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Molecular docking approach for evaluating the anticancer efficacy of Phytoconstituents from *Annona squamosa* Linn. against breast cancer

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Abstract
Medicinal plants serve as valuable repositories of diverse photochemicals, offering remedies for a wide array of human ailments, including breast cancer. Their significant role extends to both modern and traditional medicine, exerting substantial physiological effects on the human body. The therapeutic potential of herbal treatments, enriched with an array of bioactive photochemicals, proves advantageous and reduced adverse effects compared to modern pharmaceuticals. Molecular docking has emerged as a crucial tool in drug discovery due to its cost-effectiveness and increasing popularity among researchers. The present investigation focuses on exploring the potential of *Annonaceous acetogenins* from *Annona squamosa* Linn. as anti-cancer agents, particularly targeting the estrogen receptor (ERα) protein (PDBID - 3ERT). The study employs ligands such as Annomuricin, Annosquacin I, Bullatacin, and Bullatalicin to interact with the ERα protein. Encouraging results are observed in terms of binding energy and inhibition constants, indicating their potential to impede the proliferation of breast cancer by targeting the ERα protein.

Keywords: Hernia, buffalo bull, umbilical, herniorrhaphy

Introduction
Cancer contributes significantly, with a staggering count of over 14 million new cases and an annual fatality rate of 8 million (Torre *et al.*, 2015) [60]. The progression of cancer is a complex, prolonged, and multi-phase process involving a myriad of intricate factors in its initiation, development, and advancement. Simultaneously, a combination of genetic and epigenetic variations drives the continuous transformation of a normal cell into a localized tumor mass, which subsequently spreads to neighboring and distant tissues and organs (Mohammad *et al.*, 2014) [32]. The surge in mortality rates is linked to the emergence of tumor recurrences, attributed to cells becoming resistant to chemotherapy and radiotherapy (Kawasaki *et al*., 2008) [25]. Consequently, there is an urgent need to devise an effective alternative strategy for the management and assessment of cancer (Balsam Rizeq *et al*., 2020) [51].

Breast cancer, a devastating malignancy impacting millions of women and their families on a global scale, stands as the second leading cause of cancer-related deaths in women (DeSantis *et al.*, 2011; Vahid Zarezade *et al*., 2018) [14, 63]. The origin of breast cancer is often traced back to milk glands or ducts connecting lobules to nipples (American Cancer Society, 2019) [3]. Being the most prevalent cancer among women, it affects around 2.1 million women annually and contributes significantly to cancer-related fatalities in this demographic. In 2018 alone, an estimated 627,000 women lost their lives to breast cancer, accounting for roughly 15% of all female cancer-related deaths (WHO, 2020) [66], with 58% death in developing countries (GLOBOCAN, 2008) [17]. Identifying these genes provides insights into the genetic and molecular mechanisms of the disease, subsequently guiding the search for effective therapies (Toss A and Cristofanilli M, 2015) [61].

A plethora of elaborate treatment approaches are employed to improve breast cancer, including chemotherapy, therapies targeting cancer gene receptors, radiation, and surgical interventions. These are complemented by sophisticated diagnostic techniques such as radiological imaging and the identification of oncogenes and tumor markers. However, the undesirable side effects stemming from cytotoxic anticancer drugs or radiation often lead to unexpected hospitalizations and the need for additional medications to manage adverse reactions (Rashid

et al., 2016) [49]. Relatively 60-80% of the world's community nevertheless rely on traditional medicines for the therapy of common illness (WHO 2002; Patwardhan *et al.,* 2005) [65, 44] which encourages the power of herbal constituents as a data based complementary therapy for malady like cancer (Mohammad *et al.,* 2014) [32]. Flourishing interest in natural product pharmacology has led to the recognition of phytochemicals which could inhibit key cellular signaling pathways with momentous transformation observed in cancer cells (Lee *et al.,* 2013, Biba Vikas *et al.,* 2019) [28, 7].

Annona squamosa L., commonly known as sugar apple, belongs to the Annonaceae family (Raj *et al.,* 2009; Srivastava *et al.,* 2011) [47, 57]. This semi-deciduous tree is found abundantly in tropical regions of South America and the West Indies (Morton, 1987) [36] and is cultivated in both tropical and subtropical areas across the globe (Ngiefu *et al.,* 1977; Yang *et al.,* 2009a) [41, 69]. Every part of the tree is extensively employed in traditional medicine for various ailments, including cancer and parasitism (Gajalakshmi *et al.,* 2011) [16]. Since the isolation of the key compound annonaceous acetogenin in 1982 (Tempesta *et al.,* 1982) [59], a significant number of studies have been conducted on the phytochemical and pharmacological aspects of these plants (Lage *et al.,* 2014; Pimenta *et al.,* 2014; Rout & Kar 2014; Chen *et al.,* 2016) [27, 45, 53, 72]. Various phytoconstituents have been isolated from *Annona squamosa*. These include diterpenes, such as Annosquamosin A, B, C, D, E,F and G and Anonaine, Roemerine, Norlaureline, Aporphine, Norcorydine, Corydine (Yang *et al.,* 2002; Zhou *et al.,*2013, Chen *et al.,* 2015, Yadav *et al.,* 2011, Bhakuni *et al.,*1972; You *et al.,*1995; Soni *et al.,* 2012, Hopp *et al.,*1998; Hopp, 1997; Oberlies *et al.,*1997, Ndob *et al.,* 2009; Li *et al.,* 2010; Chen *et al.,* 2012a; Liaw *et al.,* 2008) [73, 74, 68, 6, 71, 55, 22, 21, 43, 40, 30, 31, 12].

Computational approaches to drug discovery have the advantage of high speed, economical and even more importantly, it can enable to raise questions that would otherwise be difficult to address experimentally (Henley *et al.,* 2017) [20]. Molecular docking is one the utmost applied virtual screening programme and has metamorphosed increasingly supplemented on account of immense growth in 3D X-ray and NMR structures (Lengauer T., Rarey M. 1996) [29]. Due to its capability to forecast the binding conformation of small molecule ligands to the accurate target binding site molecular docking is one of the most periodically used methods for structure-based drug design (Kitchen *et al.,* 2004, Rohs *et al.,* 2005, Guedes *et al.,* 2014, Agarwal *et al.,* 2015, Singh *et al.,* 2017) [26, 52, 19, 1, 54]. Auto dock is a extensile ligand- protein docking programme essentially runs as two steps procedure: the calculation of the map of interactions of the binding site with some general atom types (performed with AutoGrid) and the posing of the ligand respecting this map of interaction (performed with AutoDock). The version used in the study is Version 1.5.6, which provides paramount new features like protein residue flexibility and high quality scoring functions (PLDA, 2020) [46].

The objective of this present investigation is to elucidate the inhibitory mechanism of bioactive compounds sourced from *Annona squamosa* Linn., specifically Annomuricin A, Annosquacin I, Bullatacin, and Bullatalicin. These compounds will be examined for their potential to interact with a significant therapeutic target receptor, namely ERα (Estrogen receptor), which plays a pivotal role in both the initiation and advancement stages of breast cancer (Reetuparna Acharya *et al.,* 2019) [50].

Materials and Methods

Molecular study strategy

Ligand-protein reactions, mode of binding and binding affinity of phytoconstituents of *Annona squamosa* Linn. with estrogen receptor were determined by docking method using Autodock tools Version 1.5.6, Open Babel Version 2.4.1, Discovery Studio Visualizer v16.1.0.15350 respectively through the following steps.

Protein preparation

The atomic coordinates of the protein ERα (Estrogen Receptor) were recovered from RCSB Protein Data Bank (*https://www.rcsb.org/pdb*) (Berman *et al.,* 2000 and Deshpande *et al.,* 2005) [5, 15] with their corresponding PDB ID 3ERT respectively. Before docking analysis, the heteroatom were detached and supplemented H-atoms, assigning charges, solvation parameters and fragmental volumes to the protein was done using the automated docking tool, Autodock (Goodsell *et al.,*1996, Jones *et al.,*1997, Rarey *et al.,* 1996, Vaishali Chandel *et al*., 2020) [18, 23, 48, 64]. To analyze the synergy of determined bioactive associates, the energy was minimized using SwissPDB viewer (SPDBV) (Sonia Mann *et al.,* 2015) [56].

Binding site prediction

Discovery studio was used in the present study for exploring the possible binding sites for the target receptors and anticipating the ligand binding site which is coupled with structural cavities and pockets. (Mohankrishna Ghanta, 2018) [33].

Ligand Preparation

Chem Draw Ultra 11.0 was used for generating the three dimensional structure of ligands *viz.* Annomuricin A, Annosquacin I, Bullatacin and Bullatalicin (Mohankrishna Ghanta, 2018) [33], on the ground of canonical SMILES of the elected ligands obtained from Chem Draw, the files were transformed in to .pdb using online smiles translator. The framed 3D structures of the composites were saved in.pdb format and were eventually progressed for docking using UCSF Chimera tools (Vaishali Chandel *et al.,* 2020) [64].

Docking Analysis

Autodock Tools (ADT) version 1.5.6 and Autodock version 4.2 programmes (Morris GM *et al.,* 2009) [35] from http://www.scripps.edu/mb/olson/doc/autodock, Scripps Research Institute were used to perform this docking analysis. The non-polar hydrogen moiety were consolidated with carbon and ligand fraction were added with polar hydrogen. Intramural degrees of freedom and torsions were set. The compounds Annomuricin A, Annosquacin I, Bullatacin and Bullatalicin were docked with target protein Estrogen receptor (PDB ID: 3ERT) with ligands being flexible and protein molecules were considered as a rigid body. Individual ligand compound was given as input in the parameter meant for "ligand" and the protocol was run for each of the ligands.

Computation with grid spacing of 0.5Å was set for affinity and electrostatic mapping of all atom types existing in protein molecules were carried out using Autodock tools. For docking conformational search Lamarckian Genetic Algorithm was used with 50 GA runs having a population size of 300 with a mutation rate of 0.02 evolved for 10 generations (Morris *et*

al., 1998) [34]. A cluster inspection was based on root mean square deviation (RMSD) values, referring to starting geometry was implemented and the lowest energy conformation of the most colonized cluster was considered as the most trustable solution. The AutoGrid 4.2.3 and AutoDock 4.2.3 programs were used to produce grid maps and to obtain results Based on the predicted binding energy, the Dock score of the best poses docked into the target protein for all the tested compounds was calculated. Ligand-protein complex were sorted and evaluated by Discovery studio of the selected compounds including pose view (Mohankrishna Ghanta, 2018) [33].

Results

Table 1: Binding Modes of Phytoconstituents of *Annona squamosa* Linn against Estrogen Receptor

Protein	Compound	Run with min. Energy	RMSD (A)	Binding Energy (Kcal/Mol)	Inhibition Constant (µM)
Human estrogen receptor alpha ligand-binding domain in complex with 4-hydroxytamoxifen. (PDB ID: 3ERT)	Annomuricin A	10	35.85	-4.24	78.80
	Annosquacin I	2	32.32	-5.60	78.91
	Bullatacin	46	33.30	-4.92	246.91
	Bullatalicin	41	32.87	-5.85	51.64
	4-Hydroxytamoxifen	45	37.04	-9.02	243.47

Table 2: Surface characteristics of Small molecule (Ligands) of *Annona squamosa* Linn.

Compound	No. of Rotatable bond	No. of atoms	No. of non-hydrogen atom	No. of Vibrational degree of freedom	No. of torsional degrees of freedom	Estimated loss of torsional free energy upon binding	Center of rotation	Free energy coefficient for torsional degrees of freedom
Annomuricin A	31	48	43	138	31	+9.2473	-0.075 -0.280 -0.019	0.2983
Annosquacin I	28	47	44	135	28	+8.3524	-0.003 -0.111 -0.081	0.2983
Bullatacin	28	47	44	135	28	+8.3524	-0.104 -0.047 -0.023	0.2983
Bullatalicin	29	49	45	141	29	+8.6507	-0.024 -0.058 -0.085	0.2983
4-Hydroxytamoxifen	9	30	28	84	9	+2.6847	-0.107 -0.003 -0.015	0.2983

Table 3: Hydrophobic Interactions between Annomuricin A with Estrogen Receptor

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	346A	LEU	3.71	2413	362
2	346A	LEU	3.68	2416	364
3	349A	LEU	3.55	2429	392
4	391A	LEU	3.57	2422	804
5	424A	ILE	3.48	2419	1124
6	522A	MET	3.84	2404	2094
7	525A	LEU	3.55	2396	2126
8	526A	TYR	3.58	2399	2135
9	526A	TYR	3.15	2400	2138

Table 4: Salt Bridge Interactions between Annomuricin A with Estrogen Receptor

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	394A	ARG	4.77	✔	Carboxylate	2425, 2426

Table 5: Hydrophobic Interactions between Annosquacin I with Estrogen Receptor

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	346A	LEU	3.77	2404	365
2	346A	LEU	3.37	2411	364
3	347A	THR	3.86	2397	374
4	350A	ALA	3.18	2401	400
5	387A	LEU	3.58	2401	771
6	391A	LEU	3.72	2405	804
7	404A	PHE	3.41	2404	936
8	404A	PHE	3.49	2405	938
9	424A	ILE	3.84	2412	1124
10	522A	MET	3.90	2422	2094
11	525A	LEU	3.74	2421	2126
12	526A	TYR	3.37	2422	2135
13	535A	PRO	3.71	2430	2225
14	536A	LEU	3.22	2426	2235

Table 6: Hydrogen bond Interactions between Annosquacin I with Estrogen Receptor

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	525A	LEU	1.90	2.71	139.95	✖	✖	2431 [O3]	2125 [O2]

Table 7: Hydrophobic Interactions between Bullatacin with Estrogen Receptor

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	346A	LEU	3.44	2401	362
2	347A	THR	3.51	2395	374
3	350A	ALA	3.53	2396	400
4	383A	TRP	3.46	2391	735
5	383A	TRP	3.76	2390	733
6	424A	ILE	3.17	2409	1124
7	525A	LEU	3.10	2407	2129
8	525A	LEU	3.22	2412	2126
9	526A	TYR	3.61	2417	2138
10	529A	LYS	3.46	2420	2166
11	533A	VAL	3.98	2423	2211
12	534A	VAL	3.73	2430	2219
13	539A	LEU	3.77	2430	2268

Table 8: Hydrogen bond Interactions between Bullatacin with Estrogen Receptor

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	536A	LEU	3.14	3.90	132.40	✔	✖	2228 [Nam]	2431 [O3]

Table 9: Hydrophobic Interactions between Bullatalicin with Estrogen Receptor

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	346A	LEU	3.28	2417	362
2	347A	THR	3.01	2390	374
3	349A	LEU	3.07	2432	394
4	350A	ALA	3.87	2416	400
5	383A	TRP	3.22	2416	735
6	384A	LEU	3.39	2422	743
7	387A	LEU	3.63	2425	769
8	404A	PHE	3.99	2432	936
9	522A	MET	3.46	2405	2094
10	525A	LEU	3.54	2416	2128
11	525A	LEU	3.72	2418	2129
12	525A	LEU	3.22	2401	2126
13	526A	TYR	3.40	2402	2135

Table 10: Hydrogen bond Interactions between Bullatalicin with Estrogen Receptor

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	387A	LEU	3.10	4.06	176.42	✖	✖	2433 [O3]	768 [O2]

Table 11: Hydrophobic Interactions between 4-Hydroxy Tamoxifen with Estrogen Receptor

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	346A	LEU	3.43	2409	362
2	346A	LEU	3.25	2415	364
3	347A	THR	3.89	2400	374
4	350A	ALA	3.49	2408	400
5	383A	TRP	3.29	2398	735
6	384A	LEU	3.69	2395	743
7	384A	LEU	3.85	2394	744
8	391A	LEU	3.56	2412	804
9	404A	PHE	3.26	2414	938
10	424A	ILE	3.48	2393	1124
11	525A	LEU	3.17	2397	2128
12	525A	LEU	3.83	2401	2129

Table 12: Hydrogen bond Interactions between 4-Hydroxy Tamoxifen with Estrogen Receptor.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	351A	ASP	1.93	2.78	139.74	✖	✔	2405 [N3]	408 [O2]

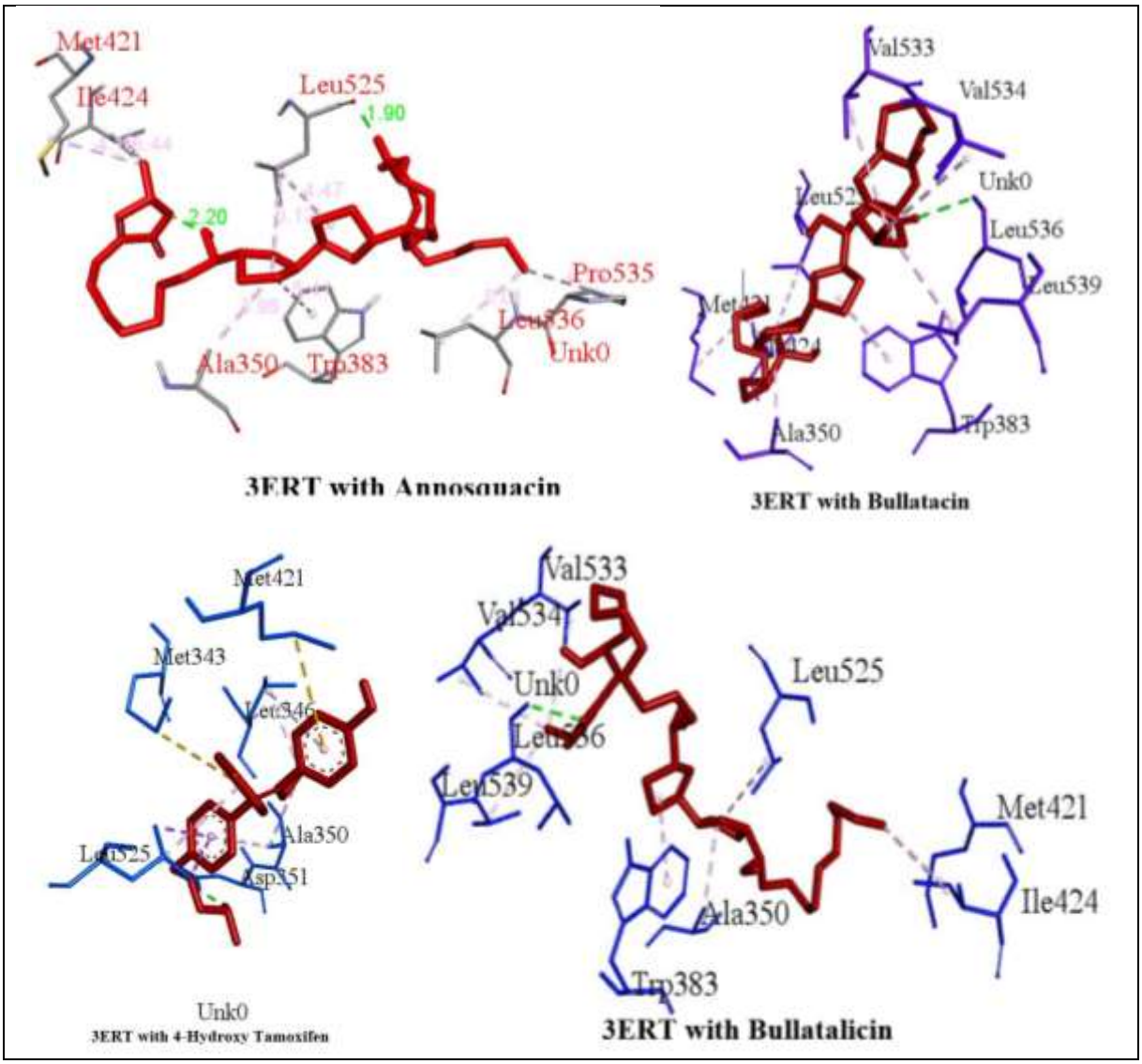

Fig 1: 3D structure of Potential binding site amino acids of Oestrogen receptor with ligands of *Annona squamosa* Linn.

Discussion

The journey of drug discovery is a prolonged, investigative, and formidable undertaking, not to mention the substantial financial resources it demands (Tufts Center for the Study of Drug Development, 2014) [62]. The concept of identifying a target, aligning it with a suitable compound possessing pertinent attributes, and eventually progressing to market availability is a laborious, exceedingly intricate, and somewhat serendipitous endeavor (Muntha, 2016; FDA, 2020) [37, 64]. To surmount these challenges, molecular docking emerges as a commendable solution, enabling the evaluation of thousands of potential hit molecules within a matter of days. Moreover, virtual screening aids in identifying novel targets for which suitable leads are not yet available (Alvarez, 2004, Natarajan Sathishkumar *et al.*, 2012) [2, 39]. Over time, this approach has gained increasing utility due to the remarkable expansion in three-dimensional X-ray and NMR structures, along with the enhanced resolutions recorded in the Protein Data Bank (PDB) (Rcsb.org, 2020) [4].

Plants belonging to the Annona genus have a long history of traditional use in treating a diverse range of ailments, encompassing both noninfectious and infectious diseases. Annona species have demonstrated potential as anti-cancer agents, although comprehensive evaluations in this regard have been limited (Nugraha, 2019, Coria-Tellez *et al.*, 2018) [42, 13]. Phytochemical analyses of seed extracts have highlighted Annonaceous acetogenins as the primary active constituents (Chen *et al.*, 2012) [11]. Previous reports have also indicated the potent anti-neoplastic activity of custard apple peels (Naik *et al.*, 2008; Joy and Remani, 2008) [38, 24].

Applying the aforementioned knowledge, we conducted docking studies utilizing Autodock tools on Annonaceous acetogenins extracted from *Annona squamosa* Linn. Subsequent to the docking process, the ligands were categorized based on their affinity to the protein receptor. Our investigation focused on the interaction between the cancerous target protein and Annonaceous acetogenins, hinging on the binding energies of the resultant complexes. The accuracy of the AutoDock outcomes was established by considering the lowest binding energy values and the formation of hydrogen bonds between the receptor proteins and acetogenins (Natarajan Sathishkumar *et al.*, 2012) [39]. The rankings of docking results were determined according to the binding energy exhibited by the acetogenins' ligands. During the docking simulation, hydrogen bond formations were observed, as illustrated in Fig. 02 and Fig.03, respectively. Moreover, a noteworthy hydrogen bond formation was observed between Annosquacin I and Bullatacin, as well as Bullatalicin, at distances of 1.9, 3.14, and 3.10, alongside a concurrent occurrence of a hydrogen

bond with 4-hydrogen Tamoxifen at a distance of 1.93.

The binding energies of Annomuricin A, Annosquacin I, Bullatacin and Bullatalicin were maximum at runs 10, 2, 46, 41 and with 4-hydroxy Tamoxifen it is 45th run. Correspondingly the RMSD, Binding energy (Kcal/Mol) and Inhibition energy (μM) includes -4.24, -5.60, -.92, -5.85 and -9.02 and 78.8, 78.91. 246.91, 51.64 and 243.47 respectively. The amino acid participated for hydrophobic interaction with Annomuricin A includes LEU346A. LEU346A, LEU349A, LEU391A, ILE424A, MET522A, LEU525A, TYR526A, TYR526A and for salt bridge interactions ARG394A. The amino acid participated for hydrophobic interaction with Annosquacin I includes Leu346A, THR347A, ALA350A, LEU391A, PHE404A, ILE424A, MET 522A, LEU525A, PRO535A, and LEU536A and with hydrogen bond includes LEU525A. The amino acid participating for hydrophobic interaction with Bullatacin includes LEU346A, THR347A, ALA350A, TRP383A, ILE424A, LEU525A, TYR526A, LYS529A, VAL533A, VAL534A and hydrogen bond participated includes LEU536A. For Bullatalicin, the hydrophobic interaction involved includes LEU346A, THR347A, ALA 350A, TRP383A, LEU384A, LEU384A, Phe4.4A, MET522A, LEU525A, LEU525A, TYR526A and hydrogen bond interaction with LEU387A. For 4-Hydroxy Tamoxifen, the amino acids participating in hydrophobic interaction includes LEU346A, THR347A, ALA350A, TRP383A, LEU384A, LEU384A, LEU391A, PHE404A, ILE424A, LEU525A and the hydrogen bond interaction between 4-Hydroxy Tamoxifen with estrogen includes ASP 351A respectively.

Based on the results all the Annonaceous acetogenins of *Annona squamosa* were found to have acceptable fastening affinity with estrogenic receptor. Thus, the expression of ERα (Estrogen receptor), which plays a crucial role in proliferation of breast cancer cells, their expression rate has been decreased considerably. Docking simulation and molecular docking studies results confirmed that Annonaceous acetogenins of *Annona squamosa* Linn are potential ligands for cancer promoting protein like ERα (Estrogen receptor).

Conclusion

Annona squamosa has been used since years for their medicinal properties, latterly, many studies have focused on the identification of cancer inhibitors from natural sources, and clinical studies have just begun. Our studies concludes that Annonaceous acetogenins, particularly Annomuricin A, Annosquacin I, Bullatacin and Bullatalicin I from natural sources from *Annona squamosa* will be effective compounds to control the overexpression of crucial protein for the propagation of breast cancer named ERα (Estrogen Receptor) establishing these agents may be used in cancer treatment. Further, Annonaceous acetogenins should be subjected to further experimental studies in order to confirm these findings.

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