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RESEARCH ARTICLE

In Silico and Pharmacokinetic Activity of Bioactive Components from *Annona muricata* Leaves Against Breast Cancer

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Abstract

Objectives: To identify the potential inhibitors isolated from *Annona muricata* leaves against the epidermal growth factor of tyrosine kinase receptor, a crucial factor involved in the development of breast cancer. **Methods:** In this study, the ethanolic extract of *Annona muricata* leaves was studied by GC-MS analysis. The functional compounds derived from the GC-MS spectrum were docked with a target molecule, the Epidermal Growth Factor of Tyrosine Kinase Receptor [PDB Id: 1M17], for breast cancer using Auto dock vina. The Bioactivities of compounds against the key role enzymes like GPCR ligand, nuclear, and enzyme inhibitor in Breast Cancer was analyzed using Molinspiration. The pharmacokinetic and pharmacodynamic attributes of the chemical compounds were studied by the Swiss ADME tool. **Findings:** The outcomes from Molecular docking proved that the bioactive compounds such as 9,19-Cyclolanost-24-en-3-ol, (3.beta.), Octadec - 9 - enoic acid, Cycloeucalenol, and Phytol could act as potentially active inhibitors against the Epidermal Growth Factor of Tyrosine Kinase Receptor in Breast Cancer. **Novelty:** The inhibitory effect of the bioactive components from the ethanolic extract of *Annona muricata* leaves against the Epidermal Growth Factor of Tyrosine Kinase Receptor through the *in silico* approach has not been explored. This research work will be the first to attempt the *in silico* mode to determine the potential inhibitors of the Epidermal Growth Factor of Tyrosine Kinase Receptor and successfully identified four bioactive compounds that down-regulate the expression of EGFR and control the proliferation of breast cancer cells.

Keywords: Drug Development; Breast Cancer; Inhibitor; Pharmacokinetics; Pharmacodynamics; Auto Docking; Bioactive Compounds

1 Introduction

Cancer is one of the main causes of death and a major obstacle to raising life expectancy. Globally, there is a significant increase in the burden of cancer occurrence and mortality rates [(1)]. These instances can be attributed to several factors, including population

growth and aging, as well as shifts in the distribution and prevalence of the primary risk factors for cancer, many of which are related to socioeconomic development[2]. The GLOBOCAN 2018 reveals that breast cancer accounted for almost 11.6% of all female cancer cases, making it the second most often diagnosed malignancy[3].

Breast cancer is one of the most prevalent tumors in women, although metastases are the primary cause of death. It is characterized by unregulated cell development that develops into a hard, painless inflammation in the breast tissue, typically in the milk-producing lobules or ducts[4]. Breast tumors are most frequently categorized by the state of three distinct cell surface receptors: the human epidermal growth factor (EGF) receptor, the estrogen receptor (ER), and the progesterone receptor (PR). However, breast cancer can potentially metastasize to distant places, including the lymph nodes and various organs, after initiating as a local disease[5].

The expression of various genes that control the survival and invasion of cancer cells is involved in this process. Due to this, pharmaceuticals or phytochemicals that alter these genes or proteins produced by these gene expressions that control cancer cell survival, metastasis, apoptosis, and invasion are crucial as drug targets in developing new medications[6,7]. Because of their importance in maintaining and promoting health, medicinal plants are now being studied extensively, focusing on identifying plants acting against cancer cells.

A. muricata leaves contain bioactive substances such as alkaloids, flavonoids, phenols, and acetogenins, which are linked to the biological activities of extracts made from the leaves. *A. muricata* leaves have yielded the isolation of over forty acetogenins, with annonacin being the most prevalent among them[8]. Alkaloids and flavonoids from the Annona species are examples of antioxidant chemicals inhibiting free radical damage connected to cancer development[9,10]. *Annona muricata* acetogenins have a unique affinity for certain resistant cells and show cytotoxic effects against cancer cells. Acetogenins from Annonaceae exhibit cytotoxic effects against cancer cells, including A549 lung cancer cells, PACA-2 pancreatic cancer cells, and colon cancer cells[11].

In order to produce new synthetic or herbal drugs, it is necessary to evaluate the pharmacokinetic properties at progressively earlier stages of the discovery process, when the number of potential compounds is high but physical sample access is constrained. The ADMET efficiency of the drug was assessed by looking at the pharmacokinetic properties and toxicity of the ligand profiles. Future medications are more likely to result from potential hits with favorable pharmacotherapeutic characteristics[12]. The findings could suggest that targeted compounds with high binding energies and a solid ADMET profile would be considered possible hits for breast cancer therapeutic development after testing in *in-vitro* experiments[13].

2 Methodology

2.1 Collection of plant leaves

The healthy leaves of *Annona muricata* were collected from Srimad Andavan Arts and Science College (Autonomous), Thiruvanaikovil, Trichy, Tamil Nadu, India. They were washed with distilled water and kept in the shade drying for 10 days. The dried powder was used for the preparation of plant extract.

2.2 Leaf extract preparation

The extracts of the *Annona muricata* leaves were obtained using the cold maceration method suggested by[14]. **100g o**f collected *Annona muricata* leaves were subjected to Soxhlet extraction using ethanol as the solvent. The ethanolic extract thus collected was allowed to be fractionated using n-butanol to predominantly obtain the phytochemical compounds. Then, the extract was filtered using Whatman No. 1 filter paper, and the solvents were removed using a rotary vacuum evaporator.

2.3 Fabrication of Molecules Involved in *in silico* analysis

2.3.1 Preparation of ligands

The 3D structure and the computed descriptors of the ligand compounds identified from the GC-MS analysis of an ethanolic extract of *Annona muricata* leaves were retrieved from the PubChem database in SMILES format (Table 1). The GCMS results of the ethanolic extract of *Annona muricata* leaves have already been published online[15]. All the atomic coordinates were changed to pdbqt set-up using Online SMILES Translator(https://cactus.nci.nih.gov/translate/), an open-source online chemical toolbox for the interconversion of chemical structures. The energy was minimized using a Universal Force Field[16].

Table 1. List of bioactive compoundsfrom the PubChem Database in SMILES format

S. No	Compounds	Canonical Smiles
01	Lauric acid	CCCCCCCCCCCC(=O)O
02	Tetradecanoic acid, methyl ester	CCCCCCCCCCCCCC(=O)OC
03	Pentadecylic acid	CCCCCCCCCCCCCCC(=O)O
04	9-Octadecenoic acid (Z)-, 9-octadecenyl ester,	CCCCCCCCC=CCCCCCCCCOC(=O)CCCCCCCC=CCCCCCCCC
05	Octadecanoic acid,2-hydroxy-1,3-propanediyl	CCCCCCCCCCCCCCCCCCC(=O)OCC(COC(=O)CCCCCCCCCCCCCCCCCC)O
06	Phosphonic acid, dioctadecyl ester	CCCCCCCCCCCCCCCCCCO[P+](=O)OCCCCCCCCCCCCCCCCCC
07	Phytol	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
08	Cycloeucalenol	CC1C2CCC3C4(CCC(C4(CCC35C2(C5)CCC1O)C)C(C)CCC(=C)C(C)C)C
09	OCTADEC - 9 - ENOIC ACID	CCCCCCCCC=CCCCCCCCC(=O)O
10	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	CC(CCC=C(C)C)C1CCC2(C1(CCC34C2CCC5C3(C4)CCC(C5(C)C)O)C)C
11	Benzoic acid, 2,5-dihydroxy-, methyl ester	COC(=O)C1=C(C=CC(=C1)O)O
12	Glyceryl 1,3-dipalmitate	CCCCCCCCCCCCCCCCC(=O)OCC(COC(=O)CCCCCCCCCCCCCCCCC)O

2.3.2 Preparation of receptor

The X-ray crystallographic structure of the Epidermal Growth Factor of the Tyrosine Kinase Receptor (PDB ID: 1M17) was retrieved from the PDB database (https://www.rcsb.org/structure/). The criteria for choosing PDBs were (a) minimum resolution and (b) conformation of the docked ligand being the same as in the crystallized structure after the redocking procedure[17,18]. The PDB files chosen for the molecular docking-based virtual screening study were processed by removing water molecules, adding hydrogen atoms, and finally prepared by Discovery Studio.

2.4 Molecular docking

The structure files of biochemical compounds (.sdf) and their target (.pdb) (Epidermal Growth Factor of Tyrosine Kinase Receptor – 1M17) were uploaded into Auto Dock Vina[19]. The target proteins were converted into macromolecules, which changed the atomic coordinates into pdbqt format. The grid box was centered on the crystal structures to perform molecular docking, and all other parameters were left as default. The docking results were screened for binding affinity, and all possible docked conformations were generated for the compound. After analyzing with Discovery Studio and PyMOL, only those conformations that specifically interact with the active-site residues of the Epidermal Growth Factor of Tyrosine Kinase Receptor (Target) were selected. Discovery Studio was employed to explore the details of covalent and non-covalent interactions, namely as follows: hydrogen bond, unfavourable donor-donor, alkyl, sigma-pi bond, carbon-hydrogen bond, and van der Waals interaction formed between the biochemical compounds and the target Epidermal Growth Factor of Tyrosine Kinase Receptor.

2.5 Drug Likeliness and Bioactivity Score

The physico-chemical properties of the ligand compounds were retrieved from the SwissADME online web server (http://www.swissadme.ch/index.php) to satisfy Lipinski's rule of five, which is essential for rational drug design[20]. The bioactive compounds showed no violation of all the five rules: not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, the molecular weight of compounds less than 500, partition coefficient (log P) less than 5, rotatable bonds less than 10, topological polar surface area (TPSA) of not greater than 140 was selected for further analysis. The bioactivity score of bioactive compounds was checked using the Molinspiration Cheminformatics web page, and the ADMET study was done using the SWISS ADME prediction tool. The bioactivity contribution will be calculated for each sub-structure of a fragment; the bioactivity for the entire molecule will then be calculated as a sum of the activity of contributions of all the fragments in a molecule. This provides a molecule activity score (a number, typically between -3 and 3). It has been recommended by Molinspiration that molecules with the highest activity score have the highest probability of being active.

2.6 ADMET predictions

Molecular descriptors are the deciding factor for the pharmacokinetic & pharmacodynamic properties and toxicity of a compound. ADMET properties predicted by the *in silico* approach determine the likelihood of compounds being used as human therapeutic agents[21]. The SWISS ADME online server calculated the ADMET properties of the bioactive compounds in the ethanolic extract of *Annona muricata* leaves. Compounds need to have a promising ADMET profile. The BBB, GIA, CYP450

inhibition, Skin Permeability, LogP, and LogD were also calculated. The SWISS ADME and MedChem predictions indicated the ability of the ligand molecules to act as a strong inhibitor against breast cancer receptors.

3 Results and Discussion

Currently, treatment options for breast adenocarcinoma are still futile owing to (a) the rapid evolution of drug-resistant forms, (b) minimal success with existing therapeutics, and (c) systemic toxicity with nontargeted therapies. Hence, using natural products to treat breast cancer has recently gained importance[22]. A few such natural products have successfully inhibited any or a few of the several pathways that trigger tumorigenesis and metastasis of breast cancer cells.

A .muricata is widely used as a traditional medicine. Parts of the *A. muricata* plant, such as the leaves, fruit, seeds, bark, and roots, have pharmacological properties. From the 49 research articles that we obtained, it was reported that its pharmacological properties included anticancer (25%), antiulcer (17%), antidiabetic (14%), antiprotozoal (10%), antidiarrhea (8%), antibacterial (8%), antiviral (8%), antihypertensive (6%), and wound healing properties because of the various compounds contained in *A. muricata*[23].

On that note, nearly twelve different phytochemical compounds were identified from the n-butanol extraction of the ethanol leaf extract of *Annona muricata* by GCMS analysis. Some compounds, such as tetradecanoic acid, n-hexadecanoic acid, hexadecanoic acid methyl ester, phytol, and octadecanoic acid, confirmed the antimicrobial and free radical scavenging activities of the leaf extract[24]. All these compounds were subjected to various *in silico* analyses to determine their potency against breast cancer cells.

For a molecule to obey "the rule of five," it must exhibit molecular weight (MW) $\leq$ 500 Da as an oral bioavailability criterium, hydrogen bond donor (HBDs) $\leq$ 5, hydrogen bond acceptor (HBAs) $\leq$ 10, and LogP (octanol-water partition coefficient) $\leq$ 5. These descriptors of oral bioavailability are important as they predict the permeability and absorption of such drug across a biological membrane such as an epithelium cell, partition coefficient value (log p), which is important in predicting intestinal absorption of such drug[25].

The physico-chemical properties of the compounds retrieved from the SwissADME tool are presented in Table 2. They exhibited drug-like characteristics based on Lipinski's rule of 5, which determines if the compound has a certain biological or pharmacological activity to make it an active oral drug in humans. The current *in silico* study was undertaken to identify efficient anti-breast cancer compounds from the *Annona muricata* leaves.

Table 2. Determination of physico-chemical properties of bio-chemical compounds using Molinspiration online tool

S. No	MW	HBA	HBD	Molecular Formula	nRotb	TPSA	milogP	nAtoms	Volume
01	200.32	2	1	C12H24O2	10	37.30	5.04	14	224.22
02	242.40	2	0	C15H30O2	13	26.30	6.36	17	275.35
03	242.40	2	1	C15H30O2	13	37.30	6.55	17	274.62
04	532.94	2	0	C36H68O2	32	26.30	10.19	38	615.81
05	625.03	5	1	C39H76O5	38	72.84	10.17	44	697.80
06	585.96	3	0	C36H74O3P+	36	35.54	10.09	40	659.92
07	296.54	1	1	C20H40O	13	20.23	6.76	21	349.38
08	426.73	1	1	C30H50O	5	20.23	7.62	31	462.17
09	282.47	2	1	C20H38O3	15	37.30	7.58	20	318.84
10	426.73	1	1	C30H50O	4	20.23	8.21	31	461.26
11	168.15	4	2	C8H8O4	2	66.76	1.63	12	144.61
12	568.92	5	1	C35H68O5	34	72.84	9.91	40	630.59

MW - Molecular Weight; HBA- Hydrogen bond acceptor; HBD - Hydrogen bond donor; nRotb - Number ofrotatable bonds; TPSA - Topological polar surface area (Å^2); milogP- Partition coefficient; S.No – Corresponds to the bioactive compounds listedin Table 1. Text in bold font (S.No 7 to 10) indicates the properties of potentinhibitors from Annona muricata leaves against breast cancer

Molinspiration was used to evaluate the bioactivity of the ligand molecules by calculating the activity against certain enzyme inhibitors (kinase, protease, etc.), GPCR ligands, ion channel modulators, and nuclear receptor ligands shown in Table 3. A molecule with a bioactivity score of more than 0.00 will likely possess appreciable biological activities. At the same time, values between -0.50 and 0.00 are expected to be moderately active; if the score is less than -0.50, it is assumed to be inactive[26].

The compound selected for the present study has a good, acceptable range of Kinase inhibitors, GPCR, Nuclear receptor ligands, ion channel modulators, and protease inhibitors. The predicted bioactivity by Molinspiration is shown in Table 3. The

Table 3. Bioactivities of compounds against the key role enzymes in Breast Cancer using Molinspiration

S. No	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear inhibitor	Protease Inhibitor	Enzyme Inhibitor
01	-0.27	-0.04	-0.75	-0.24	-0.36	+0.04
02	-0.24	-0.07	-0.51	-0.24	-0.28	-0.02
03	-0.04	+0.05	-0.42	+0.01	-0.11	+0.16
04	+0.06	-0.12	-0.11	+0.06	+0.06	+0.07
05	-0.07	-0.57	-0.36	-0.26	+0.05	-0.22
06	+0.07	-0.23	-0.09	-0.03	+0.03	-0.01
07	+0.11	+0.16	-0.32	+0.35	+0.00	+0.31
08	+0.14	+0.14	-0.37	+0.92	+0.10	+0.61
09	+0.17	+0.07	-0.22	+0.23	+0.07	+0.27
10	+0.21	+0.10	-0.40	+0.86	+0.14	+0.66
11	-0.96	-0.43	-1.02	-0.75	-1.12	-0.48
12	+0.07	-0.21	-0.12	+0.01	+0.09	+0.03

S. No- Corresponds to the bioactivecompounds listed in Table 1; GPCR ligand – [G - Protein Coupled Receptor ligand];Text in bold font (S.No 7 to 10) indicates the properties of potent inhibitorsfrom Annona muricata leaves against breast cancer

ADMET profile was analyzed using the SWISS ADME online tool to analyze the pharmacodynamic study of all the bioactive compounds to understand the action of the drug inside a host's body[27]. Based on the bioactivity scores from the SwissADME tool, four compounds were scrutinized as potent inhibitors of receptor molecules, and their efficiency was validated using molecular docking studies.

Table 4. Pharmacokinetics and pharmacodynamics properties of chemical compounds using the SWISS ADME online tool

S. No	CYP Inhibitors					Pharmacokinetics						Lipinski Rule Violations (Drug likeness)
	1A2	2C19	2C9	2D6	3A4	GIA		Log Kp (cm/s)	S+LogP	S+LogD	Diff Coeff	MlogP	
01	No	No	No	No	No	High	Yes	-4.54	4.870	2.550	2.847	0.816	Yes / 0 violation
02	Yes	No	No	No	No	High	Yes	-3.23	6.569	6.569	3.639	0.722	Yes / 0 violation
03	Yes	No	Yes	No	No	High	Yes	-3.07	6.264	3.975	3.639	0.722	Yes / 0 violation
04	No	No	No	No	No	Low	No	1.44	12.000	12.000	7.799	0.446	No / 2 violations
05	No	No	No	No	No	low	No	1.40	11.889	11.889	6.396	0.417	No / 2 violations
06	No	No	No	No	No	Low	No	2.65	10.012	10.012	7.979	0.427	No / 2 violations
07	No	No	Yes	No	No	Low	No	-2.29	7.986	7.986	5.304	0.621	Yes / 1 violation
08	No	No	No	No	No	Low	No	-1.87	9.547	9.547	6.979	0.547	Yes / 1 violation
09	Yes	No	Yes	No	No	High	No	-2.60	7.208	4.928	4.261	0.660	Yes / 1 violation
10	No	No	No	No	No	Low	No	-1.96	9.934	9.934	6.979	0.547	Yes / 1 violation
11	No	No	No	No	No	High	No	-5.86	1.491	1.477	1.031	1.114	Yes / 0 violation
12	No	No	No	No	No	Low	No	0.20	10.886	10.886	5.691	0.443	No / 2 violations

GIA – GastrointestinalAbsorption; BBB – Blood Brain Barrier; CYP – Cytochrome P450; Diff Coeff – DifferentialCoefficient, Log K_p – Skin Permeation; Log P – PartitionCo-efficient b/w Aqueous & and Water; Log D – Distribution Co-efficient.S.No – Corresponds to the bioactive compounds listed in Table 1; Text in boldfont (S.No 7 to 10) indicates theproperties of potent inhibitors from Annona muricata leaves against breastcancer

Molecular docking is used to identify the scrutinized ligand molecules {[9,19-Cyclolanost-24-en-3-ol, (3.beta.)], Octadec - 9 - enoic acid, Cycloeucalenol, and Phytol} as a potentially active phytochemical compound against the Epidermal Growth Factor of Tyrosine Kinase Receptor of Breast Cancer. The investigation of the mechanism of inhibition and identification of the critical residues of the binding pocket were analyzed.

The binding energy is used to compare and study the binding affinity of different compounds/ligands with their respective target molecule, i.e., the lower the binding energy, the higher the ligand affinity for the receptor. So, the ligand with the highest affinity can be chosen as the potential drug for further studies. Based on binding affinity, the binding energy of selected ligands compound was in an ascending pattern of -9.88, -8.77, -5.70, and -5.61 kcal/mol, respectively, as shown in Table 5.

Table 5. Binding energy of compounds against the target receptor 1M17 using the AUTO DOCK tool

S. No	Compounds	Binding Energy	Inhibitor constant	Intermol Energy	Vdw_hb_desolv Energy	Electrostatic Energy
01	Lauric acid	-4.34	654.01 (μM)	-7.63	-5.54	-2.08
02	Tetradecanoic acid, methyl ester	-5.18	160.02 (μM)	-9.06	-9.06	0.00
03	Pentadecylic acid	-5.05	198.26 (μM)	-9.23	-7.63	-1.60
04	9-Octadecenoic acid (Z)-, 9-octadecenyl ester,	-3.82	1.58 (mM)	-13.37	-13.36	-0.01
05	Octadecanoic acid,2-hydroxy-1,3-propanediyl	-2.43	16.47 (mM)	-14.07	-13.99	-0.08
06	Phosphonic acid, dioctadecyl ester	-1.35	102.0 (mM)	-12.09	-12.11	0.01
07	Phytol	-5.70	66.47 (μM)	-9.88	-9.86	-0.01
08	Cycloeucalenol	-9.88	57.52 (nM)	-11.67	-11.62	-0.04
09	OCTADEC - 9 - ENOIC ACID	-5.61	77.42 (μM)	-10.38	-9.89	-0.49
10	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	-8.77	375.54 (nM)	-10.26	-10.09	-0.17
11	Benzoic acid, 2,5-dihydroxy-, methyl ester	-4.94	238.62 (μM)	-6.13	-5.89	-0.24
12	Glyceryl 1,3-dipalmitate	-2.97	6.71 (mM)	-13.41	-13.37	-0.03

S.No– Corresponds to the bioactive compounds listed in Table 1; Text in bold font (S.No7 to 10) indicates the properties ofpotent inhibitors from Annona muricata leaves against breast cancer

After a detailed analysis of interactions, the (9,19-Cyclolanost-24-en-3-ol, (3.beta.) with 1M17 shown one hydrogen Bond (Asn818 —- O); Octadec - 9 - enoic acid with 1M17 shown alkyl bonds(Leu694, Val702, Leu820, Met769, Ala719, Lys721, Leu764, Met742); Cycloeucalenol with 1M17 shown hydrogen bond (Gln788 —- O); Phytol with 1M17 shown one Hydrogen bond (Gln767 —- O) as shown in (Figures 1, 2, 3 and 4) and Table 6. Based on docking analysis against various breast cancer receptors, phytol had slight antagonistic activity against BRCA1, BRCA2, and MDR1 compounds[28]. The analysis suggested that the binding and therapeutic property makes these bioactive components a prominent lead in developing a potential breast cancer inhibitor after being tested through *in vitro* experiments.

Table 6. Interacting amino acids with the target protein (1M17) of Breast cancer

S. No	Compounds	Non-Covalent Interactions				Covalent Interaction	
		Alkyl Bond	$\pi - \sigma$ Bond	Unfavourable Donor-Donor Bond	Carbon-Hydrogen Bond	Hydrogen Bond	Distance (Å)
01	Lauric acid	Arg752, Pro748	Nil	Lys828	Lys822, His826	Gln767	4.43
02	Tetradecanoic acid, methyl ester	Lys721, Val702, Leu764, Ala719, Leu820, Leu694, Leu768	Nil	Nil	Nil	Nil	Nil
03	Pentadecylic acid	Leu694, Leu768, Ala719, Leu820, Met769, Val720	Nil	Nil	Nil	Nil	Nil
04	9-Octadecenoic acid (Z)-, 9-octadecenyl ester,	Met742, Leu694, Leu768, Ala719, Leu820, Cys773, Lys721, Arg817, Phe699	Nil	Nil	Nil	Nil	Nil
05	Octadecanoic acid,2-hydroxy-1,3-propanediyl	Ala731, Leu723, Leu768, Lys721, Leu820, Ala719, Met769, Val702, Cys773, Leu834	Nil	Nil	Nil	Asp831	4.46

Continued on next page

Table 6 continued

06	Phosphonic acid, dioctadecyl ester	Cys773, Lys721, Val702, Leu820, Ala719, Leu694	Nil	Nil	Nil	Cys773	2.87
07	Phytol	Lys721, Met742, Val702, Leu820, Leu694, Met769	Nil	Nil	Nil	Gln767	5.19
08	Cycloeucalenol	Arg962, Ile785, Tyr789, Pro824, Pro951	Tyr789	Nil	Nil	Gln788	3.89
09	OCTADEC - 9 - ENOIC ACID	Leu694, Val702, Leu820, Met769, Ala719, Lys721, Leu764, Met742	Nil	Nil	Pro770	Nil	Nil
10	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	Lys721, Leu820, Met769, Val702, Ala719, Leu694, Phe699, Arg817	Nil	Nil	Nil	Asn818	3.64
11	Benzoic acid, 2,5-dihydroxy-, methyl ester	Met742, Leu764, Lys721, Leu820	Nil	Nil	Thr766	Thr766 Ala719 Glu738	2.89 3.60 5.06
12	Glyceryl 1,3-dipalmitate	Val702, Met769, Leu820, Ala719, Phe771	Nil	Nil	Nil	Nil	Nil

S.No – Corresponds to thebioactive compounds listed in Table 1; Text in bold font (S.No 7 to 10) indicatesthe properties of potent inhibitors from Annona muricata leaves against breastcancer

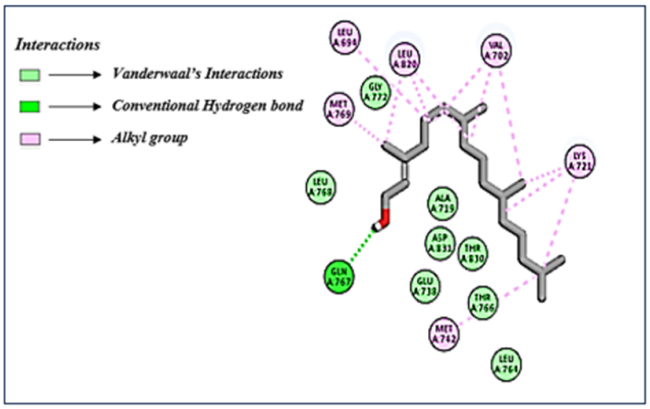

Fig 1. 3D molecular interaction view of Phytolagainst the Crystal Structure of TNF-alpha(1M17) Binding Domain

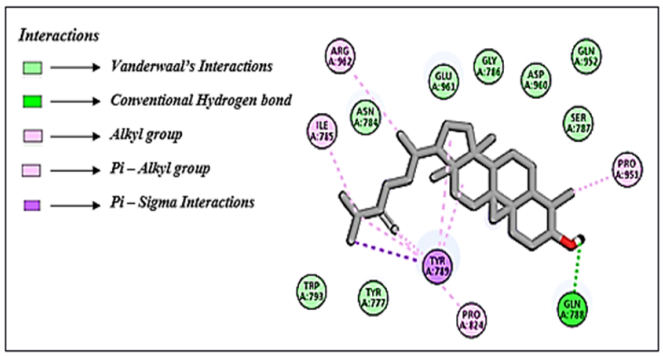

Fig 2. 3D molecular interaction view of Cycloeucalenol against the Crystal Structure of TNF-alpha(1M17)Binding Domain

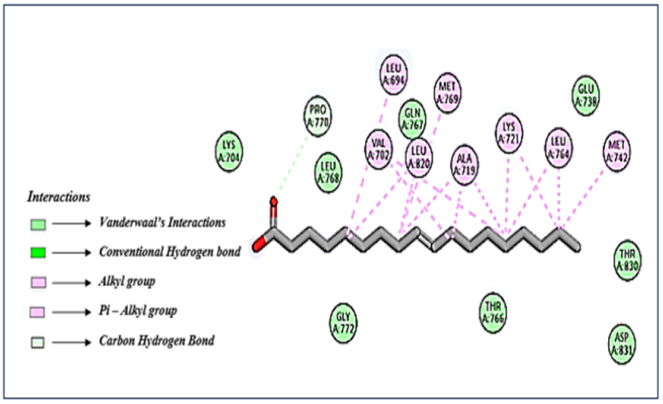

Fig 3. 3D molecular interaction view of Octadec -9 - enoic acid against the Crystal Structure of TNF-alpha(1M17) Binding Domain

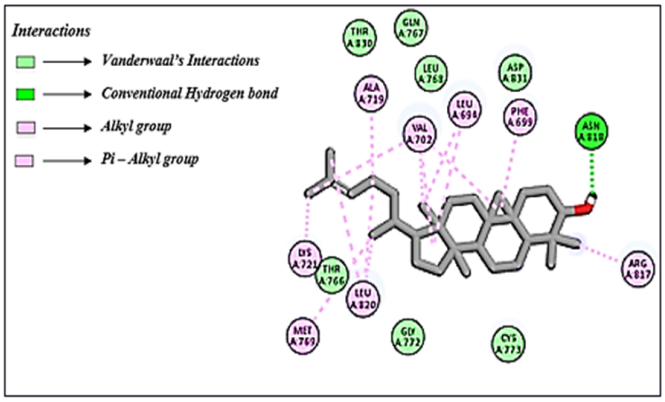

Fig 4. 3Dmolecular interaction view of (9,19-Cyclolanost-24-en-3-ol, (3.beta.) against the Crystal Structure of TNF-alpha(1M17)Binding Domain

4 Conclusion

The creation of drugs and the identification of multi-targeted inhibitors of numerous overexpressed proteins generated in breast cancer have benefited greatly from various computational methods. This study identifies four multi-functional ligands with strong binding energies to the most prevalent target proteins associated with breast cancer. Those ligand molecules include [9,19-Cyclolanost-24-en-3-ol, (3.beta.)] (-9.88 kcal/mol), Octadec - 9 - enoic acid (-8.77 kcal/mol), Cycloeucalenol (-5.70 kcal/mol), and Phytol (-5.61 kcal/mol). The ADMET profiling by the SwissADME tool and the bioactivity validation by the Molinspiration tool suggest that these molecules can act as a potential inhibitor of the TNF – α receptor molecule, which is a key molecule in the proliferation and survival of breast cancer cells. These computational methods are employed for early drug development against breast cancer after being evaluated through in-vitro and in-vivo research due to their good pharmacokinetics and pharmacodynamic properties as predicted by computational tools.

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