Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [4] March 2021 : 105-117

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

In-Silico Evaluation of Anti-Cancerous Activity of Herbal Plant Extracts

Pruthvish R[1], Gopinath S M[1], Sushma P[2], Anisha S Jain[2], Shiva Prasad Kollur[3], Chandan Shivmallu[2*]
[1]Department of Biotechnology, Acharya Institute of Technology, Bengaluru, Karnataka – 560107;
[2]Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, Karnataka – 570 015, India;
[3]Department of Sciences, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru, Karnataka – 570 026, India;

ABSTRACT
Growth factor receptors belong to the receptor tyrosine kinase superfamily and are known to control a variety of biological processes such as proliferation, apoptosis, cell growth, differentiation and survival. Since evidence suggests that these receptors are over-expressed in cancer cells, they have recently captured the attention of researchers as a promising target for cancer treatment. In recent years, computational databases and methods have been shown to be an invaluable resource for anticancer research in the field of oncology. The structural diversity of plant-derived phytomolecules with anticancer properties against receptor proteins involved in cancer signalling pathways is facilitated by molecular docking studies. Hence, in the present study the authors have made an effort to evaluate the anticancer activity of various phytocompounds against the predominant proteins of cancer, p38-α MAPK and EGFR Kinase. Among all the identified phytocompounds, saccharopine showed the highest binding affinity with the most non-bonded interactions and least binding energy score (Kcal/mol) with the binding residues of the receptors in reference to a standard drug, abemaciclib. Further research is required to isolate this phytochemical and subject it to in vitro and in vivo studies for anticancer activity to prove it as safe and potential targeted therapy in the field of oncology.
Keywords*: Proliferation, apoptosis, differentiation, databases, MAPK, EGFR*

Received 10.01.2021 Revised 23.02.2020 Accepted 09.03.2021

INTRODUCTION

Cancer has established itself to be the greatest biological enemy to mankind. Past three decades, it has been the major cause of mortality worldwide and hence has become the serious issue in the health care industry. For the year 2017, American Cancer Society estimated that about 318,420 in males and 282,500 in females will die after suffering from cancer[1]. Above mentioned is the enumeration of only United States of America in the year 2017. If we study the statistics of fast developing country like India, the trend of death rate is analogous to that of USA [2]. The fatality rate ascribable to cancer in India is nearly to 200,100 males and 195,300 women. The number quoted cancer accounts for all types of cancer [3]. It is a dreadful disease in which cells undergo several genetic and epigenetic changes and lose control over their multiplication. Henceforth, they divide in an undisciplined manner forming an indefinite mass of cells called neoplasm or tumor [4]. Cancer can crop up to almost all types of cells/tissues of the body say in oral squamous, cervix epithelial cells, breast connective tissue, etc. Depending on organs exhibiting the tumorigenesis, cancer has been noted by about hundreds of terms [5].Ayurveda has blessed us with innumerable plants which can be used to prevent breast cancer and heal the patients who have already contracted this adenocarcinoma [6]. The need is to evaluate their anti-proliferative properties and then identify the biologically active compounds which impart them this property. Once the plant is confirmed to have anti-cancer property, it can be used in the dried form to treat patients. These therapeutically important molecules have to be separated from the bulk and should be formulated to treat cancer [7]. Efficiency of these compounds can also be increased by modifying them. Proper analysis has to be done in order to get the drug which is highly potent to treat breast cancer patients [8].

Molecular Docking is a computational in-silico approach that helps in anticipating the favored orientation of a particle (ligand) when it is made to interact at the limiting pocket of a macromolecule (compound/protein) [9]. This information on direction expectation may thusly help in anticipating the limiting fondness or the strength of association between the protein and ligand. The *in-silico* molecular

docking has become a proficient procedure in the field of drug discovery revelation for structure-based medication exposure. The most famous and generally utilized programming for molecular docking purpose is AutoDock (v.4.0) [10]. It is uninhibitedly accessible open-source programming for virtual screening of small molecules to receptors and computational docking [11]. Docking contemplates was performed to get the starter information utilizing the screened phytomolecules as ligands and p38-α MAPK (p38-α mitogen-initiated protein kinase) (4FA2) and EGFR Kinase space (3W2S) as the target macromolecules. p38 mitogen-initiated protein kinase otherwise called cytokinin explicit restricting protein are serine/threonine protein kinases [12]. The human p38 MAP kinase is partitioned into four isoforms p38α, p38β, p38γ and p38δ, among which p38α is discovered to be the best-portrayed isoforms. p38 MAP kinase is dependable to phosphorylate atomic just as cytoplasmic targets. p38 MAP kinase is a signaling pathway that is capable to direct a confounded network of proteins that are associated with different capacities like cell activities, cell-differentiation, apoptosis, cell cycle capture, tumor concealment, cell senescence and cytokine synthesis [13]. In the present investigation, we have aimed to evaluate the *in-silico* anti-carcinogenic properties of *Arachis hypogea, Rhizomapolygoni*and *Anacyclus pyrethrum* against cancer.

MATERIAL AND METHODS

Protein preparation

The three-dimensional crystal structures of the target proteins, P38 alpha Mitogen-Activated Kinase and EGFR kinase domain involved in various cancer pathways were retrieved from RCSB Protein Data Bank [14]. The p38-α MAPK structure stored in the data bank had a resolution of 2.00 Å and that of EGFR kinase was reported to be of 1.90 Å. All the water molecules and pre-existing small molecules bound to the proteins were removed in prior to the molecular docking analysis. The structures of the target proteins were cleaned and energy minimized, followed by computation of Gasteiger charges and addition of polar hydrogens using PyRx 0.8 virtual screening software [15].

Protein validation

The above retrieved protein structures were further validated using software based on the conformation of the protein backbone which describes the phi, psi angles per residue. The Ramachandran plot of protein backbone determines the number of residues falling under favoured and allowed regions that include approximately 98% and 99.5% residues respectively [16]. The protein structures are considered as unsuitable if higher percentage of residues fall in outlier region of the Ramachandran plot.

Protein binding site prediction

The possible binding pockets of the proteins were obtained from CASTp 3.0 (computed atlas of surface topography of proteins) server that provides surface pockets and interior cavities of the given protein pdb file [17]. This server also measures the area and volume of the binding pockets. The residues highlighted in the sequence of the selected pocket is chosen and specified in the docking software.

Ligand optimization and ADMET studies

The 2D structure of the screened phyto constituents (ligands) of all the three plants were sketched using ChemSketch software, further these 2D format files were converted into 3D i.e. the .pdb structure files through OpenBabel 2.4.1 software by clicking on 3D coordinates option [18]. Abemaciclib is the standard drug which is already available in the market and is used to treat breast cancer is consider in our present study to compare if the ligand molecules taken from plants are showing the best activity when compared to the standard drug. The sdf file of the drug was taken from PubChem database and was converted to 3D format using OpenBabel [19]. Now the obtained ligand files are used for molecular docking studies.

ADMET studies of the ligands

The chemical absorption, distribution, metabolism, excretion and toxicity (ADMET) plays a very important role in the drug development and discovery field [20]. Any molecule to be considered as the drug should not only have the ability to inhibit the target, but must show appropriate ADMET properties. In this study, freely accessible ADMETlab web platform http://admet.scbdd.com/ is used to evaluate the drug-likeness of the selected ligands [21].

Molecular Docking

Molecular docking was performed to determine which plant compound showed the best interaction with the selected cancer targets based on their binding free energies and binding affinity and those reflecting high hydrophobic interactions and hydrogen bonds. PyRx 0.8 software was used to carry out the molecular docking analysis of the phytocompounds targeting the cancer proteins. A grid box was generated surrounding the selected binding residues of the protein to which the ligands were docked [22]. Nine poses of each ligand docked to the active site of the cancer target protein were generated and ranked based on the negative binding energy value in descending order. These compounds were further

analyzed using the visualization software, PyMOL 2.4 by Schrödinger and BIOVIA discovery studio visualizer tools [23].

Molecular Dynamic Simulation

The protein-ligand docked complexes were further analyzed *in silico* by molecular dynamic simulations using the GROMACS 2021 package [24]. To determine the conformational stability of the complex, simulations were performed for 50ns on each docked complex. The parameter and topology files for the input ligand files were generated by Prodrug server [25]. Charmm27 forcefield was applied which uses empirical and semi-empirical energy functions. The energies and coordinates were saved every 50ps for analysis. Steepest descent minimization process was utilized for energy minimization for 50,000 steps. The plots were generated and visualized by Xmgrace tool [26].

RESULTS

Protein preparation and validation

The P38 alpha Mitogen-Activated Kinase and EGFR kinase domain proteins with PDB ID 3W2S and 4FA2 respectively were visualized using UCSF Chimera a visualization software and is represented in the **Figure 1**.

The selected proteins were edited as mentioned in 2.1. section and their validation was done using PROCHECK web tool to obtain the Ramachandran Plot of favoured, allowed and disallowed regions of the proteins as shown in Figure 2.

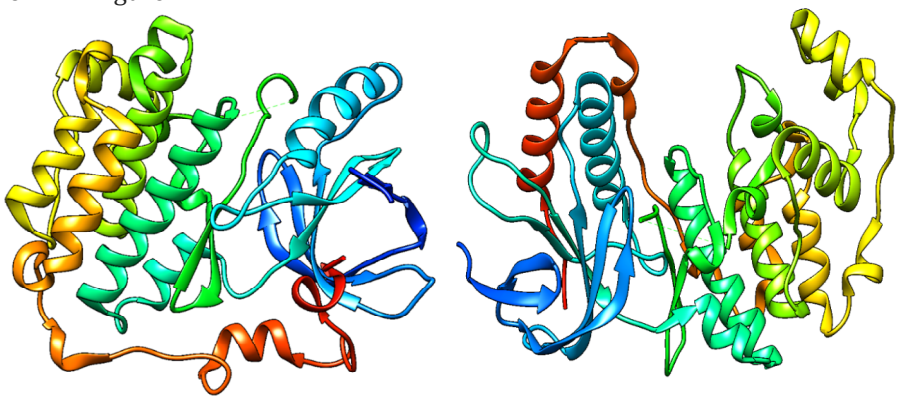


Figure 1: The 3D structure of 3WAS and 4FA2

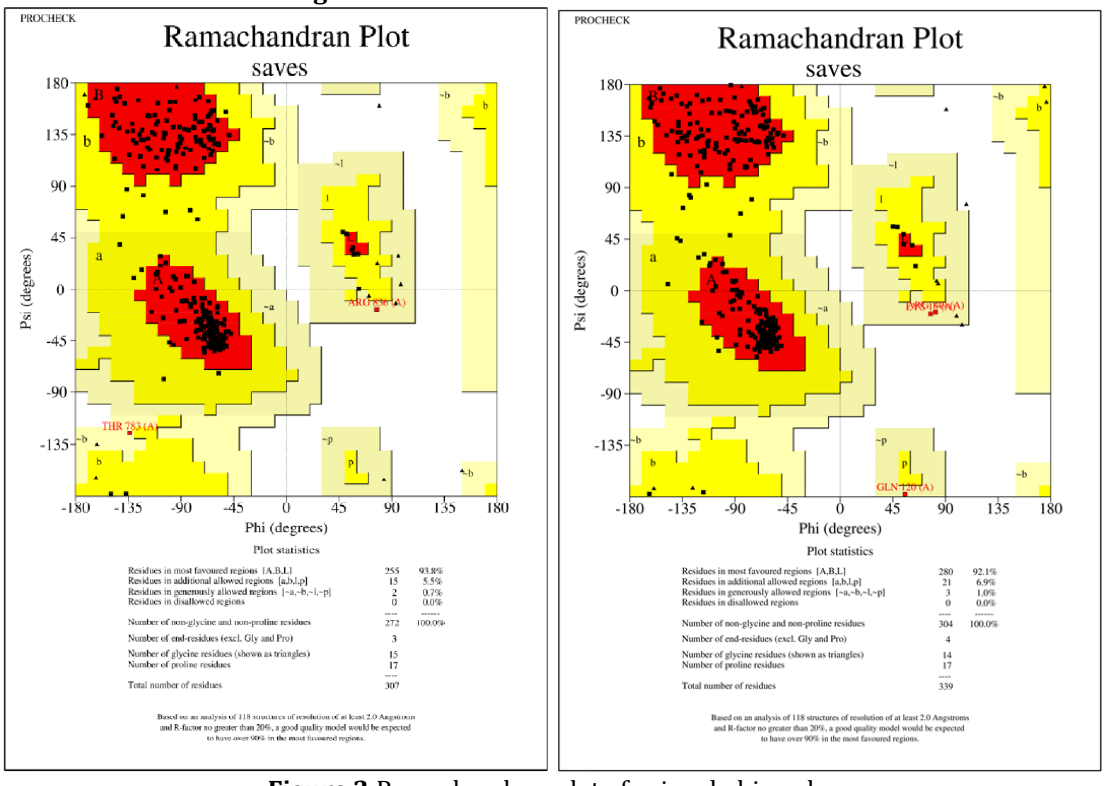


Figure 2:Ramachandran plot of psi and phi angles

Ligand Optimization and ADMET analysis

The screened phytoconstituents structures are shown in the **Figure 3** and the ADMET study graph with the upper and lower limits of all the ligands including the standard drug are represented in the **Figure 4**.

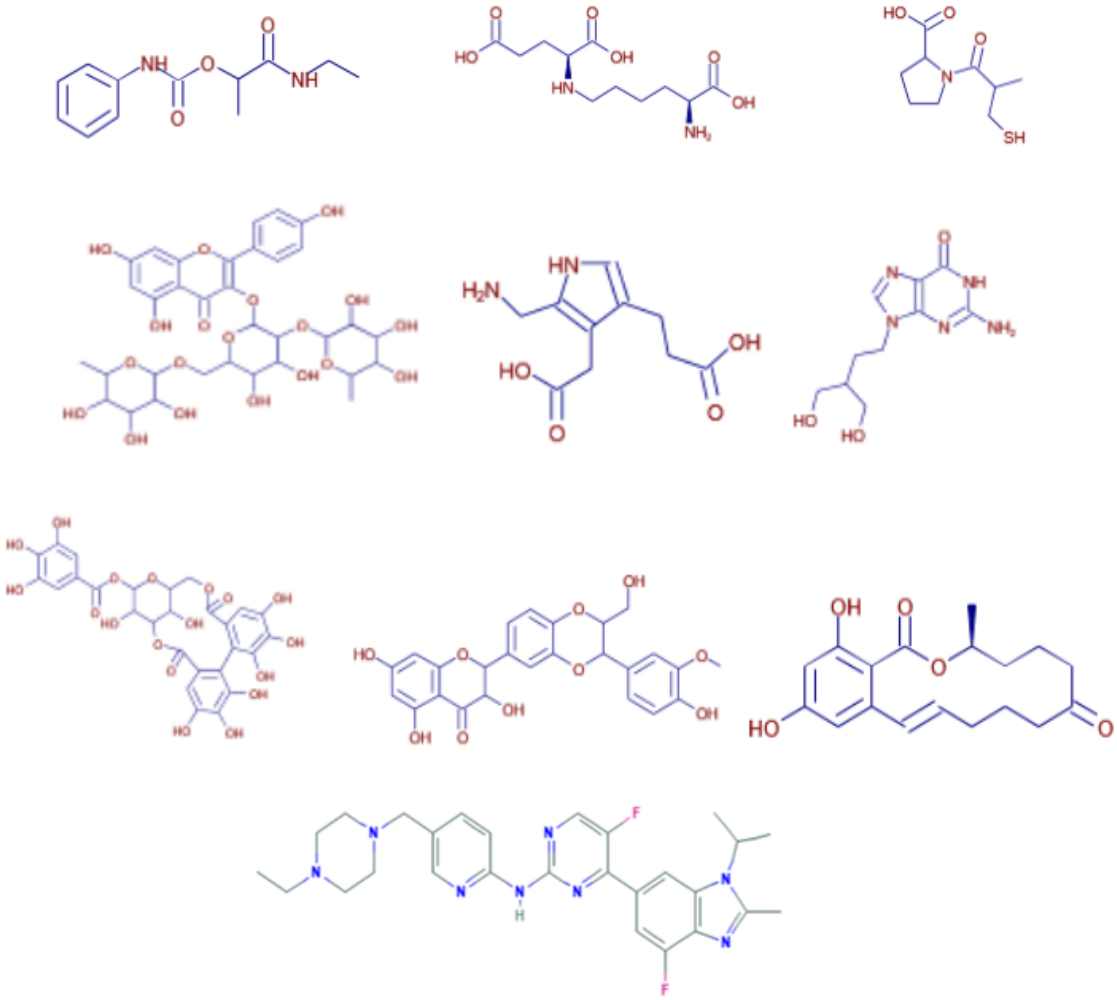


Figure 3: The structure of the screened phytoconstituents and the standard drug

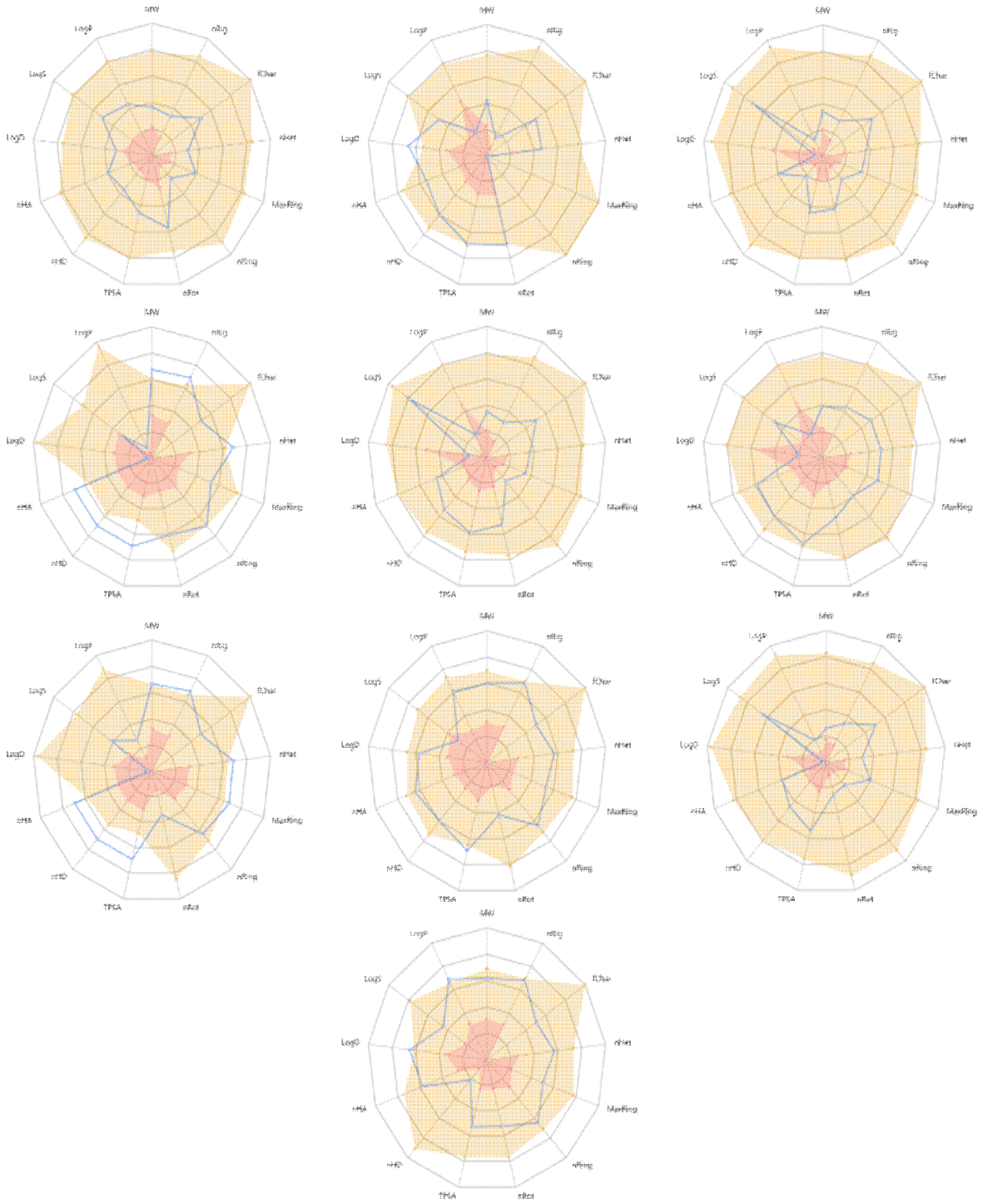


Figure 4: The ADMET analysis graphs (dark pink indicating the lower limit and light pink indicating the upper limit values) of the ligands obtained using ADMETlab

Protein Binding site prediction

The binding pocket residues of both the proteins which are responsible for the protein-ligand interactions were obtained using CASTp 3.0. Figure 5a and 6a shows the protein pockets and the highlighted part of Figure 5b and 6b shows the amino acid sequences.

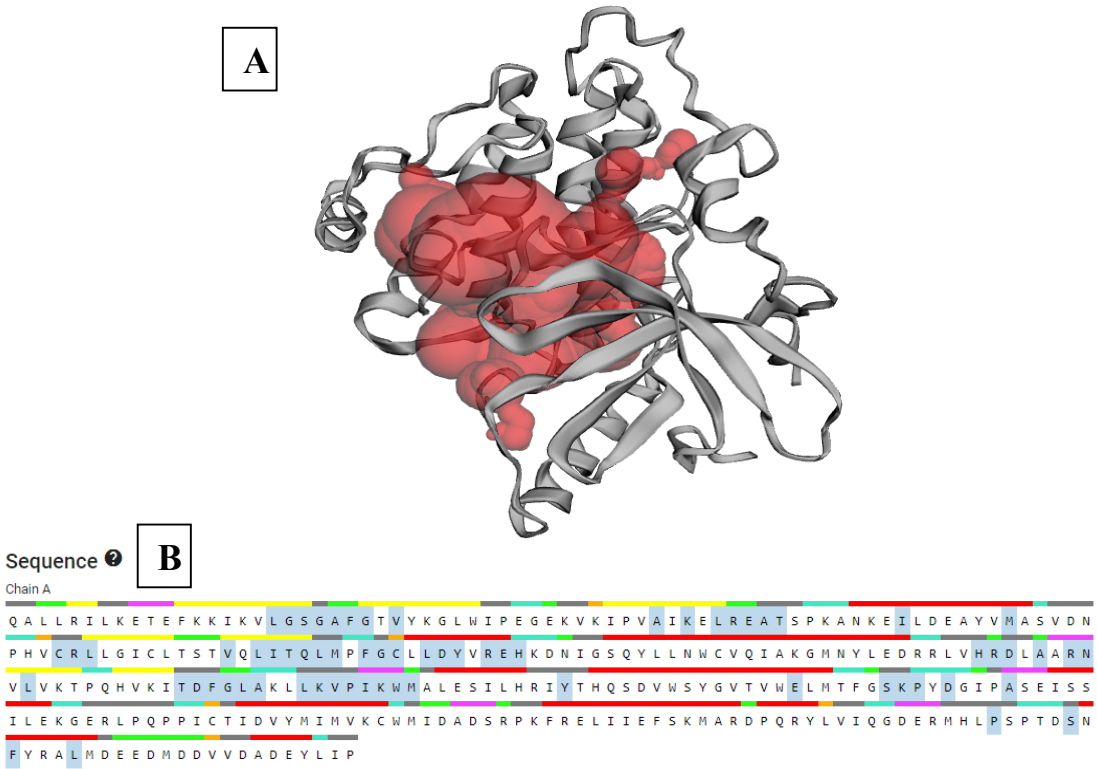


Figure 5:**A**: Red color indicating the binding pocket of 3W2S and **B**: Highlighted sequence represents the amino acid residues forming the pocket in the protein 3W2S

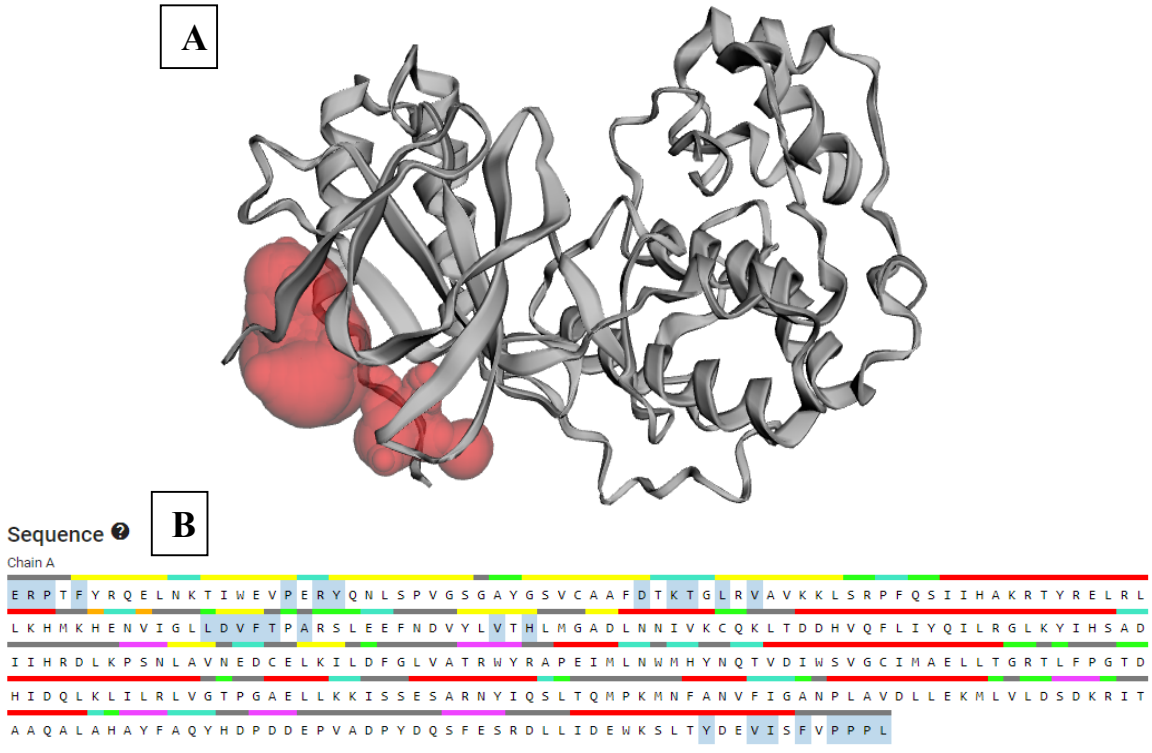


Figure 6:A: Red color indicating the binding pocket of 4FA2 and **B**: Highlighted sequence represents the amino acid residues forming the pocket in the protein 4FA2

Analysing Molecular Docking interactions

Molecular docking interaction studies were done to check the binding interactionsof the screened ligands with the selected cancer target proteins. Among the nine poses of each ligand obtained, one pose with least binding energy was selected and was visualized using PyMOL 2.4 to check its binding affinity and interaction with both the selected target proteins. The more number of hydrogen bonds and hydrophobic interactions (**Table 1**) formed with the selected amino acid residues given during the docking process confirms the more binding affinity between the protein-ligand complex. In the present study, all the screened ligands have interacted very well with the selected binding site residues of the respective proteins with good binding affinity. The Saccharopine ligand from *Arachis hypogea*with least binding affinity of -9.7 and -9.3 has shown the best binding affinity and interactions towards both the targets when compared to the standard drug Abemaciclib and other ligand molecules selected.

The 2D interactions including the amino acid residues forming both bonded and non-bonded interactions represented in different colors were taken using BIOVIA discovery studio visualizer. (**Figure 7 and Figure 8**)

Table 1: The details of bonded (hydrogen bonds) and non-bonded (hydrophobic interactions) between the respective protein and ligand

Sl No	Protein ID	Plant Name	Ligand Name	Binding affinity	N0. of H-bonds	H-bond forming amino acid residues	Hydrophobic interactions forming amino acid residues
1	**3W2S**	Standard drug	Abemaciclib	-6.2	3	MET-793, ASP-855	LEU-718, ALA-722, LYS-745, VAL-726, ALA-743, LEU-844, PHE-997
		Arachis hypogea	Carbetamide	-7.1	1	THR-854	LEU-718, VAL-726, ALA-743, LYS-745, MET-766, CYS-775, LEU-777, LEU-788, THR-790, LEU-844, ASP-855, PHE-856
			Saccharopine	-9.7	8	GLN-791, MET-793, CYS-797, THR-854, ASP-855	VAL-726, ALA-743, LEU-844
			Captopril	-5.9	3	LYS-745, ASP-855, PHE-856	VAL-726, ALA-743, MET-766, CYS-775, ARG-776, LEU-777, LEU-788, THR-790, LEU-844, THR-854
		Rhizomapolygoni	Mauritianin	-8.4	5	MET-793, CYS-797, THR-854, ASP-855	LEU-718, GLY-719, SER-720, GLY-721, ALA-722, PHE-723, VAL-726, ALA-743, LYS-745, THR-790, GLN-791, LEU-792, GLY-796, CYS-797, ASP-800, ARG-841, ASN-842, LEU-844, LEU-1001
			Porphobilinogen	-6.0	3	LYS-745, MET-793	LEU-718, VAL-726, ALA-743, ILE-744, LEU-788, THR-790, GLN-791, LEU-792, GLY-796, CYS-797, LEU-844, THR-854, LEU-788, PHE-997
			Penciclovir	-6.8	5	CYS-775, ARG-776, THR-790, THR-854, ASP-855	VAL-726, ALA-743, ILE-744, LYS-745, MET-766, VAL-769, LEU-777, LEU-788, ILE-789, LEU-844, PHE-856
		Anacyclus pyrethrum	Corilagin	-8.3	5	GLY-724, LYS-745, CYS-797, ASP-800, ASP-855	LEU-718, GLY-719, SER-720, GLY-721, PHE-723, THR-725, VAL-726, ALA-743, GLY-796, LEU-799, ARG-803, ARG-841, ASN-842, LEU-844, THR-854,

			Silibinin	-9.3	2	SER-720, ARG-841	LEU-718, GLY-719, GLY-721, PHE-723, VAL-726, ALA-743, LYS-745, THR-790, GLN-791, LEU-792, MET-793, GLY-796, ARG-841, LEU-844, ASP-855,
			Phthalamic acid	-9.2	5	LYS-745, THR-854, ASP-855, LEU-788, MET-793	LEU-718, VAL-726, ALA-743, ILE-744, LEU-777, ILE-789, THR-790, LEU-792, PRO-794, GLY-796, CYS-797, LEU-844, LEU-1001
2	**4FA2**	Standard drug	Abemaciclib	-6.8	5	LYS-53, ASP-112, SER-154, ASN-155, LEU-171	VAL-30, TYR-35, VAL-38, ALA-51, ILE-84, THR-106, MET-109, ASP-150, LYS-152, PHE-169, GLY-170, ALA-184, THR-185,
		Arachis hypogea	Carbetamide	-6.0	2	MET-78, LYS-165	LEU-75, LYS-76, LYS-79, HIS-80, GLU-81, LEU-86, LEU-87, PHE-348, VAL-349, PRO-351
			Saccharopine	-9.3	5	ALA-51, LYS-53, PHE-169, GLY-170, LEU-171	VAL-38, VAL-52, GLU-71, LEU-75, ILE-84, LEU-86, LEU-104, VAL-105, THR-106, LEU-167, ASP-168
			Captopril	-5.4	4	HIS-80, VAL-83, GLY-85, LYS-165	LEU-48, MET-78, LYS-79, ILE-84, LEU-86, LEU-87, THR-106, PRO-351
		Rhizomapolygoni	Mauritianin	-7.3	4	ARG-5, ASP-88, THR-91 PHE-348	PRO-6, PHE-8, PRO-21, ARG-23, VAL-89, PHE-90, PRO-92, ALA-93, TYR-342, VAL-345, PHE-348, VAL-349, PRO-350
			Porphobilinogen	-5.3	4	ASP-88, VAL-89, VAL-345	ARG-5, PRO-6, PHE-8, PRO-21, ARG-23, PHE-90, THR-91, ALA-93, ILE-346, PHE-348,
			Penciclovir	-6.4	4	ASP-88, THR-91, TYR-342, VAL-345	ARG-5, PRO-6, PHE-8, PRO-21, ARG-23, VAL-89, PHE-90, PRO-92, ALA-93, ARG-94, ILE-346, PHE-348, PRO-350,
		Anacyclus pyrethrum	Corilagin	-8.6	6	PRO-6, LYS-45, THR-91, ALA-93, PHE-90, PHE-348	GLU-4, ARG-5, PHE-8, PRO-21, ARG-23, ASP-88, VAL-89, PRO-92, ARG-94, TYR-342, VAL-345, ILE-346, VAL-349, PRO-350
			Silibinin	-7.8	3	THR-91, TYR-342, PHE-348	GLU-4, ARG-5, PRO-6, PHE-8, LYS-45, ASP-88, VAL-89, PHE-90, ALA-93, ARG-94, VAL-345, ILE-346, VAL-349, PRO-350
			Phthalamc acid	-8.0	4	ASP-88, VAL-89, VAL-345	ARG-5, PRO-6, PHE-8, PHE-90, PHR-91, ALA-93, ARG-94, SER-95, TYR-342, ILE-346, SER-347

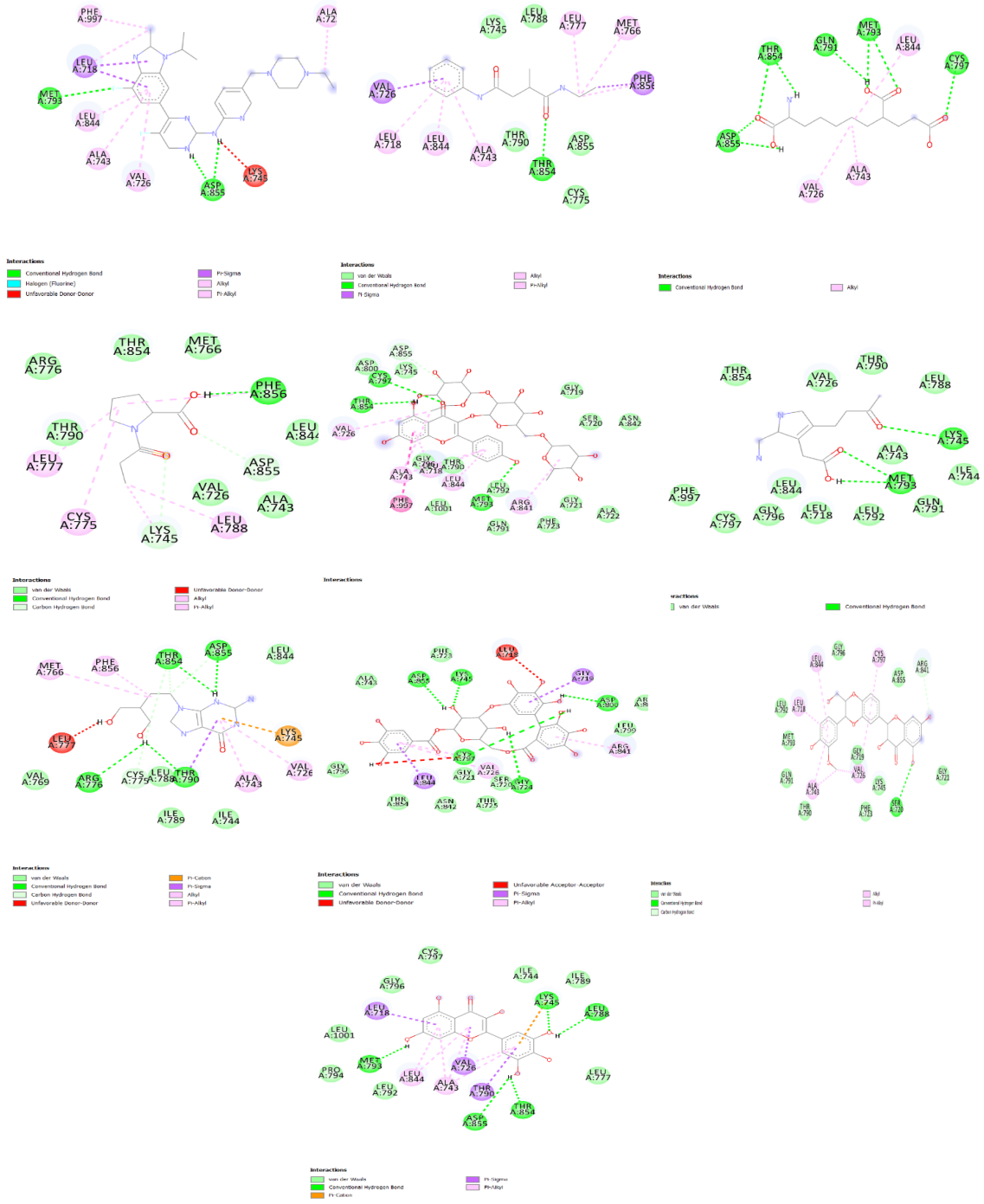


Figure 7: The 2D interactions between 3W2S with all the ligandswereanalysedusing BIOVIA discovery studio visualizer

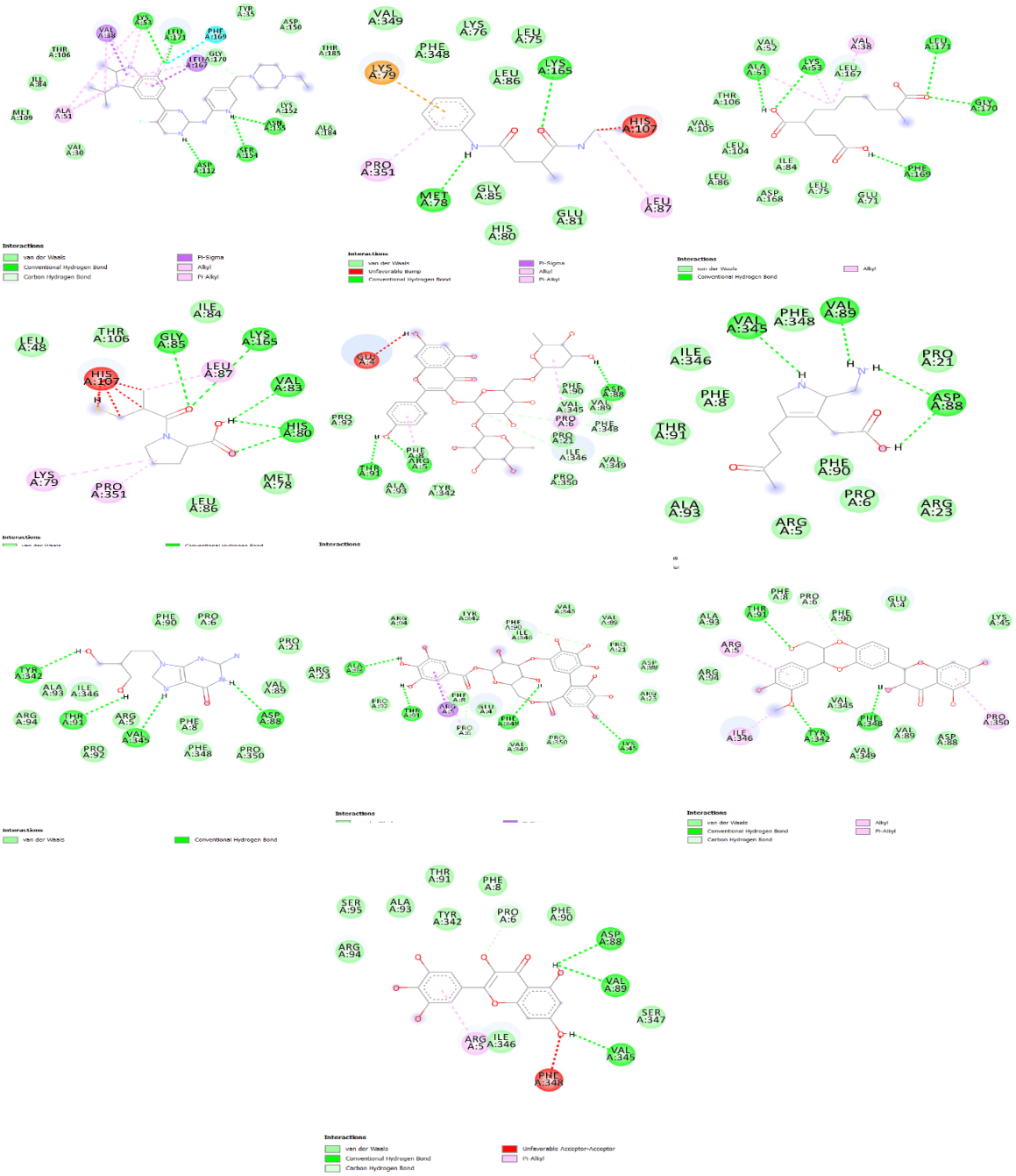


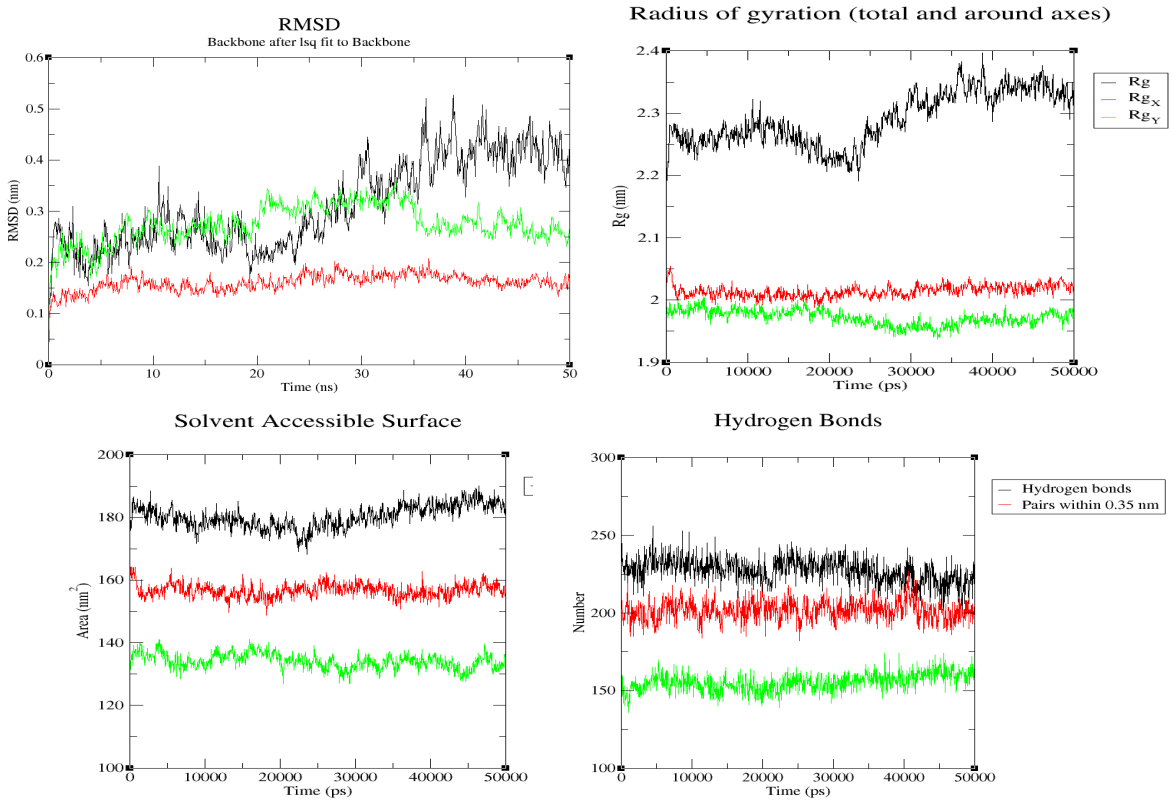
Figure 8: The 2D interactions between 4FA2 with all the ligands were analysed using BIOVIA discovery studio visualizer

Analysing Molecular Dynamics studies

After completing the docking studies, the molecular dynamics simulation was carried out to confirm if the bonded and non-bonded interactions formed between the protein-ligand complex are stable enoughwhen given different temperature, pressure and volume conditions. It was performed individually for to two different proteins, for Abemaciclib (standard drug) and Saccharopine ligand complexed with 3W2S and 4FA2. The simulation was carried out for 50 ns to ensure if the complex have confirmational stability. The movement of ligands were withing the binding pocket residues for 50 ns with comparatively less fluctuations when compared to the protein alone. The values obtained as the result of dynamics studies were analysed using XMGRACE software**(Figure 9 and 10)**to obtain the required Root Mean Square Deviation (RMSD), Solvent Accessible Surface Area (SASA) and Hydrogen bonds plots.

The RMSD plots of the proteins and docked complexes were analysed to check the deviations of the molecules and residues for about 50 ns of MD simulation. The RMSD ranges from 0.1 to 0.5 nm and 0.1 to 0.3for 3W2S and 4FA2 alone, 0.05 to 0.35 nm 0.05 to 0.35 nm for Abemaciclib-3W2S and Abemaciclib-4FA2 complex, 0.1 to 0.2 nm and 0.1 to 0.25 nm for Saccharopine-3W2S and Saccharopine-4FA2 complex which was comparatively lower (**Figure 9a**and **10a)**. The RMSD of Saccharopine complexed with both the proteins appeared to be more stable when compared with rest of the two protein and complexes.
The radius of gyration (Rg) of proteins and the complexes fluctuated up to 35000 ps and then attained equilibrium until the end of the simulation (**Figure 9b** and **10b)**.
The surface area of molecules in solvent (water) condition were analysed usingSolvent Accessible Surface Area (SASA) plots. The fluctuations of Saccharopine complexed with 3W2S and 4FA2 were least with the range of 150 to 170 nm^2 and 90 to 98 nm^2 respectively (**Figure 9c** and **10c)**.
The Hydrogen bond interactions observed during molecular docking studies between the Saccharopine complexed with both the proteins is confirmed through the simulation studies and is shown in the **Figure 9d** and **10d.**

Figure 9: A: RMSD plot, **B:** R_g plot, **C:** SASA plot and H-bonds confirmation plot of protein 3W2S alone (black color), Abemaciclib-3W2S (green color) and Saccharopine-3W2S complex (red color).



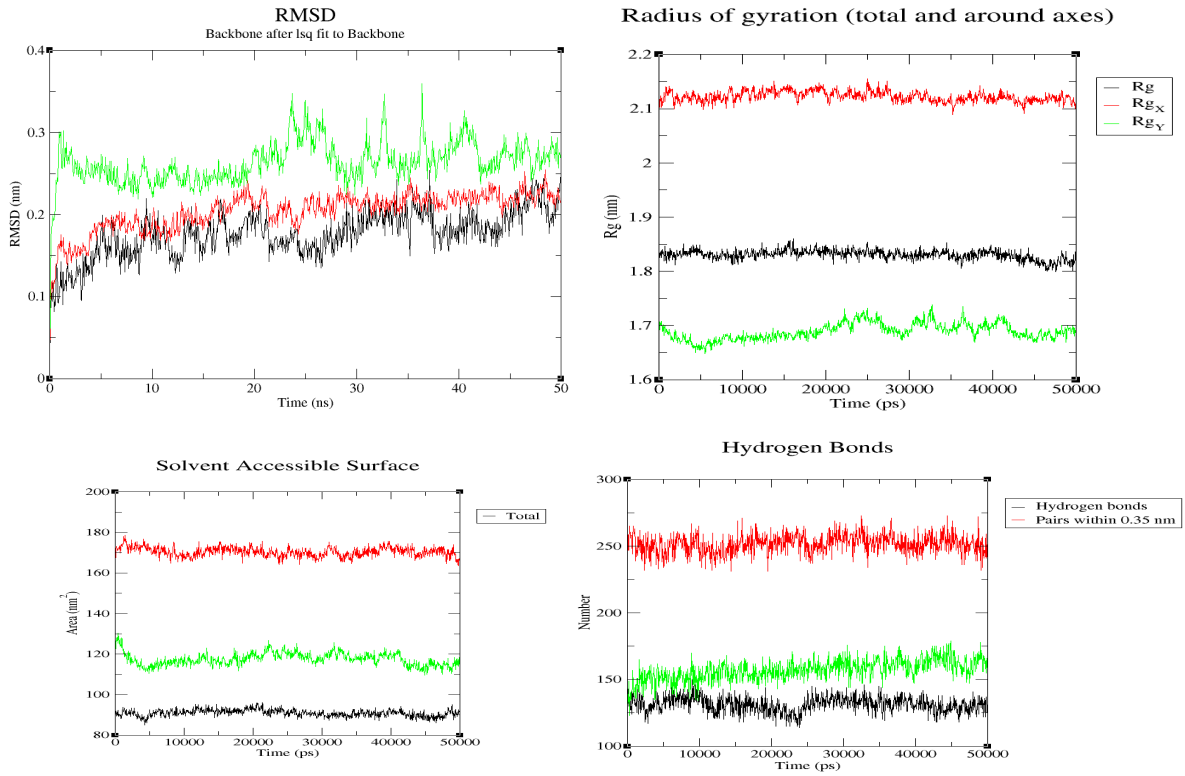


Figure 10: A: RMSD plot, **B:** R_g plot, **C:** SASA plot and H-bonds confirmation plot of protein 4FA2 alone (black color), Abemaciclib-4FA2 (green color) and Saccharopine-4FA2 complex (red color).

DISCUSSION

Cancer has established itself to be the greatest biological enemy to mankind. Past three decades, it has been the major cause of mortality worldwide and hence has become the serious issue in the health care industry [27]. The purpose of the present study is to provide an insight of how the phytoconstituents present in the plants help in inhibiting the main target proteins involved in the cancer progression. The usage of bioinformatics tools and software has laid to the development and discovery of several novel compounds having the capacity to inhibit the selected target proteins [28].

In this study, the *in silico* approaches were used to check the activity of 9 screened phytoconstituents against p38-α MAPK and EGFR Kinase proteins. Interestingly, the molecular docking results proved that the screened ligands of the plants showed extremely great binding interactions and affinity towards the target when compared to the standard drug,abemaciclib. Further, simulation of these docked protein-ligand complexes determined how stability of it in solvent system.

CONCLUSION

This study was carried out to compare and analyze the anti-cancer activity of different phytoconstituents to inhibit the predominant proteins involved in the cancer progression. All the ligands showed good binding affinity when compared to abemaciclib. Saccharopine molecule showed the least binding energy with highest binding affinity with all the active site residues of the two protein targets and thus, were considered for the molecular dynamics simulation using GROMACS. The different plots obtained after 50 ns simulation of each protein and its complexes concluded its equilibrium and stability throughout the simulations. The complete study concludes that the Saccharopine ligand taken from the *Arachis hypogea*could be used as a good alternative to the standard drugs.

REFERENCES

1. Azam SS, Abbasi SW (2013). Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. TheorBiol Med Model. 10**:**63.
2. Umesh HR, Ramesh KV, Devaraju KS (2020). Molecular docking studies of phytochemicals against trehalose–6–phosphate phosphatases of pathogenic microbes. Beni-SuefUniv J Basic ApplSci. **9:**5.
3. Acharya R, Chacko S, Bose P. *et al* (2019). Structure Based Multitargeted Molecular Docking Analysis of Selected Furanocoumarins against Breast Cancer. Sci Rep. 9:15743.

4. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 11(5):905-919.
5. Sebolt-Leopold JS (2000). Development of anticancer drugs targeting the MAP kinase pathway. Oncogene.19(56):6594-6599.
6. Olson MO (2004). Sensing cellular stress: another new function for the nucleolus?. Science Signaling.224.
7. Pearson BJ, Alvarado AS (2017). Regeneration, stem cells, and the evolution of tumor suppression. Cold Spring Harbor symposia on quantitative biology. 73:565-572.
8. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al (2000). The Protein Data Bank. Nucleic acids research.28(1):235–242.
9. Pettersen EF, Goddard TD, Huang CC, et al (2004). UCSF Chimera--a visualization system for exploratory research and analysis. J. of Computational Chemistry.25(13):1605-1612.
10. Wang W, Xia M, Chen J, Deng F, Yuan R, Zhang X, et al (2016). Data set for phylogenetic tree and RAMPAGE Ramachandran plot analysis of SODs in Gossypium raimondii and G. arboretum. Data in brief.9:345–348.
11. Junsu K, Hahnbeom P, Heo L, Seok C (2012). GalaxyWEB server for protein structure prediction and refinement. *Nucleic Acids Research*.40(W1):W294–W297.
12. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011). Open Babel: An open chemical toolbox. J Cheminform.3(33).
13. Naz A, Bano K, Akhtar N (2009). Conformational analysis (geometry optimization) of nucleosidic antitumor antibiotic showdomycin by Arguslab 4 software. *Pakistan journal of pharmaceutical sciences.* 22(1):78-82.
14. Dallakyan S, Olson AJ (2014). Small-molecule library screening by docking with PyRx. Methods Mol Biol.1263:243-250.
15. Prasad A, Shruthi G, Sushma P, Jain AS, Chandan D, et al (2020). Helicobacter pylori infection: a bioinformatic approach. International Journal of Pharmaceutical Sciences and Research.1(11):1000-15.
16. Chaudhari R, Li Z (2015). PyMine: a PyMOL plugin to integrate and visualize data for drug discovery. BMC Res Notes.8:517.
17. Dikshit R (2012). Cancer mortality in India: a nationally representative survey.*Lancet.* 379(9828):1807-16, 2012.
18. Anand P1, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research,* 25(9): 2097-116.
19. Maffei M. (2003). Dietary Supplements of Plant Origin: Nutrition and Health Allroach. CRC Press. 256.
20. Uruquiaga and Leighton (2000). Uruquiaga and Leighton 2000.*Biological Research.* 33(2):55-64.
21. Scarpa A, Guerci. (1982). Various uses of the castor oil plant (Ricinus communis L.). A review.*Journal of Ethnopharmacology.*5(2):117-37.
22. Yedjou C, Izevbigie E, Tchounwou P (2008). Preclinical Assessment of Vernonia amygdalina Leaf Extracts as DNA Damaging Anti-cancer Agent in the Management of Breast Cancer.*International Journal of Environmental Research and Public Health.*.5(5):337-341.
23. Nagamine MK, da Silva TC, Matsuzaki P, Pinello KC, Cogliati B, Pizzo CR, Akisue G, Haraguchi M, Górniak SL, Sinhorini IL, Rao KV, Barbuto JA, Dagli ML (2009). Cytotoxic effects of butanolic extract from Pfaffia paniculata (Brazilian ginseng) on cultured human breast cancer cell line MCF-7.*Experimental and Toxicologic Pathology.*61(1),75-82.
24. Anisha SJ, Sushma P, Ashwini P, Kollur SP, Shashank KP, Chandan S, et al (2020). Effect of flavonoids having anti-inflammatory and antiviral properties on the spike glycoprotein of SARS-CoV-2: An *In silico* Approach. Saudi Journal of Biological Sciences.28:1040-51.
25. Prasad SK, Pradeep S, Shimavallu C, Kollur SP, Syed A, Marraiki N, Egbuna C, Gaman M-A, Kosakowska O, Cho WC, Patrick-Iwuanyanwu KC, Ortega Castro J, Frau J, Flores-Holguín N and Glossman-Mitnik D (2021) Evaluation of *Annona muricata* Acetogenins as Potential Anti-SARS-CoV-2 Agents Through Computational Approaches. *Front. Chem.* 8:624716.
26. Kollur SP, Prasad SK, Pradeep S, Veerapur R, Patil SS, Amachawadi RG, Rajendra P, Lamraoui G, Al-Kheraif AA, Elgorban AM, Syed A, Shivamallu C (2021). Luteolin-Fabricated ZnO Nanostructures Showed PLK-1 Mediated Anti-Breast Cancer Activity. *Biomolecules*.3:385.
27. Acharya R, Chacko S, Bose P, et al (2019). Structure Based Multitargeted Molecular Docking Analysis of Selected Furanocoumarins against Breast Cancer. Sci Rep.9:15743.
28. Avinash, K.O., Sushma, P., Chandan, S., Gopenath, T.S., Kiran, M.N., Kanthesh, B.M., Kumar, J.R. (2021). In Silico Screened Flavanoids of Glycyrrhiza glabra Inhibit Cpla2 And Spla2 In Lps Stimulated Macrophages. Bull. Env. Pharmacol. Life Sci., 10(4): 14-24.

CITATION OF THIS ARTICLE

Pruthvish R, Gopinath S M, Sushma P, Anisha S Jain, Shiva Prasad Kollur, Chandan S. *In-Silico* Evaluation of Anti-Cancerous Activity of Herbal Plant Extracts. Bull. Env.Pharmacol. Life Sci., Vol10[4] March 2021 : 015-117