MOLECULAR DOCKING AND DYNAMIC SIMULATION OF ASTRAGALIN REVEALS INHIBITORY POTENTIAL AGAINST PANCREATIC LIPASE

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Abstract

Obesity has been classified as a disease that affects many people around the globe. The prevalence continues to rise each year, thus finding an effective and safe treatment as an anti-obesity drug is a major issue for researchers. At present, the only anti-obesity that gained approval for long-term treatment by the Food and Drug Administration (FDA) is orlistat. It acts by inhibiting pancreatic lipase activity. Unfortunately, it is a synthetic drug and comes with unpleasant side-effects. Hence, there is a need to search for pancreatic lipase inhibitor from natural resources. Several studies have revealed that flavonoids from *Nelumbo nucifera* leave extract showed pancreatic lipase-inhibitory activity. In this study, flavonoids from *N. nucifera* namely leucoanthocyadin, rutin and astragalin were chosen to undergo molecular docking analysis using AutoDock 4.2. Astragalin displayed the best affinity towards pancreatic lipase as compared to the other two flavonoids. Astragalin produced more hydrogen bonds and had lower free binding energy compared to orlistat. Moreover, astragalin formed a strong hydrogen bond with key amino acid Ser152 in the catalytic triad and showed good ligand recognition as it also had a strong hydrogen bond with His151, Phe215 and Arg256. Pancreatic lipase-astragalin complex underwent molecular dynamic (MD) simulation using GROMACS ver. 4. Docking simulation revealed that this complex was more stable compared to the pancreatic lipase-orlistat complex. This preliminary *in silico* result proposed that astragalin might act as an anti-obesity agent through pancreatic lipase inhibition action.

Keywords: Astragalin; *Nelumbo nucifera*; pancreatic lipase; obesity; docking.

1 Introduction

Prevalence of obesity over the past century has become a global health problem. There are more than 600 million adults and 41 million children already classified as obese [1]. Weight reduction via exercise and diet sometimes do not give persuasive result to the extremely obese patients, thus anti-obesity drugs have been developed as one of the obesity treatments due to its convenience and effectiveness. However, up to now, the only available anti-obesity drug that has been approved by FDA for long-term treatment is orlistat. This has drawn researchers' attention to find a new alternative from other resources.

Orlistat act by inhibiting pancreatic lipase. Pancreatic lipase (PL) is the main enzyme responsible for the hydrolysis of 40-70% of total dietary fats which are consumed from foods [2]. Orlistat competes with dietary fats for sites on the lipase molecules; as a result, the fat digestion will be blocked and become inefficient. Fats that are not digested will be discharged directly through feces. However, a major problem with orlistat is that it is a synthetic drug which frequently causes gastrointestinal side effects, such as diarrhea, flatulence, bloating, abdominal pain, and dyspepsia which make this drug less tolerable to the users [3].

For the last past decade, researchers had shown interest in finding the alternative of pancreatic lipase inhibitors (PLI) from natural resources with lesser side effect. So far, however, there has been little discussion about the possible use of *Nelumbo nucifera* as a possible source of PLI. *N. nucifera* leaves and petal extraction have been reported to have the anti-obesity action [4]. From the *N. nucifera* leaves extraction, it has been revealed that only flavonoids showed the most significant pancreatic lipase-inhibitory activity [5]. However, the *in silico* study on the mechanism of the studied compounds towards PL binding is still lacking. Hence, the objectives of this study are to predict the potential binding site of selected flavonoids from *N. nucifera* namely, leucoanthocyadin, rutin and astragalin on pancreatic lipase (PDBID: 1LPB) by molecular docking and to compare the stability of the best complex (astragalin-1LPB) with orlistat-1LPB complex by MD simulation.

2 Materials and Method

2.1 Materials

The computational work was run on processor Intel®Xeon (R) CPU X5450 @ 3.00GHz x 4 using the Ubuntu16.04 LTS (Xenial xerus) located at Bioinformatics Lab, Kulliyyah of Science IIUM. The molecular docking was conducted using AutoDock 4.2. All MD simulations were run with GROMACS ver. 4.

2.2Methodology

2.2.1 Molecular Docking

2.2.1.1 Receptor and Ligand Preparation

The three-dimensional (3D) crystal structure of the human PL-colipase (CL) from Protein Data Bank (PDB) with PDB-ID: 1LPB was chosen as the template for receptor. All existing ligand and water molecule were deleted prior docking. All 3D ligand structures (leucoanthocyanidin, rutin and astragalin) were derived from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) in the format of *.sdf while the 3D of orlistat (control) was derived from other database, Chemspider (www.chemspider.com) in the format of *.sdf. All .sdf files were converted to .pdb file using BIOVIA Discovery Studio.

2.2.1.2 *Blind Docking and Focus Docking*

Blind docking was run using AutoDock 4.2. Firstly, polar hydrogen atom and partial charges (Kollman charges and Gasteiger charges) were added to receptor, 1LPB and ligands which help them stabilised in binding process. Total Kollman charges added was 9.0 and total Gaisteiger charge added was -3.00. After that, PDBQT file was created as coordinate file, which consist of atomic partial charges and atom types. Pancreatic lipase was considered rigid and ligands were considered fully flexible. Thirdly, the grid maps were created using AutoGrid to pre-calculating atomic affinity potentials for each atom type in the ligand's molecule being docked. Next, the grid boxes were created for blind docking and focus docking. The spacing for blind docking Grid box was adjusted to 1.000 Å spacing with the grid box dimensions of 80 × 70 × 80 points and the x, y, z centre to -2.685, 29.851 and 38.483 points. For focus docking, the box was adjusted to 40 × 40 × 40 points and the x, y, z centre to 4.448, 27.955 and 49.675 points. After the grid box was created, the docking process was run using parameter Lamarkian genetic algorithm (LGA). The parameter employed for 100 runs to search for the best conformer of the ligands. The result was written in *dlg file for each final structure of docking conformation. The molecular docking steps were repeated thrice for each ligand to ensure software stabilization.

2.2.1.3 *Post-docking analysis*

The best conformation from each docking was extracted whereby out of 100 runs, the final structures were clustered and ranked according to the native AutoDock4.2 scoring function[8]. Firstly, the *.dlg file was opened using AutoDock 4.2 and the macromolecule which was 1LPB were loaded. Next, all final clusters of docking runs were re-clustered and represent into histograms. From the histograms, the highest cluster of conformation with the lowest binding energy is selected to determine the best docking result[8]. Next, the conformations of the ligands were analysed to select the conformation with the highest hydrogen bonds. The final conformation is then subjected to the free binding energy and inhibitor constant, *Ki*.

2.2.1.4 *Analysis of the Two Dimension (2D) of PL-Ligand Complex*

Eight conformations structure was converted from *.pdbqt file into *.pdb file for evaluation of hydrogen bonding and hydrophobic interaction between the ligands and 1LPB. The structure complex was visualised using Ligplot.

2.2.2 *MD simulation*

Astragalin-1LPB complex was chosen for MD simulation as it has the lowest free binding energy and the lowest *Ki*. Orlistat-1LPB complex was served as the control. GROMACS ver. 4 was used to perform the simulation. Both complexes were prepared for topology files creation. A cubic box was created for the complexes and water molecules were added into the box to solvate the complexes. Three Naions were added to neutralise the system followed by energy minimisation and equilibration. The simulation job was carried out over a period of 10, 000 ps. Root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and the number of hydrogen bonds of the complexes were analysed using GRaphing Advanced Computational Exploration of data (GRACE).

3 Results and Discussion

3.1 Molecular docking

The main purpose of molecular docking performed in this study was to identify a ligand that binds to pancreatic lipase binding site which consists of catalytic triad Ser152, Asp176 and His263. Besides that, molecular docking also used to identify ligands with energetically most favourable binding pose. All four aspects which are 1) free binding energy, ΔG; 2) inhibition constant, *Ki*; 3) hydrogen bond numbers and 4) hydrophobic interaction were generated and compared for those ligands. Later, the best conformations were selected from 100 docking runs. AutoDock 4.2 produced 100 conformations from 100 docking runs which have different orientation as it will try to fit into the binding site of the enzyme with the lowest energy needed. The production of conformations was then followed by scoring the individual conformations into several clusters. Each cluster has several conformations with same binding energy. The best cluster was chosen based on the highest number of conformations formed and has the lowest free binding energy. The chosen cluster number of each complex was tabulated in the Table 4.1. All the cluster number of each complex was cluster 1. For the blind docking ranking, the number of conformations formed was lower compared to focus docking due to the bigger surface of enzyme was covered. Thus, the possibility of the conformations to bind at the same place was low.

Table 1: Summary of the analysed conformations.

	Complex	*Cluster's number*	*Number of conformations formed*
Blind Docking	Astragalin-1LPB	1	2
	Leucoanthocyanidin-1LPB	1	4
	Rutin-1LPB	1	1
	Orlistat-1LPB	1	1
Focus Docking	Astragalin-1LPB	1	69
	Leucoanthocyanidin-1LPB	1	100
	Rutin-1LPB	1	12
	Orlistat-1LPB	1	21

3.1.1 Blind docking

A blind docking is a molecular docking without any assumption about the binding site of the enzyme [6]. Even though enzyme has binding site, ligand might form allosteric binding to the receptor [7].

3.1.1.1 Free Binding Energy, ΔG

The free binding energy of the four ligands and PL were recorded, and the average of the energy was calculated. Table 2 presents the free binding energy for all complexes. AutoDock 4.2 uses a semi empirical free energy force field to calculate of ΔG between ligands to protein target[13]. From the result, all the value showed negative free binding energy. The negative value for ΔG signifies a spontaneous interaction process[14]. This result suggested that all complexes underwent spontaneous reaction with PL during blind docking.

Table 2: Free binding energy analysis of each complex in blind docking. Astragalin had the lowest free binding energy correspond to the highest affinity towards PL.

Complex	*Free Binding Energy (Kcal/mol)*
Astragalin-1LPB	-5.54
Leucoanthocyanidin-1LPB	-4.71
Rutin-1LPB	-2.96
Orlistat-1LPB	-2.00

All ligand complexes showed lower free binding energy when compared with the control, orlistat-1LPB. Theoretically, if a ligand binds to a protein, the decrease in enthalpy value is due to favourable intermolecular interactions and formation of intermolecular bonds [8]. The decrease of enthalpy value contributes to the release of more heats to the surrounding. Hence, all ligand complexes showed high affinity to PL as they produced more heats which correlate with higher intermolecular interactions and intermolecular bonds. Among all ligands, astragalin showed the highest affinity towards PL as it produced the lowest free binding energy with -5.54 Kcal/mol.

3.1.1.2 Inhibition Constant, Ki

The inhibitor constant, *Ki* indicates how potent the ligand as inhibitor. It estimates the value of ligand concentration required to produce half maximum inhibition. The value of *Ki* from each complex was tabulated in Table 3. It was depicted that astragalin-1LPB complex had the lowest value of *Ki* with 87.31 µM followed by leucoanthocyanidin and rutin with 354.74 and 680.00 µM, respectively.

Table 3: The *Ki* values of each complexes using blind docking. Astragalin had the lowest number of *Ki* which correspond to the most potent ligand/ inhibitor.

Complex	***Ki (µM)***
Astragalin-1LPB	87.31
Leucoanthocyanidin-1LPB	354.74
Rutin-1LPB	680.00
Orlistat-1LPB	33, 980.00

3.1.1.3 Analysis of Protein-Ligand Complexes

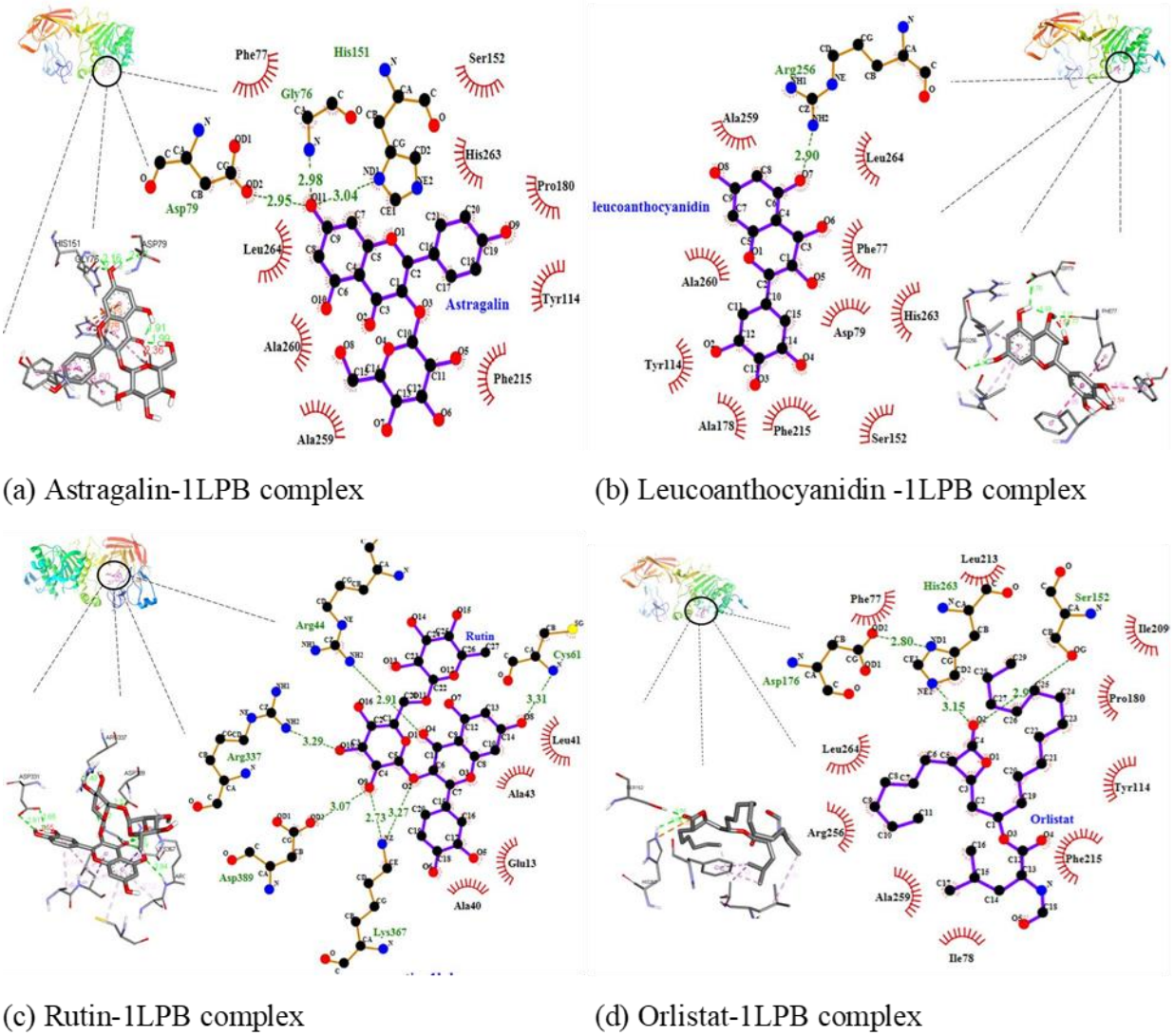
Hydrogen bond and hydrophobic interaction play critical roles in the ligand recognition and protein stability[17]. Hydrophobic interaction is also important to stabilise the hydrogen bond produced while hydrogen bonding plays an important role in increasing binding affinity of ligands to PL. Figure 1 displayed the 2D interaction between ligand and PL from blind docking.

Astragalin contacted with 12 amino acids of PL which are Ser152, His263, Pro180, Tyr114, Phe215, Ala259, Ala260, Leu264, Asp79, His151, Phe77 and Gly76 (Figure 1(a)). All of the listed amino acids contacted with astragalin by hydrophobic interactions. Binding affinity in astragalin-1lpb complex was maintained with the three hydrogen bonds that formed between Asp79, Gly76 and His151. Among these 12 amino acids, two amino acids are the catalytic triad of PL, namely Ser152 and His263. As astragalin attached to the catalytic triad of PL, it is hypothesised that the function of PL to hydrolyse lipase will be blocked by astragalin.

Cluster 1 of the leucoanthocyanidin showed that this ligand contacted with 11 amino acids residues which are Leu264, Phe77, His263, Asp79, Ser152, Phe215, Ala178, Tyr114, Ala260, Ala 259 and Arg256. Leucoanthocyanidin-1LPB complex also had hydrophobic interactions with two catalytic triads, His263 and Ser152 (Figure 1(b)).

Rutin contacted with three amino acids from PL which are Lys367, Asp389 and Arg337 with additional six amino acids from CL which are Glu13, Ala40, Leu41, Ala43, Arg44 and Cys61 (Figure 1(c). The hydrogen bonding had formed at Cys61 and Arg44 at CL and formed at Lys367, Asp389 and Arg337 at the PL. The data showed that rutin can strongly bind at site far from the binding site of PL and thus none of catalytic triad binds to the rutin in the blind docking processes. Two bindings between PL-CL complexes are Lys399-Glu45 and Asp389-Arg44 [9]. Asp389 from PL need to bind with Arg44 from CL. As rutin had bind at the place where this binding should occur, the formation of PL-CL complex will be disturbed. Rutin might compete with CL at this binding pocket. Thus, it is hypothesised that rutin might be allosteric inhibitor to PL.

As for the control, orlistat was also subjected to blind molecular docking to check the reliability of the docking methods. Orlistat interacted with 13 amino acids of PL which were Ile78, Ala259, Arg256, Leu264, Asp176, His263, Ser152, Ile209, Phe77, Leu213, Pro180, Tyr114 and Phe215 (Figure 1(d)). The complex had strongly bound to the binding site of PL using hydrogen bonds which consist of Ser152, His263 and Asp176. From previous study, orlistat-1LPB complex produced three hydrogen bonds by the end of docking which were Phe77, Ser152 and His263 [10].



(a) Astragalin-1LPB complex (b) Leucoanthocyanidin -1LPB complex

(c) Rutin-1LPB complex (d) Orlistat-1LPB complex

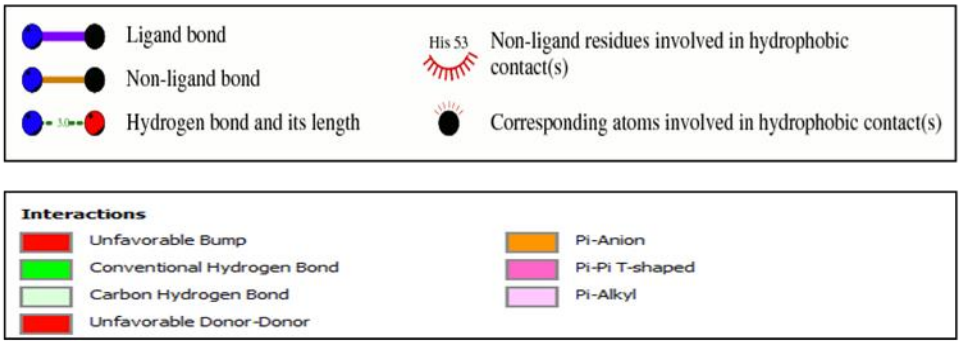


Figure 1: The 2D analysis of all complexes for blind docking using Ligplot. Astragalin and leucoanthocyanidin bound at the binding site of PL while rutin bound between PL and CL.

3.1.2 Focus Docking

Focus docking approach is a molecular docking with an assumption about the binding site of the enzyme. Focus docking was applied as a further term to the scoring function and has been shown to get better results of docking evaluation [11].

3.1.2.1 Free Binding Energy

Table 4 presents the result obtained from the calculation of free binding energy for each complex in focus docking. From the result, all the free binding energy showed negative value except for rutin. The lowest free binding energy value was astragalin-1LPB complex compared with the other three ligands. It showed that astragalin had the highest affinity to 1LPB, thus, the best PLI candidate. On the other hand, the positive value of free binding energy in rutin signifies unnatural interaction process or termed as unfavourable binding [12]. Rutin absorbed energy during the ligand and protein interaction and this reaction was not energetically favourable. Orlistat-1LPB complex had higher free binding energy if compared to astragalin-1LPB and leucoanthocyanidin-1LPB complexes.

Table 4: Free binding energy analysis of each complex in focus docking.

Complex	*Free Binding Energy (Kcal/mol)*
Astragalin-1LPB	-8.36
Leucoanthocyanidin-1LPB	-7.25
Rutin-1LPB	+9.82
Orlistat-1LPB	-3.51

3.1.2.2 Inhibition Constant, Ki

The value of *Ki* from each complex was tabulated in the Table 5. The value of *Ki* from astragalin-1LPB showed the lowest value of *Ki* when compared to other complexes with 0.853 µM. This value was only 0.001% of the value of *Ki* from orlistat-1LPB complex. It had shown that the most potent flavonoid is astragalin. The other flavonoid that showed lower percentage of *Ki* when compared to the orlistat was leucoanthocyanidin-1LPB with 0.006%. As for rutin, AutoDock 4.2 cannot compute the *Ki* as this ligand had unfavourable binding with the 1LPB as this ligand preferably bind 1LPB in allosteric manner.

Table 5: The *Ki* values of each complexes using focus docking.

Complex	*Ki (µM)*
Astragalin-1LPB	0.853
Leucoanthocyanidin-1LPB	5.040
Rutin-1LPB	-
Orlistat-1LPB	82,765

3.1.2.3 Analysis of Protein-Ligand Complexes

The 2D analysis result from focus docking was presented in Figure 2. As displayed in Figure 2(a), Cluster 1 of astragalin had interactions with 16 amino acids of receptors which are Pro180, Ala178, Ser152, Phe77, Asp79, Trp85, His 151, His75, His263, Gly76, Ala260, Leu264, Arg256, Ile78, Ala259 and Phe215. Out of 16 amino acids, five of them interacted with astragalin using hydrogen bonds which are Ser152, Asp79, His151, Arg256 and Phe215 with normal hydrogen distance. It is a well-established fact that Ser152 is in the primary residue that is vital in lipolytic activity. This finding indicates that astragalin might have good inhibitor characteristics towards PL. Then, this complex also showed high stability as it also produced many hydrophobic interactions as it involved 11 amino acids from binding pocket including His263, another catalytic triad.

The 2D analysis of Cluster 1 leucoanthocyanidin showed that this compound have contacted with 12 amino acids of PL which consist of Phe 215, Ala 178, Pro 180, Tyr 114, Ser 152, Phe 77, Gly 76, Asp 79, His 75, His 151, His 263 and Ala 260 (Figure 2(b)). The hydrogen bonds were produced at three amino acids which are Gly 76, Asp 79 and His 151. Previous study reported that, zinc04104767- 1LPB complex had produced two salt bridges with the Arg256 and Asp79 residues in the binding pocket of PL [10].

The result of analysis between Cluster 1 of rutin-1LPB complex is shown in Figure 2(c). The hydrophobic interactions were observed on 11 amino acids which are Leu153, Ala178, Ile209, Pro180, Leu264, His263, Ala259, Ala260, Ile78, His151 and Gly76. In addition, there were also four amino acids interacted with rutin via hydrogen bonds which are Phe77, Tyr114, Phe215 and Ser152. The hydrogen bond was shown between Ser152 with =O of rutin. However, rutin was found to split into two due to the force energy from the intermolecular energy. This is proportional with the production of positive value of free binding energy of rutin in focus docking. This strengthen the fact that rutin is mostly possible to bind at location other than binding site which specifically between PL and CL.

Figure 2(d) shows the analysis of orlistat-1LPB. There were 16 amino acids that interacted with the control which were Gly76, Pro180, Tyr114, Ile209, Ala178, Ser152, Glu179, Ala260, Phe215, Arg256, Asp79, Leu264, His263, Ile78, His151 and Phe77. All the amino acids interacted via hydrophobic contact except Ser152 and Phe77 that contacted with hydrogen bonds. Focus docking on the control compound showed that this compound have good interaction with Ser152. Orlistat or also called tetrahydrolipstatin is a potent inhibitor of gastrointestinal lipases [13] that contains a β-lactone structure which is responsible for irreversible inhibition [14].

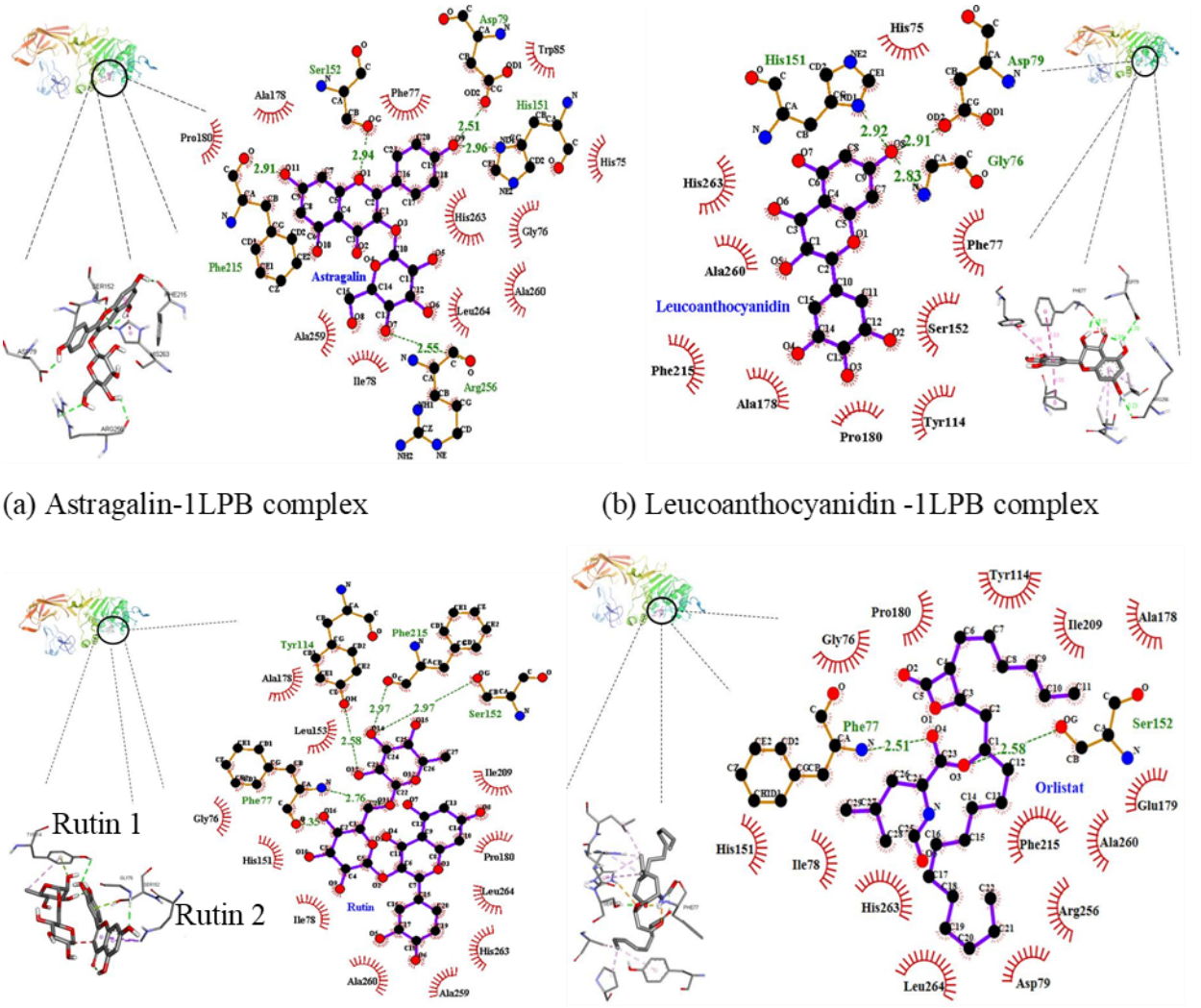


Figure 2: The 2D analysis of all complexes for focus docking using Ligplot. Astragalin bound closely to the PL binding site while rutin could bind at same place as astragalin but with unfavourable energy.

3.1.3 *Comparison between Blind and Focus Docking*

3.1.3.1 *Free Binding Energy*

It is well-known that the lower the free binding energy the higher the affinity of the ligand with the protein [15]. According to both blind and focus docking, astragalin showed the highest affinity as it produced the lowest free binding energy. Figure 3 presented the comparison of free binding energy between blind and focus docking of three flavonoids present in *N. nucifera* leaves and orlistat (control). The free binding energy between these two methods showed the same pattern. Astragalin-1LPB complex constantly showed the lowest free binding energy, followed by leucoanthocyanidin, rutin, and orlistat. However, in focus docking, rutin had positive value due to the breakdown of the ligand.

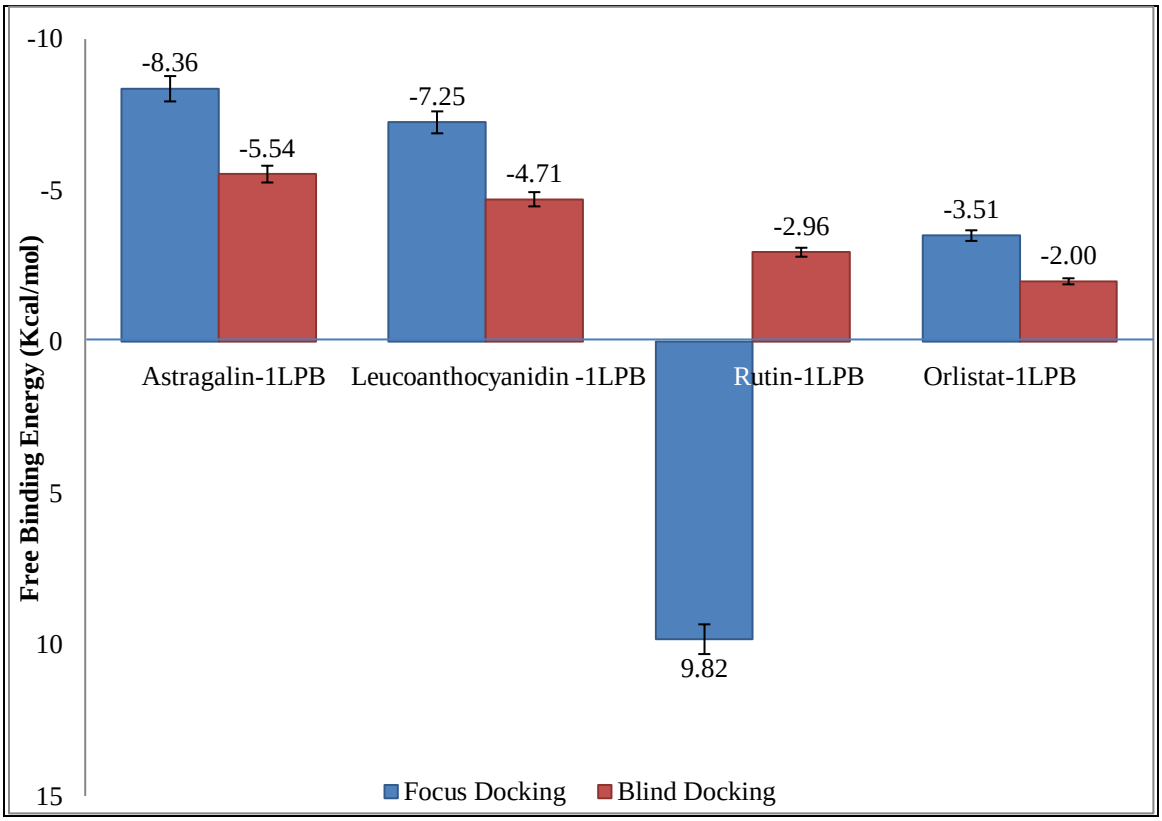


Figure 3: Free binding energy of ligands using different approaches of molecular docking (n=3).

3.1.3.2 *Inhibitor Constant, Ki*

Figure 4 shows the significant difference between the values of *Ki* between the complexes. The trend of *Ki* from two different docking was similar and the result showed significant gaps between astragalin-PL and orlistat-PL complex. The lowest *Ki* value was shown in astragalin-PL complex followed by leucoanthocyanidin, rutin, and orlistat.

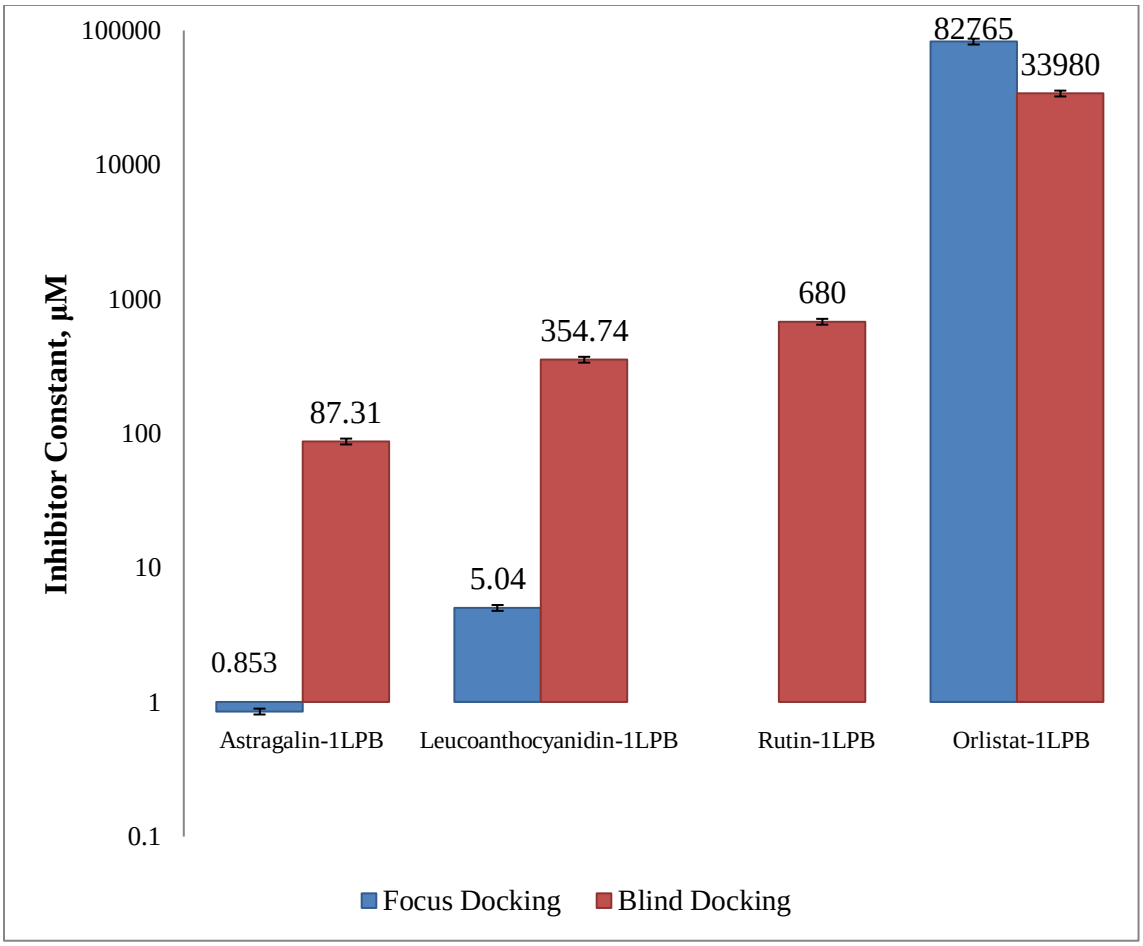


Figure 4: The inhibitor constant, *Ki* for each complex (n=3).

3.1.3.3 Number of Hydrogen Bond and Hydrophobic Interaction

To measure the interaction that occurred between all selected ligands and PL, the bar graph in Figure 5 was observed and analysed. The total number of these two main interactions between ligands and receptor showed that astragalin-1LPB complex had almost the same quantity of interaction when compare with orlistat. Astragalin produced the highest number of hydrogen bonds in focus docking and formed a good hydrophobic interaction with 1LPB. The rank of hydrogen bond among all complexes from the highest to the lowest is arranged as follows: astragalin-PL = rutin-PL ≥ leucoanthocyanidin ≥ orlistat-PL. The number of residues bind to each ligand was listed in Table 6. The number of hydrogen bond indicates the affinity of the ligand towards PL. Orlistat-PL complex shows the highest hydrophobic interaction when compared with other complexes. This interaction helps orlistat to stabilise at the binding sites of PL.

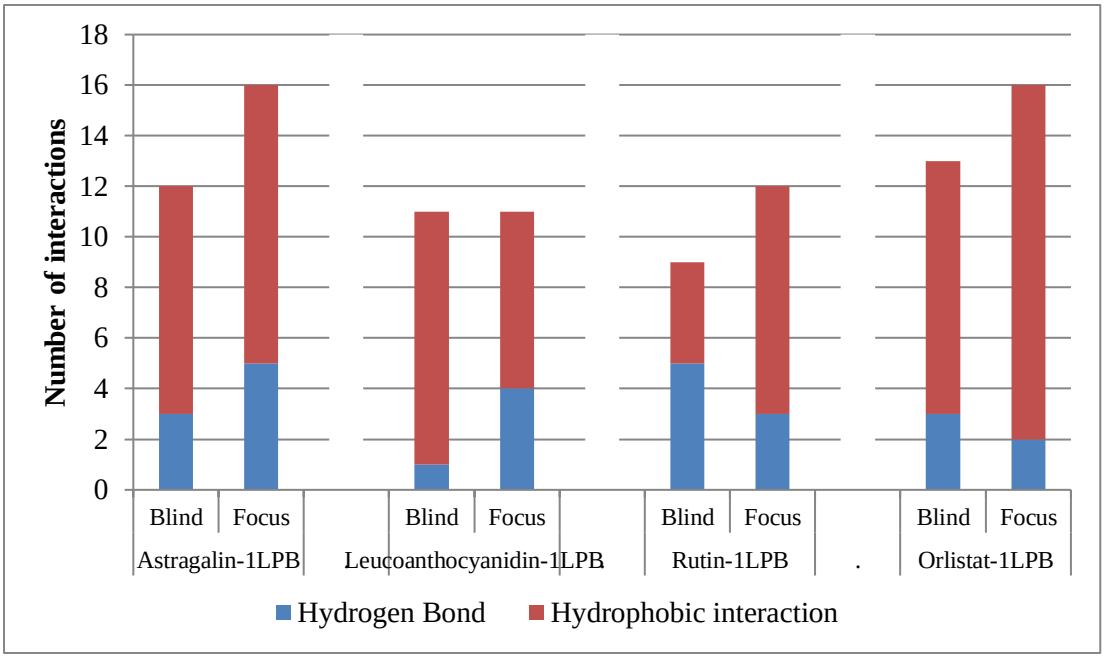


Figure 5: The number of hydrogen bond and hydrophobic interaction of each complex.

Among all the complexes that had been formed, only three ligands can bind to Ser 152 which was astragalin-1LPB complex, rutin-1LPB complex and orlistat-1LPB complex. However, only astragalin can bind with the lowest free binding energy compared to orlistat and rutin. Furthermore, rutin can only bind to amino acids with additional energy from outside of the complex. This result showed that astragalin is the best PLI compared to other ligands.

Table 6: The important residues which bind to ligand using hydrogen bonds.

Complex	*Blind docking*	*Focus docking*
Astragalin-1LPB	Asp79, Gly76, His151	Ser152, Asp79, His151, Arg256, Phe215
Leucoanthocyanidin-1LPB	Arg256	Gly76, Asp79, His151
Rutin-1LPB	Cys67, Arg44, Lys67, Asp35	Ser152, Phe77, Tyr114, Phe215
Orlistat-1LPB	Ser152, His263, Asp 176	Ser152, Phe77

3.2 MD Simulation

3.2.1 RMSD

Astragalin-1LPB complex from focus docking was chosen to study the complex stability in comparison with orlistat-1LPB complex (control). The value of RMSD calculated by GROMACS evaluated the deviation of the complexes from the original starting structure over the course of the 10, 000 ps simulation. The stability of the ligand-PL complex in its dynamic mode is important to indicate the suitability of the ligand to bind with the enzyme. Plots of the standard deviations of the Cα RMSD of both complexes shown in Figure 6 suggested that both complexes were stable over the trajectory of 10, 000 ps simulation. The average Cα RMSD for astragalin-1LPB complex was 3.0 Å ± 1.0 Å, whereas orlistat-1LPB complex was 3.5 Å ± 1.0 Å. Findings from previous study showed that if the RMSD fluctuated too much, it will represent by the range motion more than ~3Å which indicate asymptotic behaviour of the protein [16]. The higher the RMSD value means the longer the deviations of the backbone. From the starting

point, both of complexes only fluctuated in the range of 1.0 Å only which means the protein was stable. Other than that, both complexes have a plateau state starting at 6000 ps. This means that both complexes were stable at 4 Å, but the orlistat-PL complex stable a little bit faster that astragalin-PL.

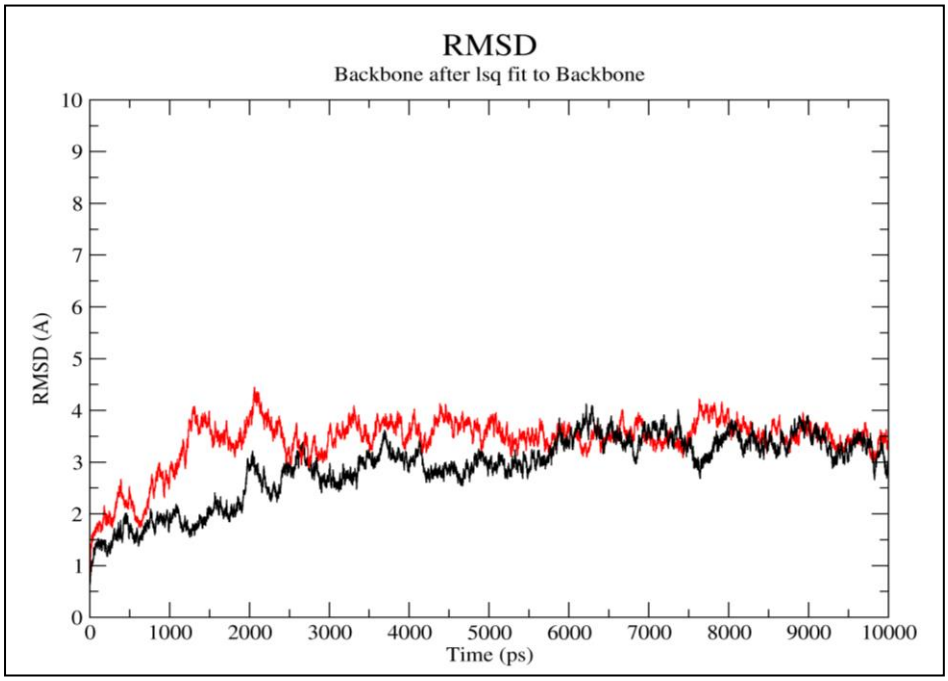


Figure 6: RMSD of the 1LPB Cα atoms vs. time for the astragalin-1LPB (black) and orlistat-1LPB (red).

3.2.2 RMSF

RMSF is an important parameter to find the flexibility of both complexes for each atom of the complexes involving altogether 449 amino acids. The raw data of the RMSF was then visualized using the Xgmr and produced line graph as illustrated in Figure 7. The Cα RMSF value derived from the MD simulations for the astragalin-1LPB and orlistat-1LPB complexes were calculated to assess the backbone flexibility. The RMSF for the astragalin-PL and orlistat-1LPB complexes were 2Å ± 1.5Å and 2Å ± 1Å, respectively. This finding showed that there is no significant difference from both complexes, and it can be deduced that both complexes have good bone flexibility. It was also observed that the backbone of Cα RMSF of astragalin-1LPB was more stabilized at 1-350 residues than orlistat-1lLPB. The RMSF graph for astragalin-PL complex was less fluctuated compare to astragalin-1LPB complex. Interestingly, both complexes had fluctuation at Pro410 which is one of the rigid amino acids. The reason behind the movement of this amino acid is because this amino acid located at T-turn of tertiary structure. This was supported by a finding from Sudi et al., 2014 [17]. According to the author, Pro265 of D-2CP-DehD protein and L-2CP-DehD protein complexes were also fluctuated to a greater extent ~32.5Å because Pro265 was one of the atoms from carboxyl terminal residue. This knowledge can give interesting idea on how actually the ligands move into the binding sites which might involve some contacts with the loops of PL.

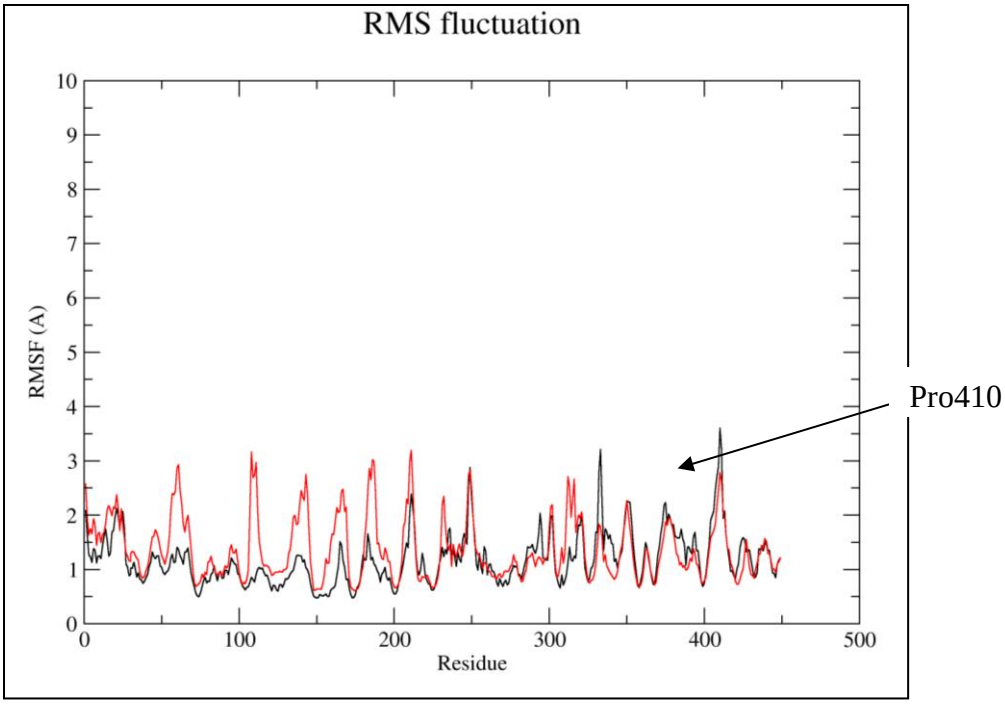


Figure 7: RMSF of the 1LPB Cα atoms vs. the number of atoms for the astragalin-PL and orlistat-PL complexes. Astragalin-1LPB is represented as black line while orlistat-1LPB is represented as red line.

3.2.3 Number of hydrogen bonds

The number of hydrogen bonds formed throughout simulation by 1LPB and two ligands were shown in Figure 8. The number of hydrogens produced for each ~1ps was recorded. There were changes in the hydrogen bonding networks in which for astragalin-1LPB complex, the numbers of hydrogen bonds were stabled at 2 bonds and it is almost constantly produced along dynamic conditions. This data showed that astragalin was maintained at its location and hold tightly by the hydrogen bonds. In the case of orlistat-1LPB, these changes led to a slightly weaker H-bond network with one to two average hydrogen bonds. There are also moments (~4000ps, 6,000ps 8,000ps) where hydrogen bonds had been disappeared between orlistat-1LPB and at this moment orlistat is hypothesized was held by hydrophobic interactions. This detachment of orlistat from the binding site might affect the overall binding ability.

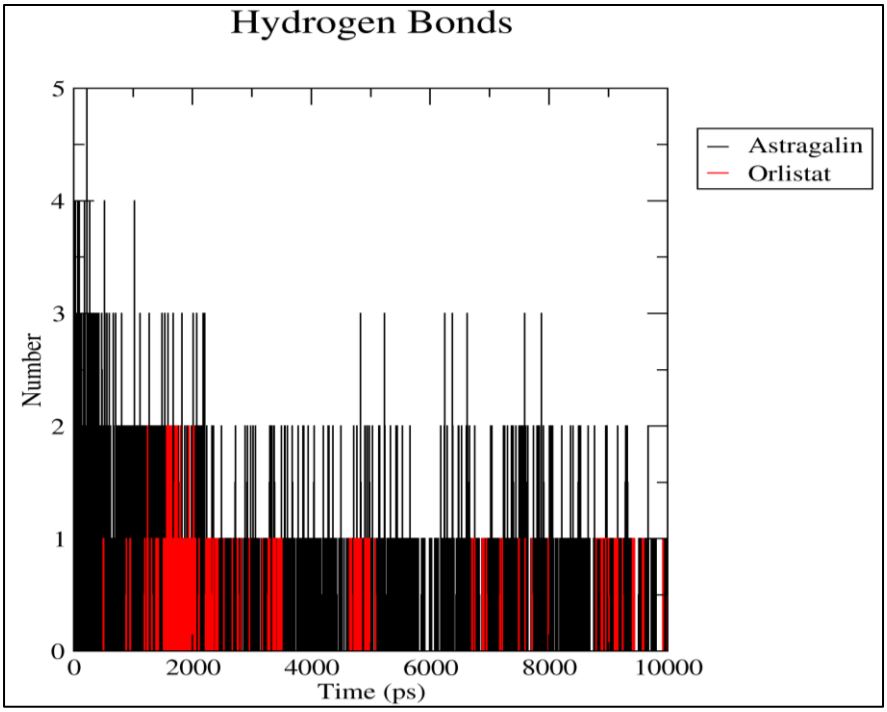


Figure 8: Number of apparent hydrogen bonds vs. time for the astragalin-1LPB (black) and orlistat-1LPB (red).

4 Conclusion

In conclusion, the potential of three flavonoids from *N. nucifera* namely astragalin, leucoanthocyanidin and rutin to inhibit PL were successfully unveiled via molecular docking. Molecular docking displayed astragalin as the best PLI compared to the other two flavonoids. Astragalin had the highest affinity towards PL, and it bound to the active site Ser152. Astragalin docked to PL with the lowest energy compared to the other two flavonoids. Moreover, astragalin showed the lowest *Ki* value and gave the highest number in hydrogen bonds. MD simulation over the course of 10, 000 ps displayed the stability astragalin-1LPB complex whereby it showed constant atomic deviations and fluctuation, together with the stable formation of the hydrogen bonds. Overall, these results proposed that astragalin might be a potential PLI. Further *in vitro* and *in vivo* experimental investigation is recommended for further confirmation of the molecular docking result.

Conflicting Interest

The authors declare they have no conflicting interest.

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