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Comparative Docking Analysis of Vitexin and Tamoxifen with EMT Marker

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Abstract **: Epithelial Mesenchymal Transition (EMT), is an evolutionary conserved developmental program, has been implicated in carcinogenesis and confers metastatic properties upon cancer cells by enhancing mobility, invasion and resistance to apoptotic stimuli. When EMT occur the connection between the cells were broken, their cytoskeleton was rearranged, and the ability of migration, invasion and apoptosis can be changed. Therefore it is of great significance for tumour invasion and metastasis via inhibition of EMT. Cancer metastasis is a significant target in clinical treatment. The present comparison study focuses on molecular docking analysis of vitexin - a well known flavone glucoside isolated from the roots of *Vitex negundo* and drug tamoxifen on EMT marker trans membrane protein E-cadherin. The results of molecular docking analysis determines the binding capacity of ligand on receptor, ability of stable complex formation and the role of ligand in modifying the action of receptor protein and shows that the vitexin can able to interact with E-cadherin in regulating the EMT process on controlling cancer progression.**

Keywords : E-cadherin, Vitexin, Tamoxifen, EMT, Autodock, Docking.

I. INTRODUCTION

Docking is a molecular modelling technique that is used to predict how a protein (enzyme) interacts with small ligand molecules (Cichero *et al*., 2013). Docking is useful for predicting both the strength and type of signal produced during molecular interaction. Molecular docking is one of the most frequently used methods in structure based drug design, due to its ability to predict the binding conformation of small ligand molecule to the appropriate target binding site (Meng *et al.*, 2011). Cadherins are transmembrane proteins that mediate cell - cell adhesion in animals (Priest *et al*., 2019). By regulating contact formation and stability, cadherins play a crucial role in tissue morphogenesis and homeostasis. The present work was performed as a comparative study, the docking is performed with a single transmembrane protein E-cadherin with vitexin and tamoxifen as ligands. Vitexin is isolated from the roots of *Vitex negundo* plant. *Vitex negundo* is also known as Chinese chaste tree. Tamoxifen is the oldest and most-prescribed selective estrogen receptor modulator (SERM) and it is approved by the U.S. Food and Drug Administration (FDA) to treat the patient in the early stages of breast cancer and prescribed for women who are in higher risks of getting breast cancer (Binkhorst *et al.*, 2015). The efficiency of binding of ligands with E-cadherin is observed and it is performed using autodock vina software and visualization of both the ligands with E-cadherin is done with pymol and discovery studio (Ballante *et al*., 2016).

II. EXPERIMENTAL PROCEDURE

A. Protein Preparation

The three dimensional structure file of the drug target was selected from the structure retrieval tool. Protein sequence was selected from uniprot, and checked for its function, subcellular location and sequences. The FASTA format is obtained and the FASTA sequence is further used in Rasmol software for its three dimensional structure. The target protein is E-cadherin, a transmembrane protein which is unavailable in FASTA sequence in uniprot, so swiss homology modelling is preferred for structure retrieval (Vrevan *et al.*, 2014). Swiss model assists and guides the user in building protein homology models at different levels of complexity. Successful model building requires at least

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one experimentally determined 3D structure (template) that shows significant amino acid sequence similarity with the target sequence (Sainy & Sharma, 2017). To visualize the 3D structure, which is obtained from swiss model, rasmol is used. Rasmol is a program for molecular graphics visualization. (Hauser & Windshugel, 2015). All water molecules were removed and Gasteiger charges were checked. To initiate docking process, it is important to note that the structures used in docking should be in PDB format and all the hydrogen atoms are to be removed. E-Cadherin has A, B and C aminoacid chains, for accurate results either of the two chains were removed, only A chain is selected and docking is performed (Mostashari-Rad *et al*., 2019). The prepared protein target E- cadherin is further used for the docking process.

B. Ligand Preparation

The selected ligands are vitexin and tamoxifen. Vitexin is an apigenin flavone glycoside, which is obtained from the roots of *Vitex negundo*, it is used as an anticancer, anti-inflammatory, anti-analgesic agents. Tamoxifen is a drug used to treat breast cancer patients in the initial stages of cancer. These ligands are taken from Pubchem database (Vitexin - Compound ID - 5280441, Tamoxifen Compound ID - 2733526) (Kim *et al.,* 2015). The structures are then designed using ACD Chemsketch software (Salma Jamal & Abhinav Grover 2017). The ACD Chemsketch gives the three dimensional structure of the ligands. From this software, the SMILES format of the structure was obtained. The open babel software is used to convert the SMILES format which is obtained from ACD Chemsketch software. The conversion of ligand structure from SDF to PDB format, was carried out using open babel software (Yoshikawa *et al.,* 2019). The SMILES format which is taken from pubchem database are pasted in the molinspiration software and it is used to calculate the compound property. Molinspiration includes some of the log values like mi log value, TPSA (Topological polar surface area), which is related to intestinal absorption, the molecular weight of the compound and the number of violations were also calculated (Singh & Gupta, 2013). The SwissADME (Absorption, Distribution, Metabolism, Elimination) is similar software like molinspiration, where the SMILES format are taken from the pubchem database and it is used in swiss ADME, to predict the bioavailability, physiochemical property, Lipinski filter of the desired ligands. If the values of these parameters are normal, if there is zero violations and the other mentioned properties has a good score. Then the ligands are considered to be utilised in docking and it signifies its clinical properties to be used as drug. The prepared ligand is further used for docking analysis (Sharma *et al.,* 2016).

C. Protein-Ligand Interaction

Autodock is the one of the best studies of automated docking tools. The software is used for the modelling flexible small drug molecules binding to the target proteins of known structure. Genetic algorithms are used for checking the conformational search. Auto dock is a user friendly tool to perform blind docking, where the location of binding is not known. Molecular docking is performed using the Autodock tools 4.2. graphical user interface, which generates different confirmations of protein ligand complex customized in the order from lowest to highest binding free energy (ΔG) (Shah *et al.,* 2018). The autodock is performed using E-cadherin, as a protein target and vitexin and tamoxifen are ligands. The input files - the protein, the ligand, the grid are prepared for docking procedure. The grid is prepared with the specific active sites of both the ligands, so that the protein binds to the active site of the ligand. After the preparation of protein and ligand, the autogrid function is performed. Before performing the autodock function, the docking file is prepared with the lamarckian algorithm. (Gorgulla *et al.,* 2020). The docking procedure is divided into two steps autogrid and autodock. The grid box is used to select the specific sites in protein and with that active site the ligands gets bound. After setting the grid box the autogrid is allowed to run. To get the output some of the parameters like program path file and Lamarckian algorithm are calculated (Ciemny *et al*., 2018).

D. Pymol and Discovery Studio Visualization

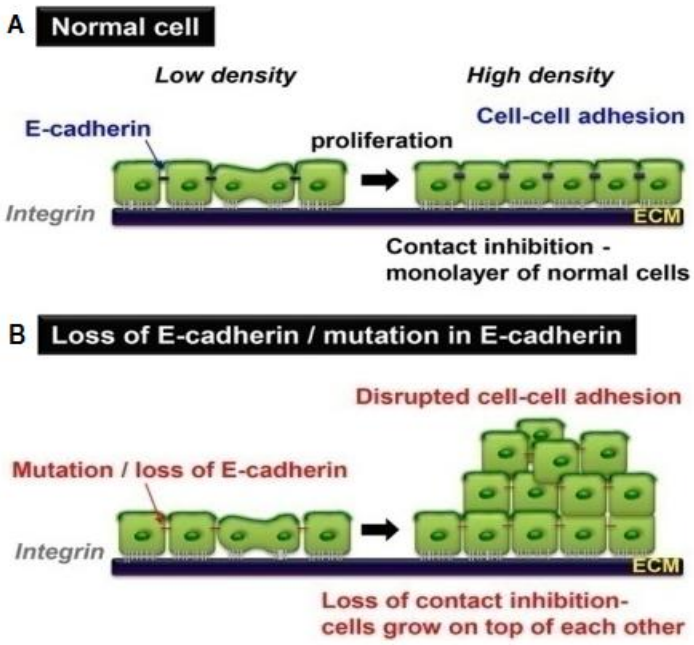
Pymol software is used to visualize the receptor-ligand complex which has obtained from docking. Biovia discovery studio also can be used to visualize protein- ligand interactions through 2D and 3D views. (Fasnacht *et al*., 2014).

III. RESULTS AND DISCUSSION

A. Structure of E-Cadherin

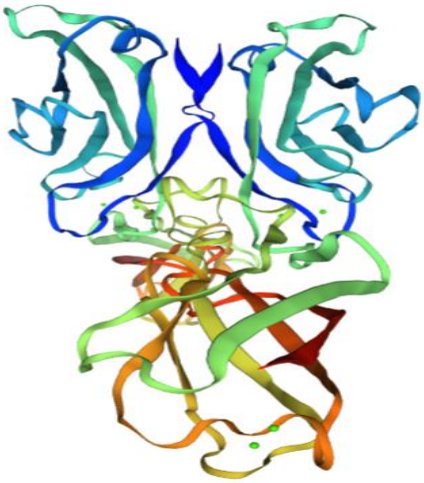
E-cadherin is a trans membrane protein, which mediates cell-cell adhesion. 'E' represents epithelial cells, which is responsible for regulating the contact formation and stability. Thus E - cadherins are helpful in tissue morphogenesis and homeostasis (Fig.1)**.** Because mesenchymal cells are responsible for development of bones, tendons and it helps in repairing of skeletal muscles. In case of cancer progression EMT transition plays a primary role in cancer metastasis. So, to inhibit metastasis (spreading of cancer cells) of mammary cancer to the secondary site, there is a need to inhibit the conversion from epithelial cells to mesenchymal cells. Preliminary docking analysis was done with selected E - cadherin as a target protein (Fig..2) using the ligands vitexin and tamoxifen. (Adu-Gyamfi *et al.,* 2021; Zaidel-Bar, 2013).

Fig 1. Role of E - Cadherin.



A - Cell - cell adhesion. B - Cells gets disturbed and there is a loss of contact inhibition, further leads to advanced stages of cancer.

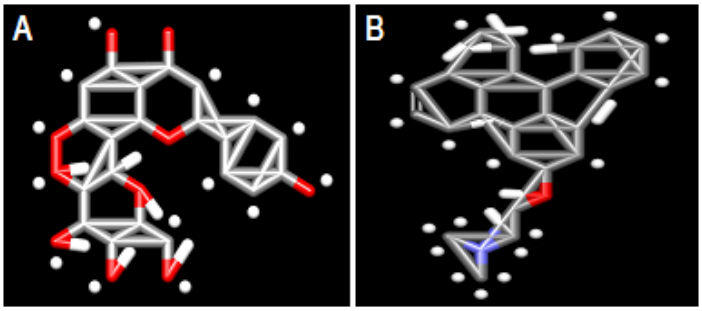
Fig 2. Three dimensional structure of E- cadherin.



B. Structure of Vitexin and Tamoxifen

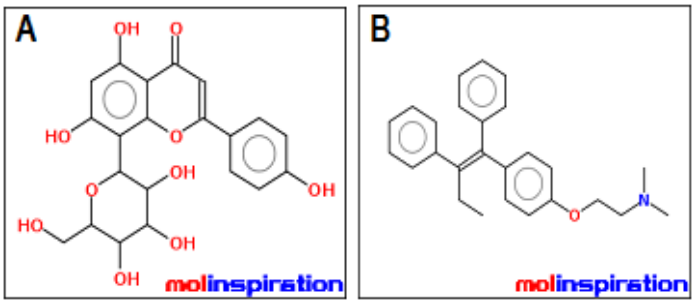
A comparative analysis of the selected ligands vitexin and tamoxifen (Fig 3 and Fig 4) were performed. The Molinspiration and swiss ADME is used to predict the properties of these ligands. Molinspiration is to predict the ligand properties like log p (partition coefficient) values, number of atoms, number of violations, Topological Surface Area (TPSA) etc. (Shagufta & Ahmad, 2018).

Fig 3. Three Dimensional structure



A – Vitexin, B - Tamoxifen .

Fig. 4. Two Dimensional structure



A – Vitexin, B - Tamoxifen

The results depicted in Table 1 details the properties of the selected ligands vitexin and tamoxifen which clearly indicates that the vitexin has more surface area than tamoxifen which reflects in total number of atoms and their molecular weight. (Chua *et al.*, 2013).

Table 1 The compound property prediction of Vitexin and Tamoxifen by Molinspiration.

Compounds Property Prediction	Molinspiration Property Engine	
	Vitexin	Tamoxifen
miLOGP	0.52	6.06
TPSA	181.04	12.47
Natoms	31	28
MW	432.38	371.52
nON	10	2
nOHNH	7	0
Nviolations	1	1
Nrotb	3	8
Volume	355.20	376.13

miLOGP - molinspiration LOG Prediction, TPSA - Topological Polar Surface Area, Natoms - Number of atoms, MW- Molecular Weight, nON- number of hydrogen bond acceptor, nOHNH - Number of hydrogen bond donor, Nviolations - Number of violations, Nrotb - Number of rotatable Bonds.

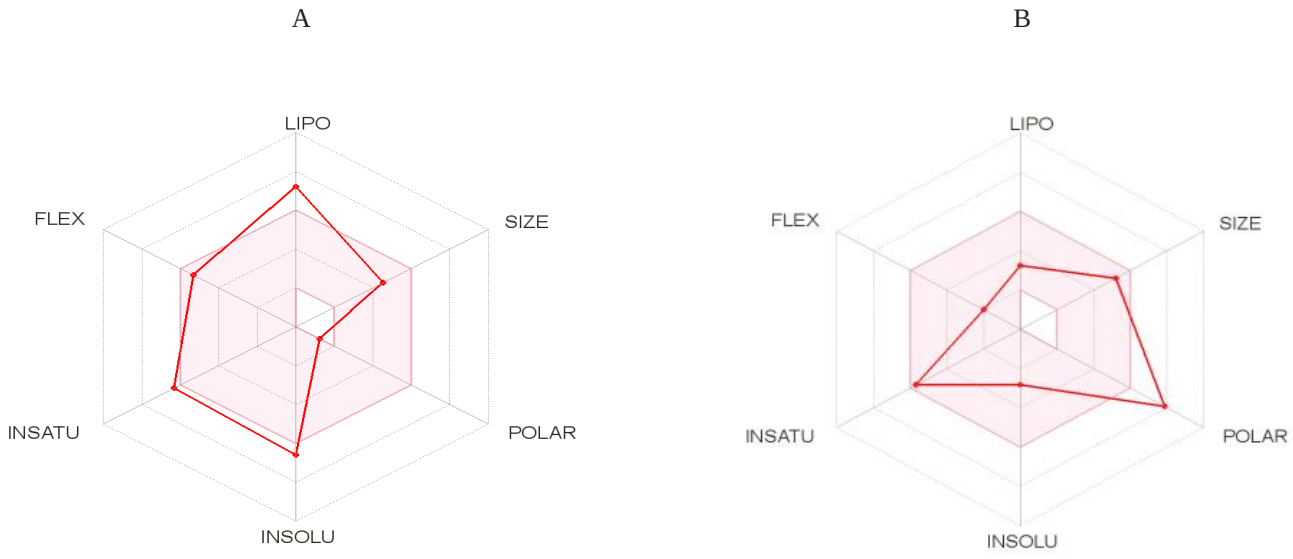
The results obtained from molinspiration property prediction and bioactivity prediction, it was clearly evident that on comparison with tamoxifen, vitexin possess good structural arrangement with convenient hydrogen bond acceptor and wide surface area can able to easily bind and interact with the target protein. Table II, indicates the kinase inhibitor which is responsible for the compounds to binds with cancer protein should be 0.18 or above. Vitexin has the kinase inhibitor activity at 0.19 and tamoxifen has -0.01, which is very less to bind with the cancer protein (Duffy *et al.*, 2015). The results, states that the vitexin has a good structural flexibility and it has good intestinal absorption, which is calculated by Nrotb (rotatable bonds) and TPSA respectively. The enzyme inhibitor capacity of vitexin is 0.46 and tamoxifen is 0.32. From this result, it is again proven that vitexin have good binding capacity with the selected protein target. Swiss ADME tells about the description of compounds. Vitexin has zero violations and it has 3 rotatable bonds and it has a good Lipinski score and bioavailability score (Fig 5).

Table II The bioactivity prediction score of Vitexin and Tamoxifen.

Molinspiration Bioactivity Score	**Vitexin**	**Tamoxifen**
GPCR ligands	0.13	0.30
Ion channel modulator	-0.14	0.00
Kinase inhibitor	0.19	-0.01
Nuclear receptor ligand	0.23	0.57
Protease inhibitor	0.03	0.04
Enzyme inhibitor	0.46	0.32

GPCR - G Protein Coupled Receptor.

Fig 5. Bioavailability radar A. Vitexin and B. Tamoxifen



Pink zone depicts suitable physicochemical space for oral bioavailability for Vitexin (LIPO indicates lipophilicity in terms of XLOGP3, SIZE indicates in terms of molecular weight, POLAR indicates polarity in terms of topological polar surface area, INSOLU depicts insolubility in water in terms of log S scale, INSATU refers to saturation as per fraction of carbons in the sp3 hybridization and finally FLEX indicates flexibility as per rotatable bonds.

C. Docking Analysis

The three dimensional molecular interaction of vitexin and tamoxifen were identified with E-cadherin on docking process. The grid box is designed to bind with the specific site. For autodocking the Lamarckian genetic algorithm is selected for ligand conformational searching. The results obtained are detailed in Table III & Table IV

Table III Docking score of protein - ligand complex

S.No.	Conformation Parameters	Vitexin	Tamoxifen
1.	Binding Energy	-15.29	-10.54
2.	Ligand Efficiency	-0.49	-0.38
3.	Inhibitory Constant	6.22	18.91
4.	Intermolecular Energy	17.38	12.33
5.	Van der waals Interaction	17.47	10.39
6.	Electrostatic Energy	0.1	-1.94
7.	Torsional Energy	2.09	1.79
9.	Unbound Energy	5.89	54.39
10.	Amino acids	ASP257 ASN258 LYS259 ALA289	ARG222 ALA289 ASN294 ALA298

Table IV The distance and interacted amino acid residues of E-cadherin with vitexin and tamoxifen.

E-Cadherin (Protein)	AA Residue	Distance (A)	Docking Energy (kcal/mol)
Vitexin (ligand)	ASP257	1.77	-15.29
	ASN258	1.72	
	LYS259	2.68	
	ALA289	4.40	
Tamoxifen (ligand)	ARG222	3.71	-10.54
	ALA289	4.24	
	ASN294	3.08	
	ALA298	5.12	

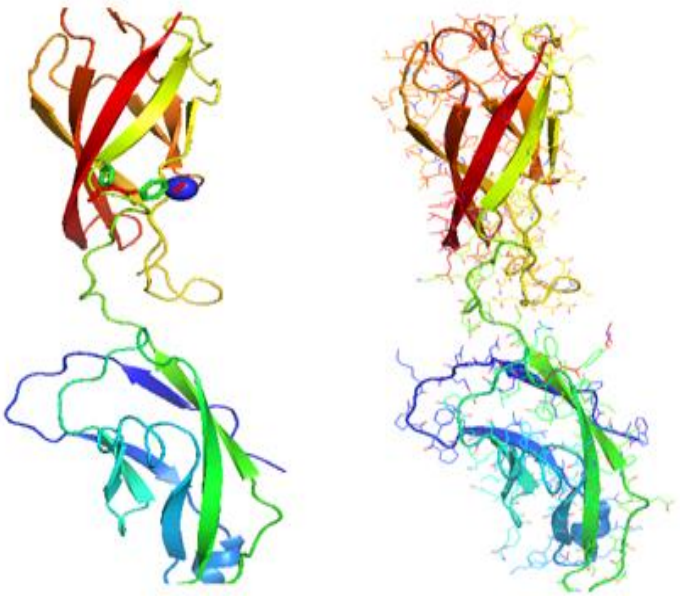
A preferable docking configuration was chosen based on the lowest empirical binding free energy and the most frequent cluster. AutoDock results are ranked according to the highest negative binding free energies and corresponding Root Mean Square Deviation (RMSD) values from the experimentally determined binding site. AutoDock shares functional commonalities, including the global optimization of the scoring function, pre-calculation of grid maps, and the pre-calculation of distant dependent pair-wise energetic between each atom type. However, they employ a different scoring function and algorithms to obtain binding free energies and should be considered as different programs (Trott & Olson, 2010).

D. Visualization of Protein-Ligand interaction

The docked protein-ligand complex visualized using BIOVIA Discovery Studio visualizer, and the distances of interactions are calculated. The best-docked ligand models were selected according to the lowest binding energy. Two and three-dimensional conformational structures of the ligand-protein complexes were visualized using BIOVIA Discovery Studio Visualizer v.4.5 (Systemes, 2017; Qasaymeh *et al.,* 2019) to investigate the binding modes. Both the ligand binds at the target protein by non-covalent interactions, such as, H-bonding, alkyl, alkyl-π, π-π, π-σ, and van der Waals interactions. Simplified visualization is illustrated in 2D, which displays the H-bonding, van der Waals forces, carbon-oxygen dipole-dipole interaction, alkyl-pi interactions, T-shaped pi-pi stacking, and pi-pi stacking. Docking analysis is less laborious, easy to perform, and yield quick results than classical drug designing techniques. Docking based drug discovery approaches is a successful methodology to develop small novel herbal-based drugs to solve various clinical complications. AutoDock Vina docking enrolls better-performing speedy analysis and elucidates better result accuracy (Meng *et al.*, 2011).

The pymol visualisation image of vitexin and tamoxifen, the ligand binding efficiency was calculated as -0.49 and -0.39 for vitexin and tamoxifen respectively (Fig. 6).

Fig. 6. The pymol visualization of E-cadherin with Vitexin and Tamoxifen



The three dimensional interactions and two dimensional interactions of vitexin and tamoxifen with E-cadherin clearly indicates that the vitexin formed four conventional hydrogen bonds with the binding site of E-cadherin through the side chains of the amino acids - ASP257, ASN258, ALA289, LYS259, and the distance among the amino acids were calculated as 1.77, 1.72, 2.68, 4.40 A° respectively. Similarly the drug tamoxifen also effectively interacted the target protein E-cadherin through the amino acids interacted are ARG222, ALA289, ASN294, ALA298, the distance among the amino acids were found to be 3.71, 4.24, 3.08, 5.12 A° respectively. (Fig. 7 & Fig. 8). The docking results obtained from the comparison of E-cadherin with vitexin and tamoxifen, it is clearly evident that, vitexin is a efficient ligand interact with the trans membrane protein target E-cadherin, which have the significant role in cancer metastasis and other molecular interaction studies with of similar EMT marker proteins such as N-cadherin, valentine, snail will further confirm the their inhibitory role on EMT. The overall results of the present investigation will benefit for the drug development and provides good pharmacological effect in the treatment of mammary cancer.

Fig. 7 3D Interactions of E-cadherin with Vitexin and Tamoxifen

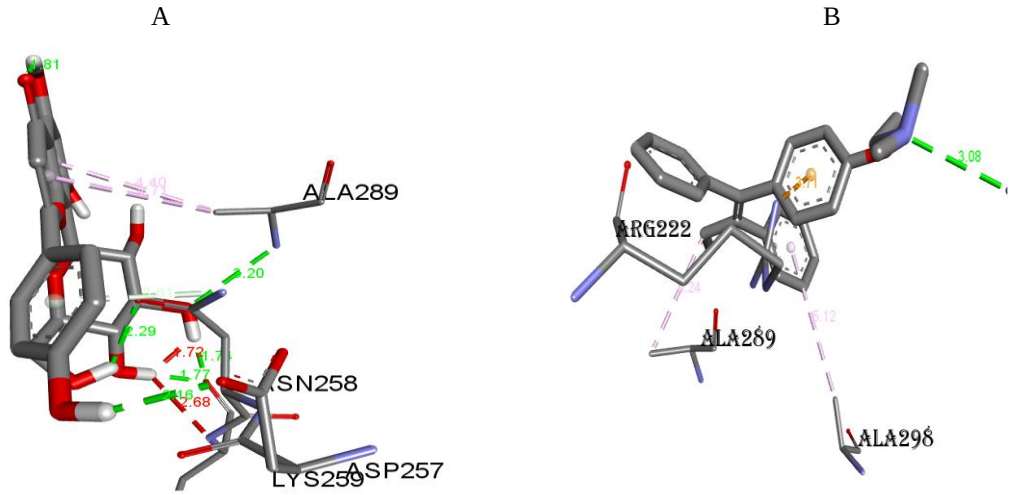
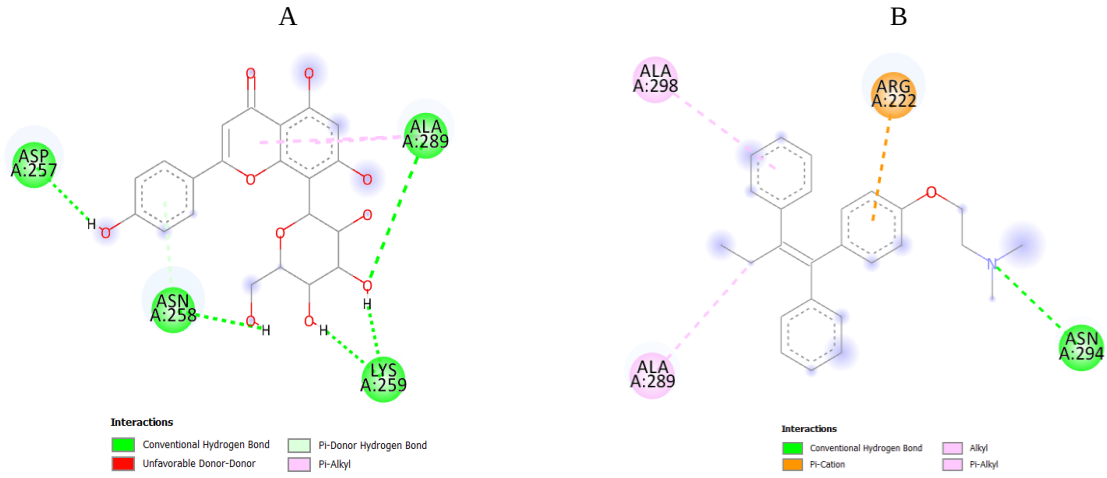


Fig. 8 2D Interactions of E-cadherin with Vitexin and Tamoxifen



A - Vitexin bound efficiently with the protein interacting through the amino acids – ASP257, ASN258, LYS259 and ALA289, with their hydrophobic residues (Green colour). **B -** Tamoxifen interacted with the protein through the amino acids ARG222, ALA289, ALA298 and ASN-294 with their hydrophobic residues (Green colour), polar residues (light purple), basic residues (blue ring) and acidic residues (orange). Difference in solvent accessible surface area for the selected ligands for the protein residue is plotted as blue shadow. Dashed arrow denotes (green) the H-bonding forming with the residue side chain.

IV. CONCLUSION

The results of the present study conclude that, the vitexin is considered as an efficient anti-metastatic phytochemical, and the compared docking analysis with the drug tamoxifen, it was clearly understood if vitexin is chosen as the drug for anti metastacy, it will surely inhibit the spreading of cancer cells in breast carcinoma. If inhibition is done at the time of conversion of cancer cells from epithelial to mesenchymal transition, the spreading can be minimized. From this docking analysis, it is clearly evident that, vitexin acts as a good ligand and gets bind to E-cadherin, showing a good binding ability and minimize the metastatic activity of cadherin mediated EMT. As the present study is a preliminary analysis, further *in vitro*, *in vivo assays* can be carried out for the future development of vitexin as a pharmaceutical drug against cancer.

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