydroxybenzoic acid	-5.3	-5.9
26	Coumarin	-6.2	-5.9
27	Butyl laurate	-5.6	-5.3
28	Squamolone	-4.8	-4.7
29	Hydroxyhydroquinone	-5.1	-4.9
30	Methyl 2-furoate	-4.3	-4.1
31	3-formyl-4-hydroxy-2h-pyran	-4.6	-4.8
32	Annocherine A	-7.2	-7.9
33	7-Methylinosine	-6.8	-6.8
34	Phloroglucinol	-4.9	-4.6
35	gallic acid	-5.5	-6
36	7,8-Dihydroparasiloxanthin	-3.2	-2.3
37	Demethylspheroidene	-6.5	18.2
38	Dihydroxylycopene/ OH-Rhodopin	-5.2	25
39	Dielsiquinone	-8.1	-8.1
40	(Z)-Tamarindienal	-4.3	-4.3
41	Annobraine	-7.3	-10
42	benzoic acid	-5.2	-5.6
43	3,4,3',4'-Tetrahydrospirilloxanthin	-6.3	30.9

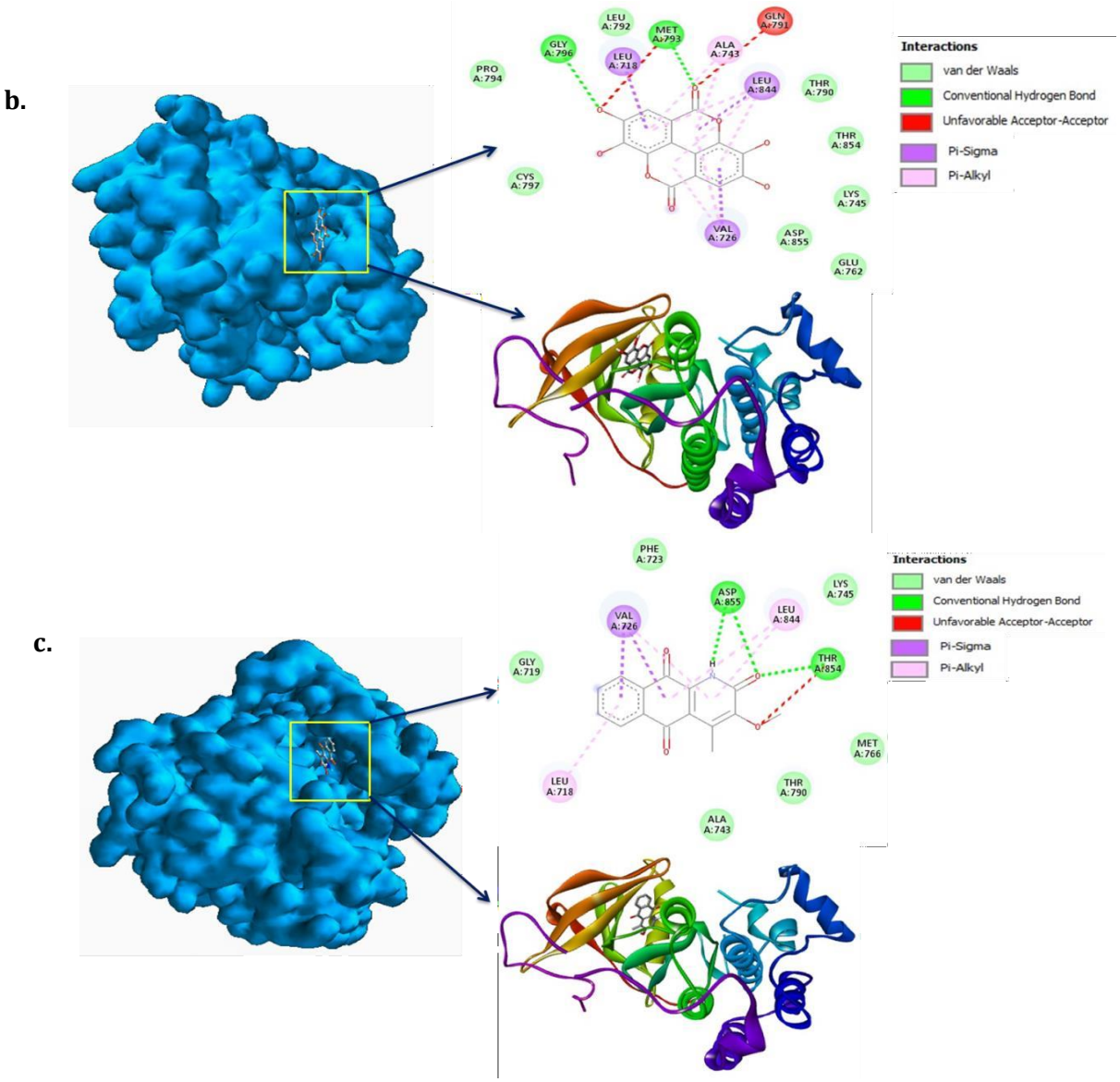
a.





[Figure 3: Molecular docking analysis of phytochemical against 3ERT; a: Piceatannol 3-O-glucoside,b: Ellagic acid, c: Dielsiquinone]





[Figure 4: Molecular docking analysis of phytochemical against 2J5F; a: Piceatannol 3-O-glucoside,b: Ellagic acid, c: Dielsiquinone]

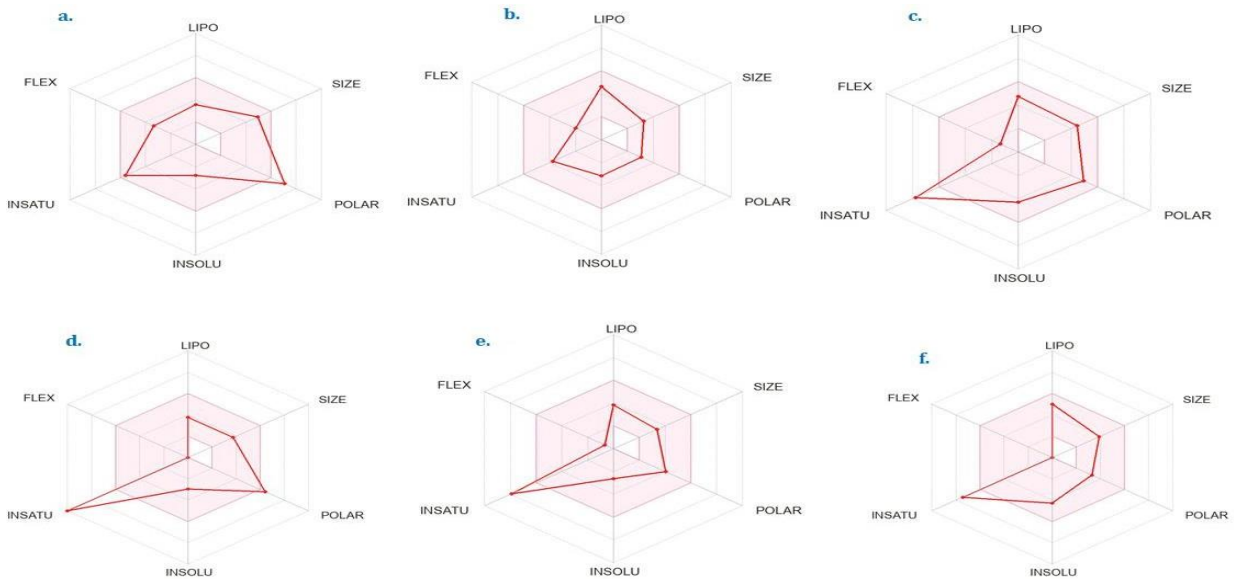
Molecular docking was conducted for 43 compounds, evaluating their binding affinities against the target compound. Among them, six compounds exhibited notably higher binding affinities and were selected for further investigation. These compounds are Piceatannol 3-O-glucoside, Cochlearine, Gerberinol, Ellagic acid, Dielsiquinone, and Annobraine. Specifically, 3 phytochemical's 2D and 3D interactions among the six phytochemicals are illustrated in Figure 3 against 3ERT and Figure 4 against 2J5F. These figures provide insights into the molecular interactions between phytochemical and its respective target compounds, shedding light on potential mechanisms of action and therapeutic relevance.

3.4. Drug likeness of selected phytochemicals:

The results detailing the drug likeness properties for each compound are presented in Table 4. The drug likeness properties of six compounds with promising binding affinity for breast cancer targets were meticulously analyzed. This examination primarily focused on physicochemical properties, pharmacokinetic properties, and various drug likeness parameters established by Lipinski, Ghose, Veber, Egan, and Muegge. Additionally, particular attention was paid to the bioavailability radar of phytochemicals identified from the CSM plant extract. Among these compounds, Dielsiquinone exhibited exceptional drug likeness properties, as all its values fell within the optimal range across different parameters of drug likeness. Consequently, Dielsiquinone was deemed suitable for molecular dynamics (MD) simulation, highlighting its potential as a promising candidate for further investigation in breast cancer therapy.

[Table 4: Drug likeness properties of selected phytochemicals identified from CSM plant extract]

Properties		Compound					
		Piceatannol 3-O-glucoside	**Cochlearine**	**Gerberinol**	**Ellagic acid**	**Dielsiquinone**	**Annobraine**
Physicochemical properties							
Molecular weight		406.38	261.32	364.35	302.19	269.25	317.29
X log P3		0.73	2.63	2.8	1.1	1.18	3.25
TPSA (Å)		160.07	49.77	100.88	141.34	76.23	55.84
Log S (ESOL)		-2.8	-3.15	-4.28	-2.94	-2.63	-4.29
Fraction Csp3		0.3	0.53	0.14	0	0.13	0.16
Rotatable Bonds		5	3	2	0	1	0
Pharmacokinetic properties							
BBB		No	Yes	No	No	Yes	Yes
HIA		Low	High	High	High	High	High
PGP		No	No	No	No	No	No
Log K_p		-8.26	-6.03	-6.53	-7.36	-5.93	-7.1
Bioavailability Score		0.55	0.55	0.55	0.55	0.55	0.55
Druglikeness properties							
Lipinski	violations	1	0	0	0	0	0
	follow	Yes	Yes	Yes	Yes	Yes	Yes
Ghose	violations	0	0	0	0	0	0
	follow	Yes	Yes	Yes	Yes	Yes	Yes
Veber	violations	1	0	0	1	0	0
	follow	No	Yes	Yes	No	Yes	Yes
Egan	violations	1	0	0	1	0	0
	follow	No	Yes	Yes	No	Yes	Yes
Muegge	violations	2	1	0	0	0	0
	follow	No	Yes	Yes	Yes	Yes	Yes

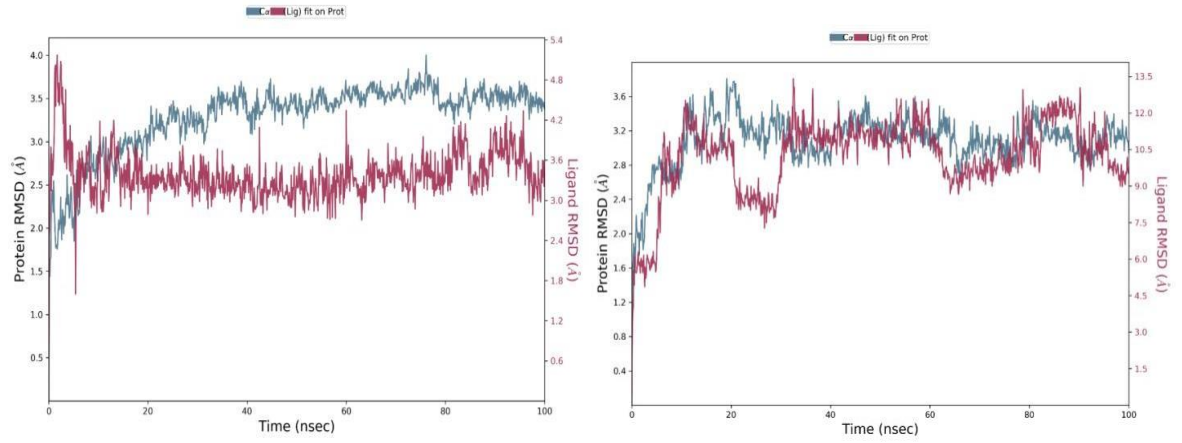


[Figure 5: Bioavailability radar of phytochemicals identified from CSM plant extract; a: Piceatannol 3-O-glucoside , b: Cochlearine, c: Gerberinol, d: ellagic acid, e: dielsiquinone, f: Annobraine]

3.5. MD Simulation:

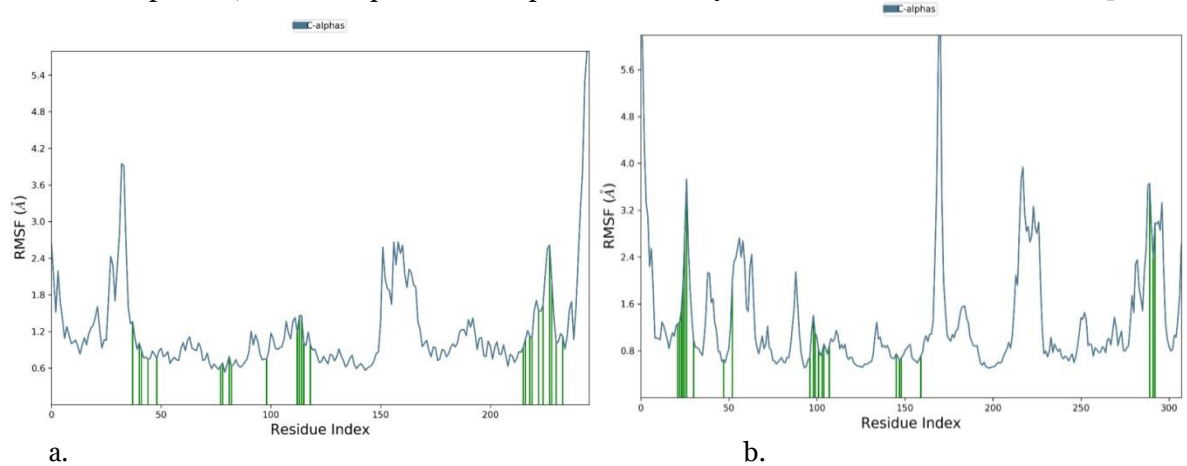
The root-mean-square deviation (RMSD) primarily computes the average distance of the simulated protein-ligand complex. Therefore, RMSD values for protein (Cα) and ligands fit proteins (Figure 6) via screened ligands (dielsiquinone') was extrapolated from 100 ns simulation trajectories. The Cα atoms in 3ERT, 2J5F complexed with all three screened ligands exhibited mean deviations (< 4Å), which is acceptable for small globular proteins. Reasonably, the Cα atoms of 3ERT docked with Dielsiquinone revealed a relatively stable RMSD value within 2.0 Å to 3.5 Å from 30 ns to 80 ns, followed by slight elevation and state of equilibrium with a calculated mean of 3.28 Å at the end of the simulation.

RMSF value of the protein-ligand complex was analyzed. Based on MD trajectories, the higher peaks of the residues are in loop regions, N- and C-terminal zones (Figure 7) showed the stability. The stability of the selected ligands binding against the target protein showed low RMSF values. The secondary structure features including alpha-helices and beta-strands were predicted throughout the simulation. Secondary Structure Elements graph was plotted against the residual index to calculate the distribution across the protein structure.



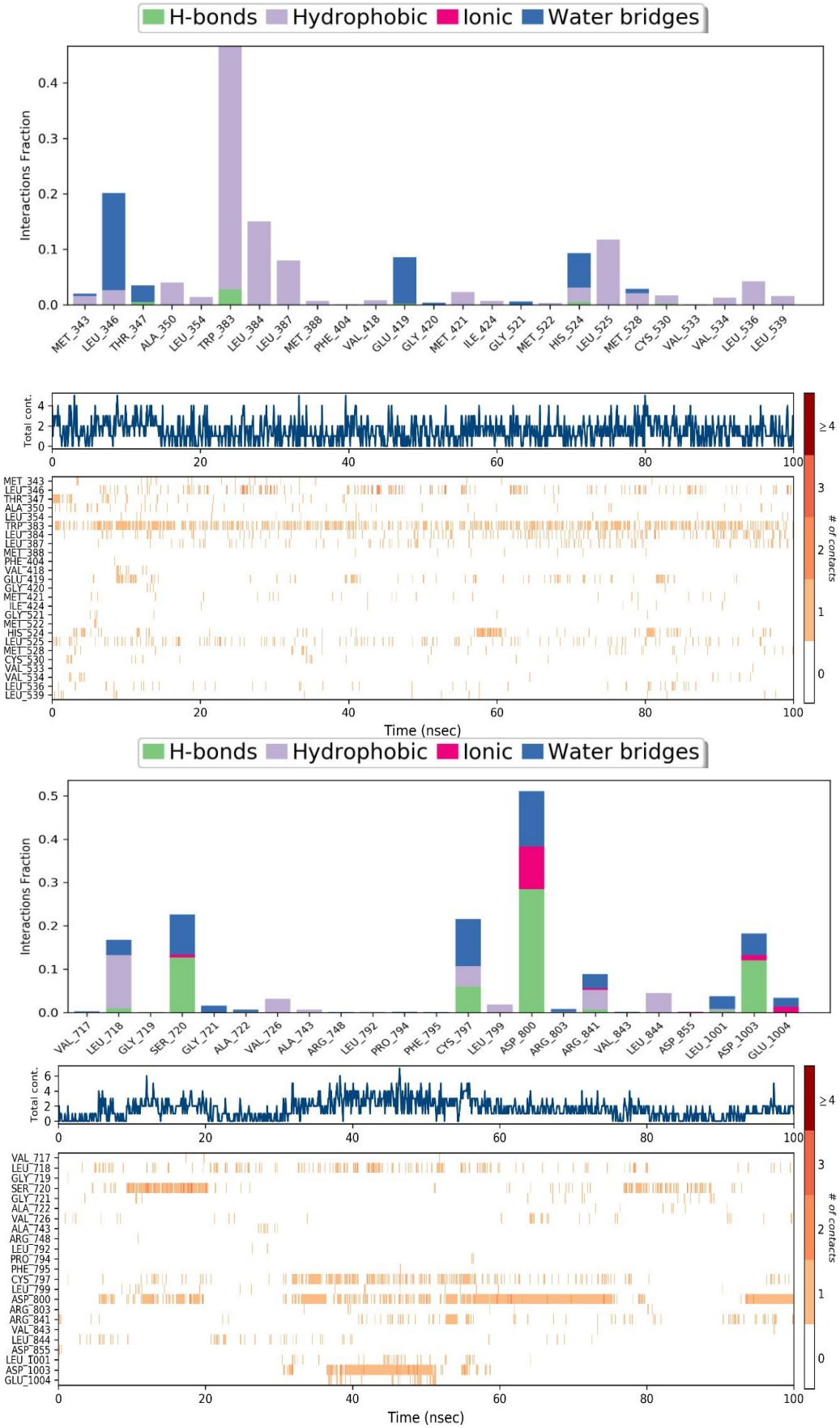
a b

[Figure 6: Calculated RMSD values for alpha carbon (Cα) atoms of 3ERT (a) and 2J5F(b) protein and Ligands fit on protein, viz. Dielsiquinone were plotted with respect to 100 ns simulation interval]



a. b.

[Figure 7: Root Mean Square Fluctuation (RMSF) of the target protein 3ERT (a) and 2J5F (b) residues complexed with the selected ligands, via Dielsiquinone]



(Figure 9: Protein-ligand contact histogram and Protein-ligand contact heatmap throughout trajectory of target protein 3ERT(a) and 2J5F(b) residues complexed with the selected ligand, Dielsiquinone)

The hydrogen bonds constituted the vast majority of the significant ligand-protein interactions (Figure 8). The hydrogen bonding was observed with certain residues. The ligand-protein interactions were also critically observed over the course of the simulation analyses. The molecular contacts and interactions (H-bonds, hydrophobic, ionic, and water bridges) showed the interaction between the target proteins and the selected ligands (Figure 8). Molecular docking analyses and MD simulation analyses revealed the interactional residues of the selected ligands and the receptor proteins.

4. Conclusion:

In conclusion, breast cancer remains a significant global health concern, prompting the exploration of alternative treatments with fewer adverse effects. This research delved into the potential anti-cancer properties of plant extracts from *Annona squamosa* L. seeds. Through meticulous analysis utilizing GC-MS and LC-MS techniques, 43 compounds were identified within these extracts. Molecular docking studies targeting proteins 3ERT and 2J5F unveiled six compounds, notably Dielsiquinone, with promising binding affinities. Remarkably, Dielsiquinone exhibited superior drug likeness properties, leading to further evaluation via molecular dynamics (MD) simulation, which suggested its potential as a potent anticancer agent *in silico*. This study underscores the promise of natural compounds, particularly Dielsiquinone, in the development of effective breast cancer therapeutics, paving the way for future research and drug discovery endeavors in this critical area.

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