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Research articles

In silico and Molecular dynamic studies of natural flavonoids for their inhibitory pattern against Interleukin-6 (1ALU) and TNF-α (5MU8) for management of ulcerative colitis

Manish Kumar Gupta, Khairunnisa Kalathil*, B Rajinikanth

Jaipur National University, Jaipur, Rajasthan, India

ABSTRACT

The search for a prospective lead chemical is a time-consuming and complicated procedure that necessitates a lot of money, patience, and labour. Humans have been using phytochemicals, especially secondary metabolites, for this purpose since ancient times, and they are still on the hunt for even source for drug discovery. Natural flavonoids including rhamnetin, eupatorin, and primuletin are involved in the treatment of numerous biological diseases. The research focuses on molecular docking of 10 flavonoid compounds with the Interleukin-6 (1ALU) and TNF-α (5MU8) to assess the binding affinity at the binding location with the highest binding affinity. The flavonoid-protein complex with the highest binding affinity and interactions was studied using molecular dynamics modelling. With the Interleukin-6 (1ALU) and TNF-α (5MU8), the flavonoid naringin had the lowermost binding energy of 9.8 Kcal/mol. It took 20 nanoseconds to complete and yielded satisfactory results. The rhamnetin, eupatorin, and primuletin residues are more successful at maintaining flavonoid stability against Interleukin-6 (1ALU) and TNF-α (5MU8), according to the overall results of our simulation. These expected results will serve as a starting point for more investigation into the significance of their drug-likeliness properties in the management of ulcerative colitis.

Keywords: Ulcerative colitis, Flavonoids, IL-6, TNF –α.

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Correspondence: Khairunnisa Kalathil*, ✉ babysameer@gmail.com

School of Pharmaceutical Sciences, Jaipur National University, Jaipur.

INTRODUCTION

Intestinal epithelium is responsible for absorption of nutrients, while also being responsible for defending the body from harmful pathogens, microorganisms, and contaminants.[1]. Inflammatory disorders, also known as ulcerative colitis, is a long-term idiopathic intestinal inflammatory disease.[2]. The deterioration of the mucosal and submucosal layer, as well as inflammation and a different clinical symptom such as inflammation, rectal bleeding, ulceration in the colon, stomach cramps, losing weight, and illness, are all symptoms of ulcerative colitis.[3].

Several etiopathogenic features involved in the growth of ulcerative colitis, with genetic issues, cigarette smoking, immune disturbances, environmental, dietary, and microbial pathogens.[4]. It starts in the colon and spreads up to the rectum, accompanied by fistula, granuloma, and fissures. The pathogenesis of UC is unknown, but it is thought to be causing intestinal epithelial cell dysfunction. Further, an inequity among the pro-inflammatory cytokines as Interleukin-6, TNF-alpha, Interferon and anti-inflammatory. [5,6].

Flavonoids are polyphenolic in nature that are present in plants. In plants it is responsible for the colour and order of flowers.

It has identified over 8000 flavonoids to date, the majority of which are found in the cells or surfaces of various plant tissue organs. Flavonoids are noticed in a large range of edible plant species and are considered essential human dietary constituents. [7,8]. Flavonoids most abundant and widely distributed classes of secondary metabolites, and they are extremely important to mankind, not only because of their role in plant coloration, but also because they contain many physiologically active members. The evidence of flavonoid's reliable and consistent positive effects, such as inflammation and cancer prevention, has sparked a lot of research.[9].

MATERIAL AND METHOD

Molecular docking protocol

An approach for predicting the binding affinity of protein-ligand complexes is molecular docking. The tertiary assembly of human Interleukin-6 and TNF-α (PDB ID: 1ALU, 5MU8) was obtained from protein databank. The eupatorine, primuletin, rhamnetin are ligands were obtained from PubChem database. CASTp server used to identify the activity site of the proteins. The interaction in-between the protein-ligand complexes were interpreted by Discovery studio visualize. [10, 11].

Preparation of ligands

The flavonoids downloaded from the PubChem and it was re-drawn using ACD/chemsketch. The re-drawn flavonoid structures are incorporated in the Maestro, Schrodinger and employed LigPrep module to prepare the ligands. The structures are processed with the default parameters in addition to the OPLS-2005 force field and all ligands were maintained at pH 7 ± 2. [12, 13].

Molecular Dynamics

The Desmond module was used to conduct molecular dynamics simulations of protein-ligand complexes in order to determine their conformational stability. The protein-ligand combination was solvated using the TIP4P water model, and the box volume was reduced. The simulation system's total charge was compensated by introducing Na+ (+8) and Cl- (-10) ions. The pressure and temperature were maintained at 300K. The system was minimized using the OPLS 2005 force field. The final stage of molecular dynamics production was studied 20 ns for the all the complexes.[14].

RESULT AND DISCUSSION

Molecular docking analysis and Protein – ligand interactions

Three flavonoid analogs were docked with human INTERLEUKIN-6 and TNF-ALPHA (PDB ID: 1ALU, 5MU8). This study is help to identify the binding affinities between the protein and ligand complexes. The binding sites of the target proteins 1ALU (95GLU, 96VAL, 98LEU, 99GLU, 116GLN, 120LYS, 141PRO, 144ASN) and 5MU8 (139PRO, 142LEU, 144PHE) respectively. Polar hydrogen and Gasteiger charges were added to the targeted protein and saved as pdbqt format and ligand also prepared for molecular docking. The grid size was set to 20 × 20 × 20 xyz points with grid spacing of 0.375 Å and grid center was designated at dimensions (x, y, and z): 23.556, 14.917, -1.944 for 5MU8 and 8.893, -23.173, 14.183 for 1ALU.

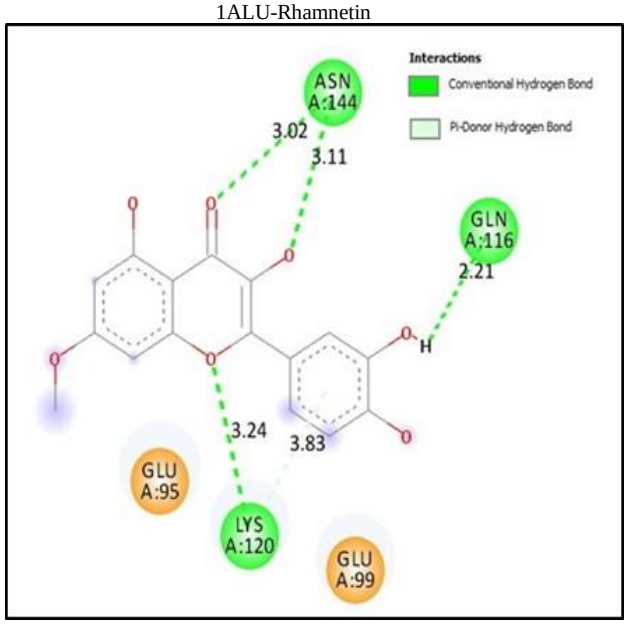
For more interaction analysis, the docking poses with the lowest bonding energy were selected and suitably matched with the target protein structure. From the docking results, Eupatorine is showing good binding energy to the target proteins but no hydrogen bond interactions present to the binding sites. Rhamnetin and Primuletin shows good binding energies and good hydrogen bond interactions compare to eupatorin. Table 1 and figure 1 shows the binding energies and interactions between the protein - ligand complexes.

Table1. Binding energies between the protein-ligand complexes

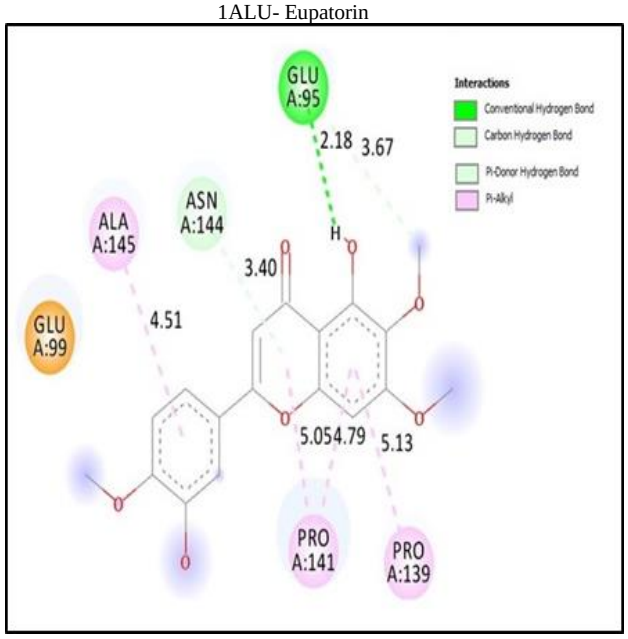
Protein-Ligand complexes	Binding Energy (kcal/mol)	No of H-Bonds
1ALU-Rhamnetin	-6.5	4
1ALU- Eupatorin	-5.7	1
1ALU-Primuletin	-5.8	5
5MU8-Rhamnetin	-6.9	2
5MU8-Eupatorin	-6.4	0
5MU8-Primuletin	-5.8	2

Figure 1. 2D interaction of Rhamnetin, Eupatorin, Primuletin with 1ALU and 5MU8

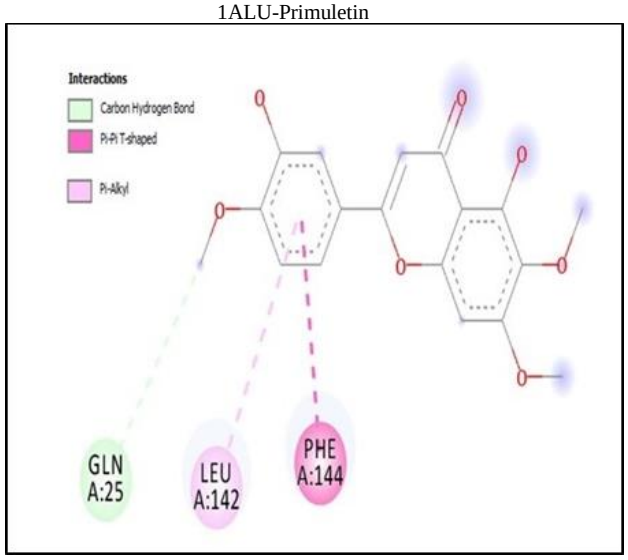
1ALU-Rhamnetin



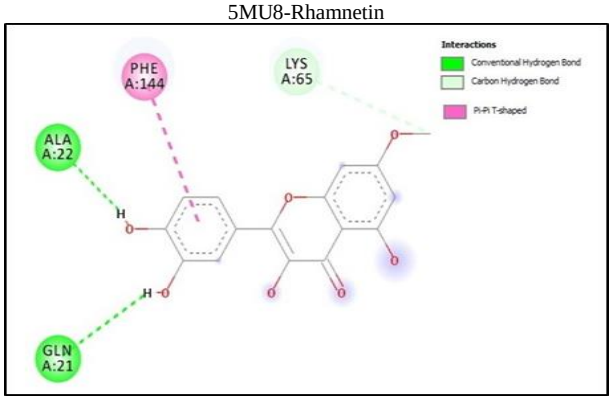
1ALU- Eupatorin



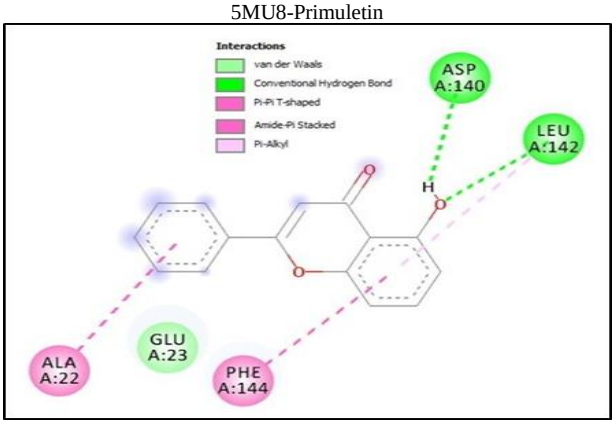
1ALU-Primuletin



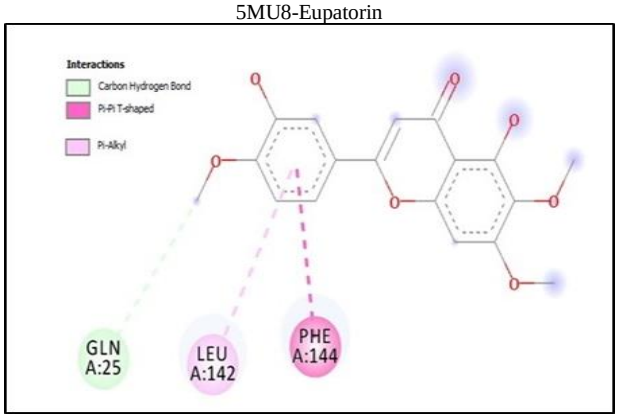
5MU8-Rhamnetin



5MU8-Primuletin



5MU8-Eupatorin



Phytomedicine is becoming increasingly popular among UC patients. Fifty percent of UC patients prefer complementary and alternative therapies, such as herbal treatment. Herbal medicine is associated with low acceptability and apparent incompetence of modern therapy in UC patients. [15, 16].

For centuries, flavonoids have been utilized as a herbal medicine for antimicrobial, anticancer, antioxidant, antiseptic etc. By utilizing these classical molecules in conjunction with a computer-aided drug prediction approach, it is possible to anticipate potential medications for treatment of UC.[17].

Figure 1: RMSD of Rhamnetin and 1ALU throughout the simulation

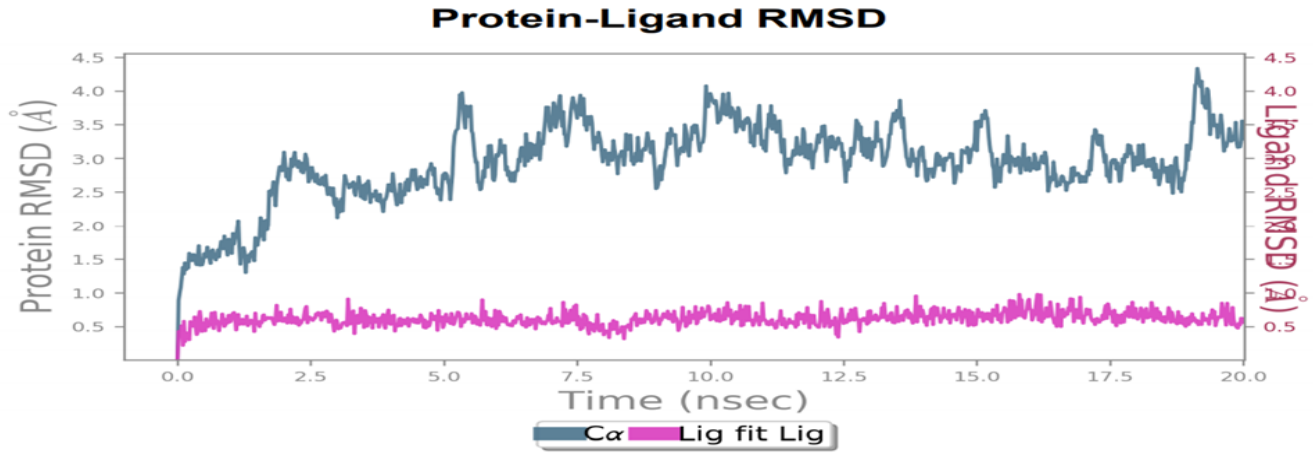


Figure 2: RMSD of Eupatorin and 1ALU throughout the simulation

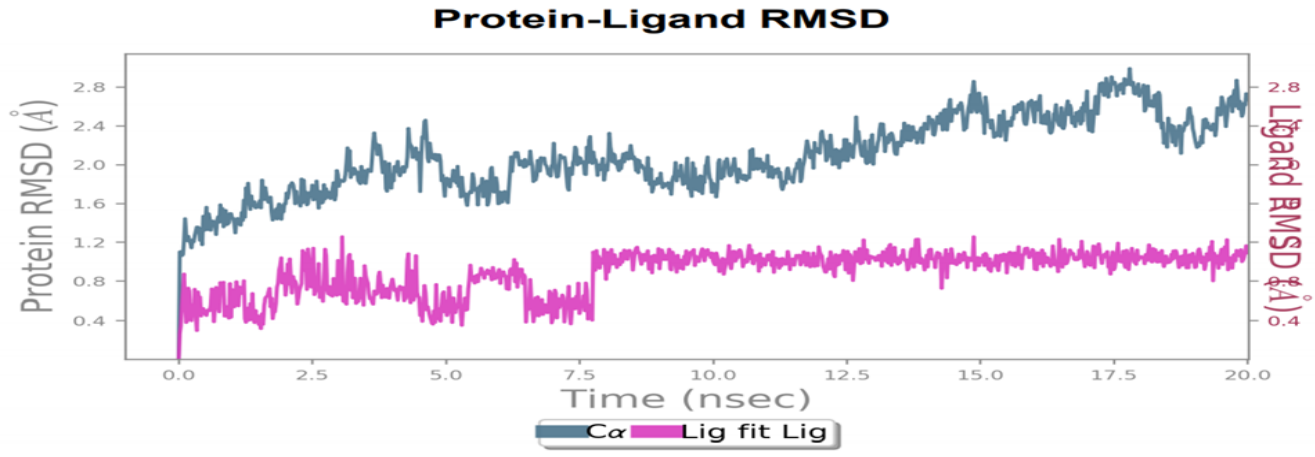


Figure 3: The figure represents the RMSD of Primuletin and 1ALU throughout the simulation

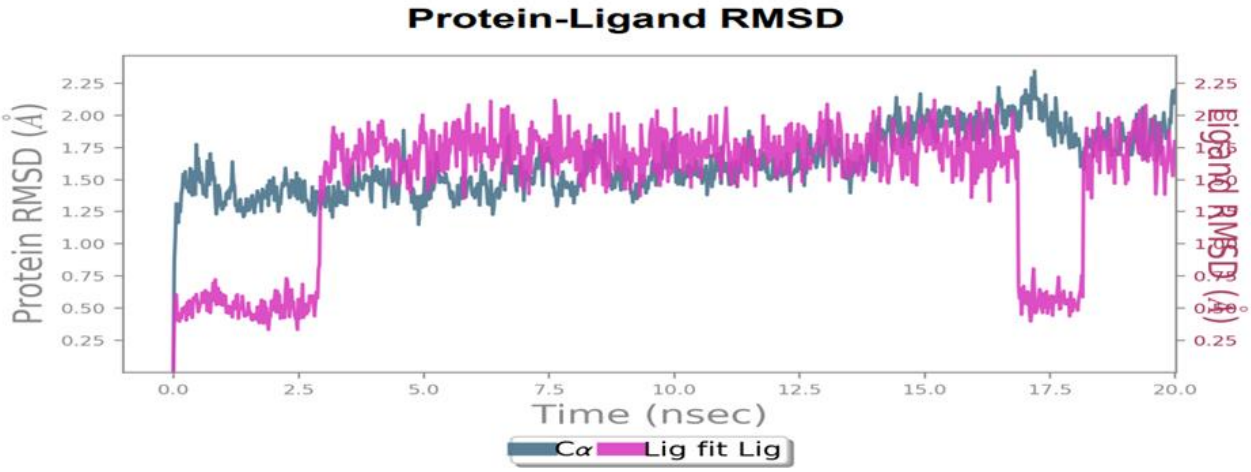


Figure 4: The figure represents the RMSD of Rhamnetin and 5MU8 throughout the simulation

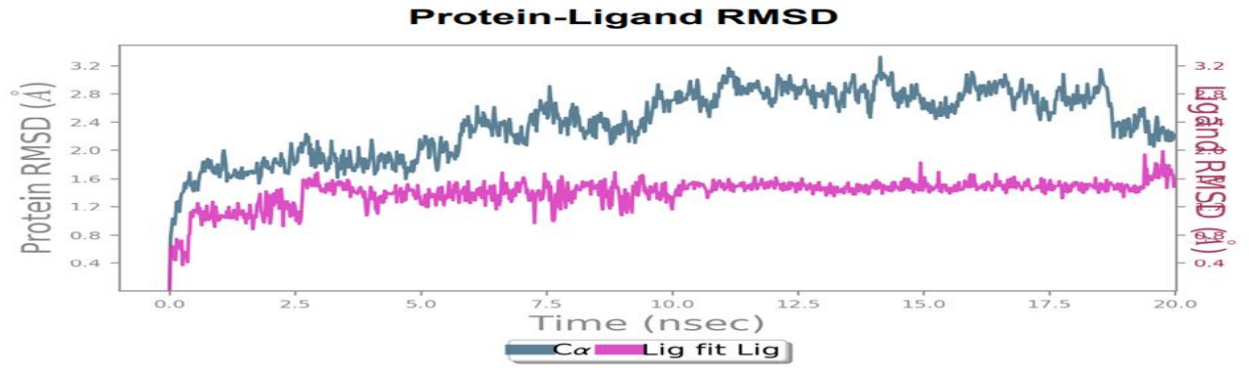


Figure 5: The figure represents the RMSD of Eupatorin and 5MU8 throughout the simulation

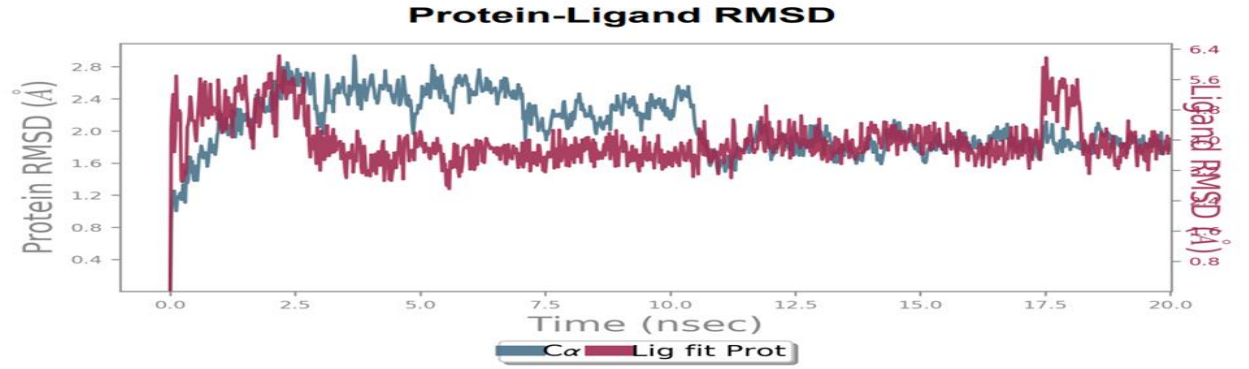
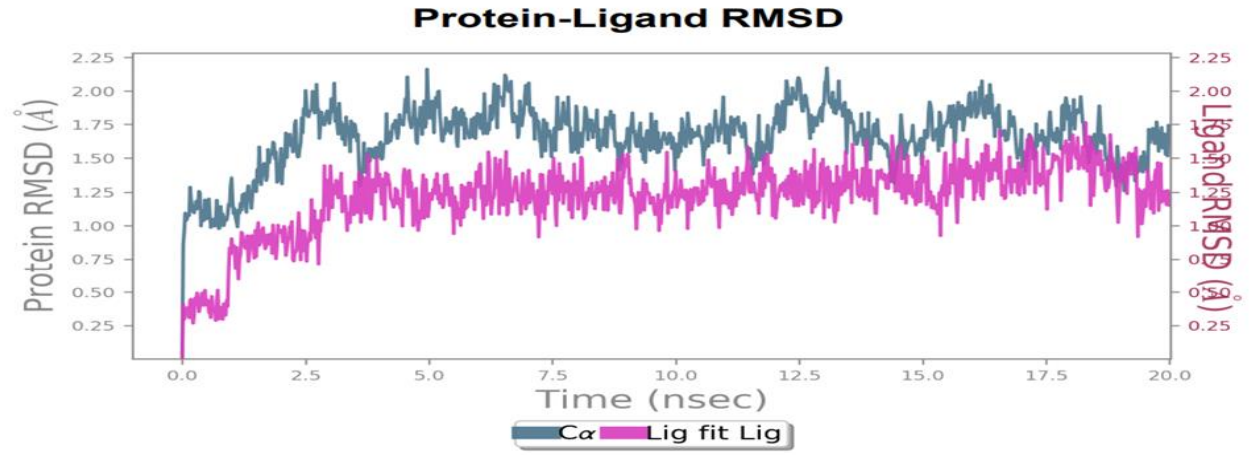


Figure 6: The figure represents the RMSD of Primuletin and 5MU8 throughout the simulation



The figures above depict the RMSD evolution of a protein (Figures. 2, 3, 4, 5, and 6). (Y-axis on the left) After aligning all protein molecules on the coordinate system backbone, the RMSD based on atom selection is calculated. Monitoring the protein's RMSD throughout the simulation can provide insights into its structural conformation. The RMSD evaluation can indicate whether or not the model has regenerated, which suggests that the simulation's oscillations near the end are centered on some thermal average structure. For tiny, globular proteins, changes of the order of 1-3 A° are quite acceptable. On the other hand, larger changes suggest that the protein's structure is changing dramatically during the simulation. Additionally, it is critical that your simulation converges, which means that the root mean square error values settle towards a constant number. If the protein's RMSD is still increasing or falling on average at the end of the simulation, your system has not stabilized, and your modelling may be too brief for meaningful analysis. The larger RMSD variation in the complex's molecular modeling result indicates instability, while the small RMSD variation indicates stability.[18].

The root means square deviation (RMSD) of the ligand (right Y-axis) reveals its stability in regard to the protein and its binding pocket. 'Lig fit Lig' provides the root mean square deviation (RMSD) of a ligand in the preceding map that has been aligned and calculated only on the basis of its reference conformation. This RMSD value quantifies the ligand atoms' internal variations.

Figure 7: Rhamnetin atoms connections with the 1ALU residues

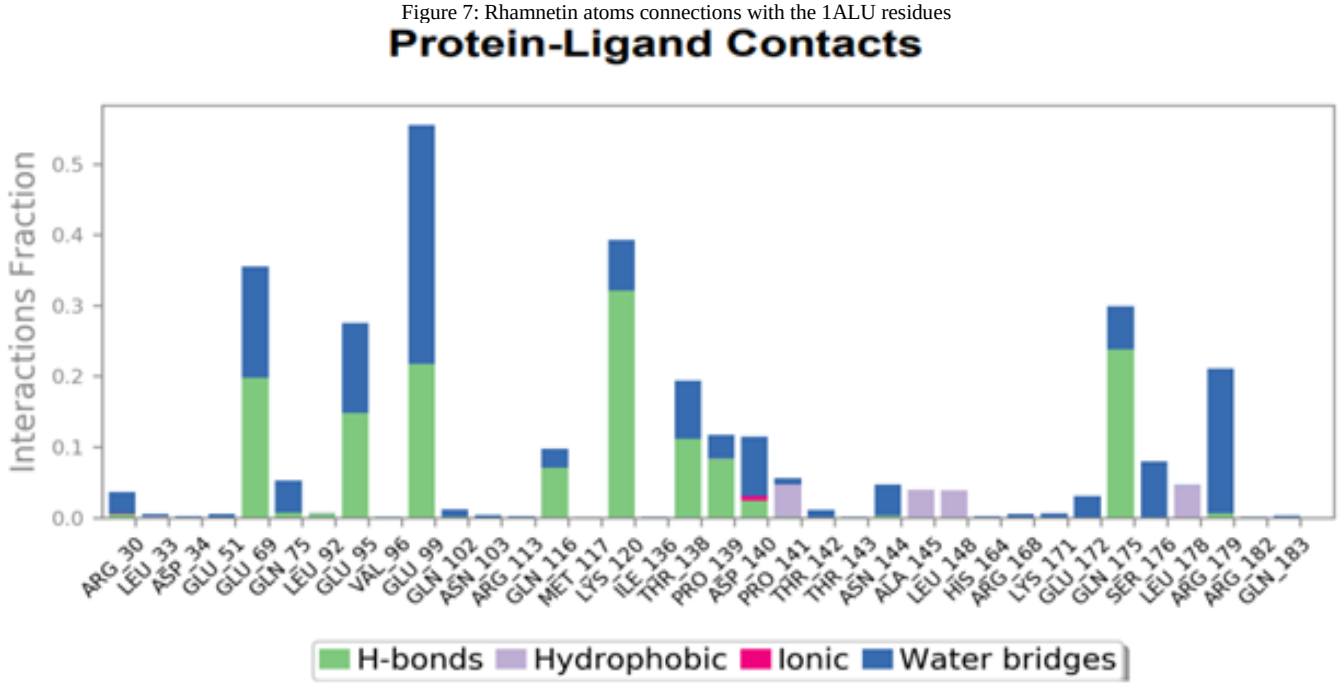


Figure 8: Eupatorin atoms connections with the 1ALU residues

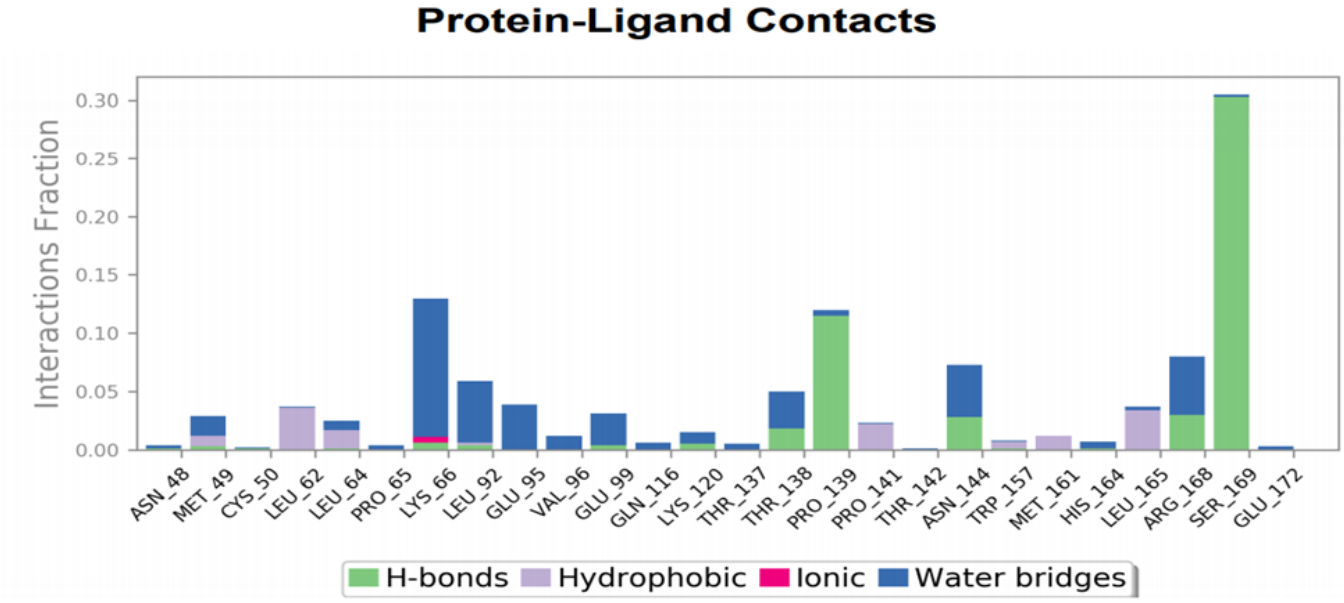


Figure 9: Primuletin atoms connections with the 1ALU residues

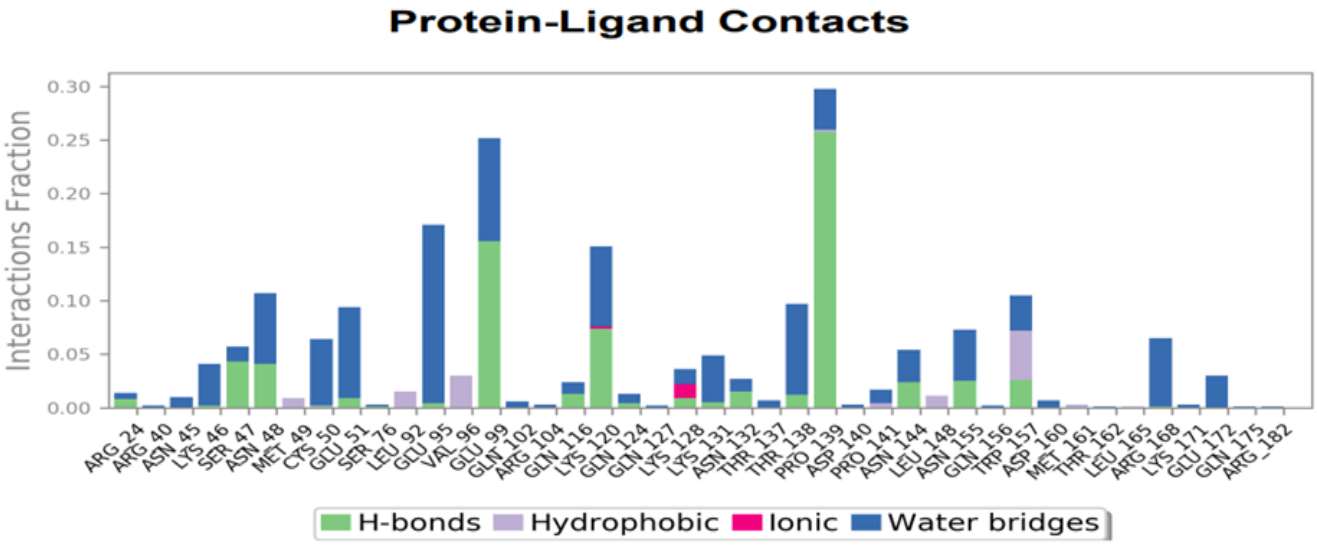


Figure 10: Rhamnetin atoms interactions with the 5MU8 residues

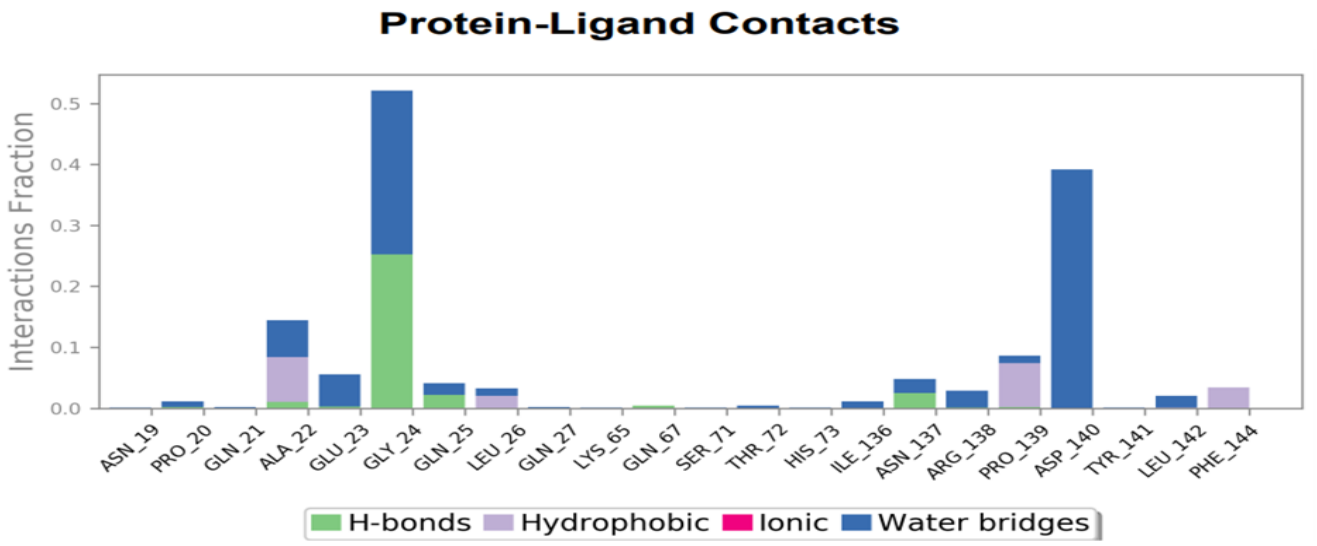


Figure 11: Eupatorin atoms interactions with the 5MU8 residues

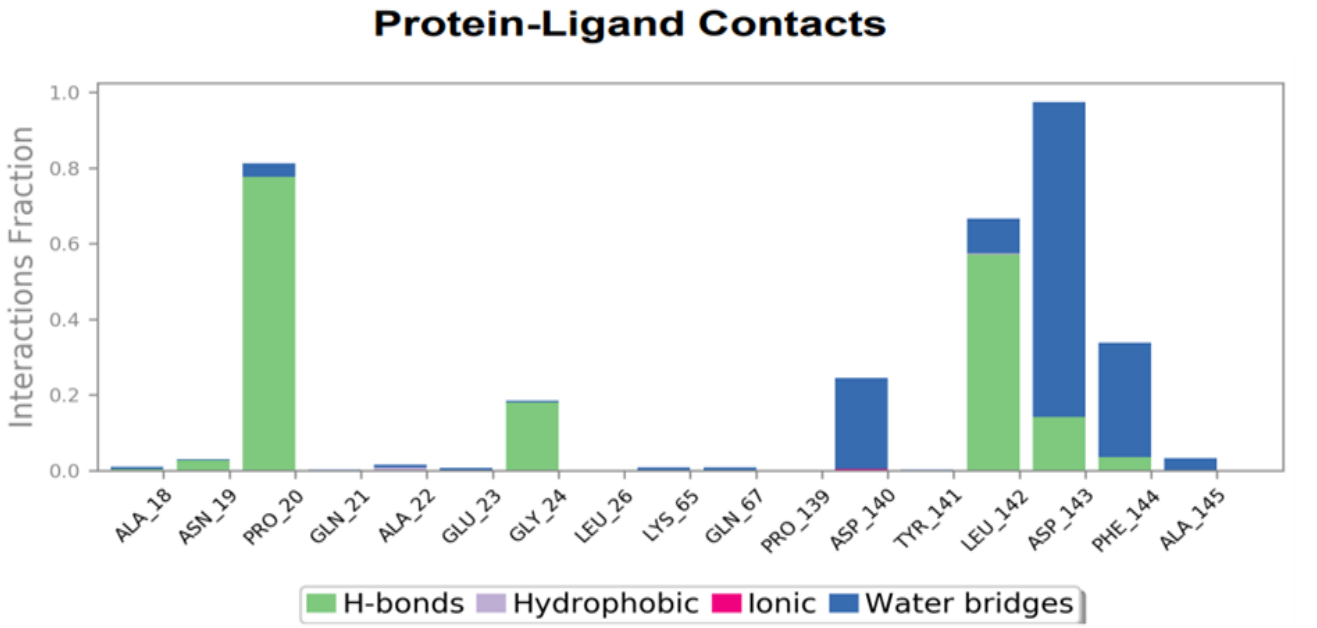
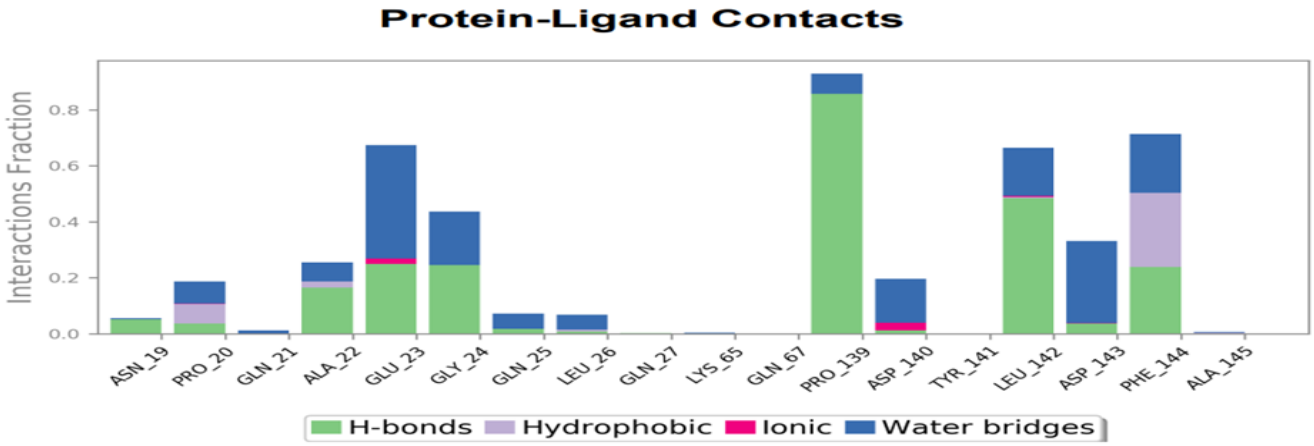


Figure 12: Primuletin atoms interactions with the 5MU8 residues



DISCUSSION

Throughout the simulation, protein interactions with the binding site can be observed. As seen in the diagrams, these interactions can be classified and summarized by form (7, 8, 9, 10, 11 and12). Hydrogen Bonds, Hydrophobic, Ionic, and Water Bridge interactions are the four forms of protein-ligand interactions.

There are hydrophobic interactions all over the binding site. Hydrophobic interactions are important in physiological processes because they increase the binding affinity and bioactivities of complex molecules while also stabilizing the protein-ligand environment. An orderly cage of water molecules surrounds hydrophobic regions. [19].

The 'Simulation Interactions Diagram' panel can be used to discuss more complex subtypes of each interaction form. The stacked bar charts are normalized over the duration of the trajectory: for example, a value of 0.7 indicates that the specific relationship is sustained for 70% of the simulation time. Because certain protein residues can make multiple contacts of the same subtype with the ligand, values above 1.0 are possible.

Stability conformation of protein- ligand complexes

The molecular dynamic simulation was done for protein-ligand complexes to identify the stability conformations. The protein-ligand complexes RMSD (root mean square deviation) plot is provided in Figure and the RMSD results showed that primuletin -targeted protein complexes succeed stable kinetics in simulations. Eupatorin, Rhamnetin complexes shows unstable kinetics.

Molecular docking analysis

Molecular Dynamics Simulations of the protein-ligand complex were carried out using Desmond, Schrodinger, LLC, NY, the USA for the duration of 20 ns. To explore the stability in a solvated system, we have performed molecular dynamics simulations for Rhamnetin, Eupatorin, Primuletin, and the closer binding energy was observed for the 1ALU and 5MU8 from the molecular docking and DFT analysis against Rhamnetin, Eupatorin, Primuletin. Simulation analysis, the RMSD of the Cα and the corresponding ligands during the productive phase relative to its starting structure are shown in figure 1 to 6. At 50 PS intervals, MDS trajectories were stored and simulation stabilities were calculated using the root mean square deviation (RMSD) of the proteins and ligands over time. The RMSD was estimated for C-alpha, which contains both a carboxyl and an amino group, whereas the RMSD was determined for heavy atoms in the case of ligand.

CONCLUSION

The measured binding energies from density functional theory studies for 1ALU (95GLU, 96VAL, 98LEU, 99GLU, 116GLN, 120LYS, 141PRO, 144ASN) and 5MU8 (139PRO, 142LEU, 144PHE) were found to be in good accordance with the interaction affinity obtained from docking studies in this analysis. The overall results of our simulation show that the Rhamnetin, Eupatorin, and Primuletin residues are more effective at maintaining flavonoid stability against 1ALU and 5MU8. These projected outcomes will serve as a foundation for further research into the importance of their drug likeliness characteristics in the treatment of ulcerative colitis.

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