In Silico Docking of Epicatechin, Corilagin and Quercetin as Potential Pancreatic Lipase Inhibitor for Obesity Treatment

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Abstract:

Background: Obesity is a complex disease, caused by an imbalance between energy intake and energy consumption in the human body thus leading to one of the prominent diseases that affect the world. The statistic of obesity continues to increase worldwide every year due to many factors. Therefore, the major concern of today's public health is to find an effective and safe treatment as an anti-obesity drug. Pancreatic lipase (PL) plays an essential role in the digestion of dietary lipids. Therefore, the primary target of the drug is to inhibit the activation of the PL enzyme. Orlistat is the only anti-obesity drug issued by the Food and Drug Administration (FDA) which is potent and specific in action. However, this drug had shown some adverse effects on the gastrointestinal tract. Thence, the alternative solution from the natural origin as orlistat substitutes are in demand. In this study, natural compounds namely quercetin, epicatechin, and corilagin were identified as the potential anti-pancreatic agents.

Materials and Methods: Molecular docking was done to assess the binding affinity of the phytochemicals. Both blind docking and focus docking were conducted. Blind docking was performed with no assumption of the potential binding site. While focus docking, focusing on the region covering the catalytic triad comprises Ser152, His263 and Asp176 which are the key residues for lipid absorption.

Result: The result shows that the epicatechin-1lpb complex has the best potential as a PL inhibitor since it recorded the lowest average free binding energy (-8.66 Kcal/mol) and formed hydrogen bonds at pockets of the active sites (Ser152, His263 and Asp176). Epicatechin also yielded the highest number of hydrophobic interactions and the lowest K_i value which further stabilized the ligand complexes and strengthened the binding affinity.

Conclusion: Thus, this preliminary i*n-silico* result proposed Epicatechin as the best candidate as a PL inhibitor agent.

Keywords: Pancreatic lipase, Lipase inhibitor, obesity, epicatechin, docking

Introduction:

Obesity can be defined as inequality between energy intake and output energy that leads to excessive Body Mass Index (BMI) over 30 kg/m^2 (Kumar et al., 2013). Obesity also is frequently linked with many serious health diseases for instance diabetes, heart disease, colorectal cancer, hyperlipidemia and atherosclerotic cerebrovascular disease (Zhang et al., 2014). Obesity has become the leading cause of preventable death. In Malaysia, diseases related to obesity are rising and ultimately become the fundamental cause of death.

Currently, Food and Drug Administration (FDA) has issued orlistat as the most competent drug for obesity treatment. Orlistat acts as a pancreatic lipase (PL) inhibitor (Kim et al., 2016). Gastric and PL are two substantial enzymes that are involved in the hydrolyzation of fatty acid. The absence of these enzymes resulted in unhydrolyzed lipids in the system which are required for losing weight. Despite the glory it brings, this drug is also struggling with detrimental effects such as steatorrhea (fat and oily stool), abdominal pain, digestive problems and diarrhea as reported (Filippatos et al., 2008).

Considering the aftereffect, better substitutes are sought to replace orlistat. Researchers had shown great interest in multiple compounds to replace orlistat. Few compounds were mentioned including quercetin, epicatechin and corilagin. So far, multiple researches were performed on the benefits of the compound, but limited studies mentioned the potential of the compound as a PL inhibitor. Thus, *in-silico* docking was performed on the selected compounds and the results were observed (Abdulkhaleq et al., 2017; Islam et al., 2013)The objective of this study is to identify the binding site of the selected phytochemicals namely quercetin, epicatechin and corilagin.

Materials and Methods:

Receptor and Ligand Preparation

The computational work was run on Intel ® Core ™ i7-3612QM CPU @2.10 GHz x 64 using Microsoft 10 and Asus X450EA with processor AMD A4-5100 APU with Radeon ™ HD Graphics @ 1.55 GHz x64 using Windows 10 Pro. The molecular docking was conducted using AutoDock 4.2.

In this study, the receptor selected is the human PL-colipase (CL) loaded from Protein Data Bank (PDB) registered under PDB-ID: 1lpb. All existing ligands and water molecules were deleted before docking was performed. 3D Ligands included quercetin, epicatechin and corilagin were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the format of *.sdf while the 3D of orlistat which acts as the positive control was derived from database Chemspider (http://www.chemspider.com/) in the format of *.sdf. Then all the *.sdf files were converted into a .pdb file via Smiles Translator and Structure, a cloud conversion service provider (https://cactus.nci.nih.gov/translate/).

Blind Docking and Focus Docking

Blind Docking was run using AutoDock 4.2. Firstly, polar hydrogen atoms and partial charges (Kollman charges and Gasteiger charges) were added to receptors, 1LPB and ligands to stabilize the atoms. Total Kollman charges added was (9.0) and total Gasteiger charges added was (-3.00). PDBQT files were then created as coordinate files. Consequently, the grid maps were then created using AutoGrid to pre-calculate atomic affinity potentials for each atom type in the ligand molecules before 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 with the grid box dimensions of 80 x 70 x 80 points and the x, y and z centre to -2.685, 29.851 and 38.483 points. While for focus docking, the box was adjusted to 0.375 Å with the grid box dimensions 40 x 40 x 40 points with x, y and z centre to 4.448, 27.955 and 49.675 points. After the grid box was created, the docking process was run using the parameter Lamarckian Genetic Algorithm (LGA). The parameter employed for 100 runs to identify the best conformer of the ligands. The result was obtained in the .dlg file for each final structure of docking conformation. The molecular docking was also repeated thrice.

Post-docking analysis

Subsequently, the result received in the .dlg file, the file was opened using AutoDock 4.2 and the macromolecules of 1lpb were loaded. The final clusters of docking runs were re-clustered and represented into histograms. From the histograms, the highest cluster of conformation with the lowest binding energy was selected to determine the best docking result. Next, the conformation of the ligands was analyzed to select the conformation with the highest hydrogen bonds. Apart from the binding energy, inhibition constant (K_i value) and hydrophobic interaction were also analyzed.

Analysis of the 1LPB-ligand complex

Four conformations structure was converted from *.pdbqt file into *.pdb file for evaluation of hydrogen bonding and hydrophobic interaction between the ligand and 1LPB. The structure was visualized using LigPlot+. The 3D structures were visualized using YASARA.

Result and Discussion:

Molecular docking was performed in this study to identify the ligands that bind to the catalytic triad which are Ser152, His263 and Asp176. Besides, the goal of docking is to identify the energetically most favorable binding pose (Li et al., 2016). Four parameters observed are free binding energy, ΔG, inhibition constant, K_i value, hydrogen bond numbers and hydrophobic interaction (Madeswaran et al., 2012).

Blind docking: Free binding energy, ΔG

Blind docking was performed to identify the possible binding sites and modes of peptide ligands by examining the entire surface of protein targets (Gandhi et al., 2019) . It is performed without any assumption of the binding site (Hetényi & Van Der Spoel, 2006). All four parameters were observed and analyzed. The implementation of the semi-empirical free energy force field by AutoDock 4.2 led to the ability to evaluate the conformations. The free binding energy of the complexes for blind docking was recorded and the average of the energy was obtained. Morris et. Al, 2012 explained that the force field was parameterized using the known structure and inhibition constant, K_i of many protein-inhibitor complexes. Therefore, the binding energy was observed to determine the affinity of the ligand-protein complex. Table 1.0 presents the free energy of all the ligands' interaction with PL. Free binding energy, ΔG of all complexes showed negative reading, indicating stronger binding to PL.

Based on Table 1.0, the result of docking for four complexes showed all compounds had average negative free binding energy. All three ligands showed lower readings of free binding energy compared to the control of the study, Orlistat. This indicates the compounds have a better binding affinity compared to orlistat. Epicatechin recorded the lowest average binding energy (-6.25 Kcal/mol) while orlistat showed the highest (-2.43 Kcal/mol). The standard deviation (SD) for all compounds is <0.05 which is statistically significant.

Table 1: The analysis of free binding energy of each complex in blind docking

Complex	1st trial	2nd trial	3rd trial	Average (Kcal/mol)	Standard Deviation
Quercetin-1LPB	-5.99	-5.94	-5.95	-5.96	0.02
Epicatechin -1LPB	-2.97	-2.99	-2.97	-2.98	0.01
Corilagin -1LPB	-6.25	-6.22	-6.28	-6.25	0.03
Orlistat-1LPB	-2.41	-2.45	-2.43	-2.43	0.02

Blind docking: Inhibition constant, *Ki*

Another parameter measured is the inhibition constant (K_i). K_i indicates the potency of the inhibitor. K_i also reflects the binding affinity and inhibitory effect on the target (Aamir et al., 2018). Therefore, the smaller the K_i, the greater the binding affinity. This parameter is significant as the indicator for the drug dose to be used.

Based on Table 2.0, the value of K_i for epicatechin-1lpb showed the lowest average value (26.35 µM). While quercetin-1lpb and corilagin-1lpb recorded the K_i average values of 42.58 µM and 6590.00 µM respectively. Selected compounds showed lower average values of K_i compared to Orlistat-1lpb that averaged at 16 450.00 µM. This result shows epicatechin-1lpb has the best potential as the inhibitor of PL. The SD of the K_i for quercetin and epicatechin are <0.05 while corilagin and orlistat recorded >0.05 which is statistically insignificant.

Blind docking: Analysis of protein-ligand complexes

The formation of hydrogen bond (H-bond) and hydrophobic interactions are crucial in stabilizing the protein structures. Varma et. Al ,2010 stated that these weak intermolecular interactions give a significant effect on the binding affinity between the ligand-protein complex which enhances the drug efficacy. H-bond interaction is known to be significant in facilitating protein-ligand binding as it assists in protein folding, protein-ligand interaction and catalysis. It promotes ligand binding affinity by displacing protein-bound water into the bulk's solvent by breaking bonds with water and generate a new bond with selected ligands (Zheng & Polli, 2010). The H-bond and hydrophobic interaction were analyzed using LigPlot+ and presented in 2D structures.

Table 2: The K_i values for each complex for blind docking.

Complex	1st trial	2nd trial	3rd trial	Average (Kcal/mol)	Standard Deviation
Quercetin- 1LPB	42.58µM	42.57µM	42.58µM	42.58µM	0.006
Epicatechin -1LPB	26.34µM	26.35µM	26.35µM	26.35µM	0.577
Corilagin -1LPB	6590µM	6590µM	6591µM	6590µM	0.006
Orlistat- 1LPB	16448µM	16751µM	16450µM	16450µM	1.53

Table 3: Free binding energy analysis of each complex for focus docking.

Complex	1st trial	2nd trial	3rd trial	Average (Kcal/mol)	Standard Deviation
Quercetin- 1lpb	-7.34	-7.34	-7.33	-7.34	0.005
Epicatechin -1lpb	-8.69	-8.67	-8.63	-8.66	0.03
Corilagin -1lpb	-8.17	-8.15	-8.14	-8.15	0.01
Orlistat- 1lpb	-5.17	-5.17	-5.16	-5.17	0.005

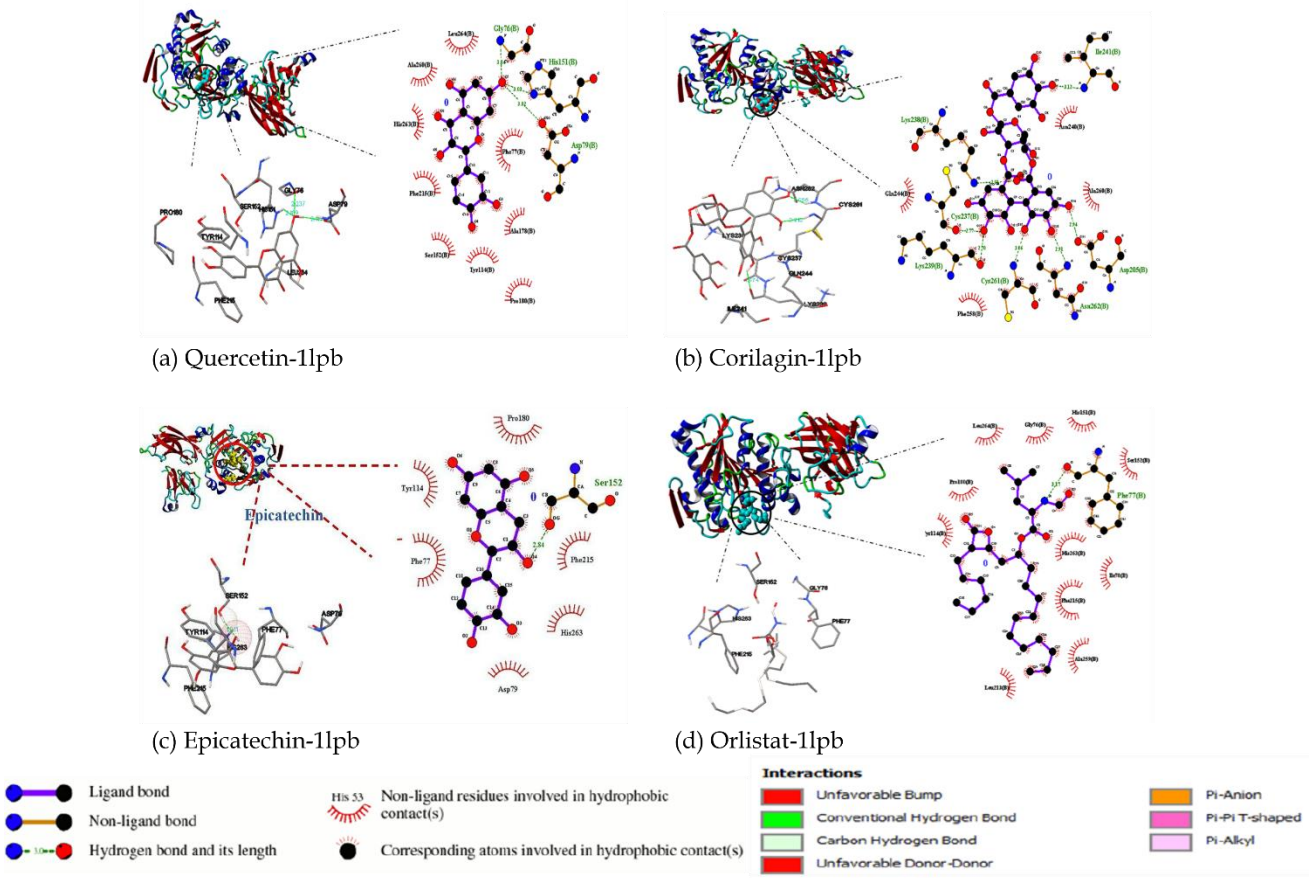


Figure 1: The 2D analysis of all complexes for blind docking using LigPlot+

Figure 1 represents blind docking, quercetin (a) contacted 12 amino acids of PL which were Ser152, His263, Phe215, His151, Gly76, Asp79, Phe77, Ala78, Tyr114, Pro180, Ala260 and Leu264. All the listed amino acids contacted quercetin using hydrophobic interactions including two of the catalytic triad which was Ser152 and His263. While, three H-bond formed at His151, Gly76 and Asp79. The oxygen atom of quercetin formed H-bond with the nitrogen atom at Gly76 and His151 with a distance of 3.04 and 3.03 Å respectively and formed another H-bond with the oxygen atom at Asp79 a distance of 3.02 Å.

For the intermolecular interactions of the corilagin-1lpb complex (b), this ligand contacted 11 amino acids of residue which were Ile241, Asn240, Ala260, Asp205, Asn262, Cys261, Phe258, Cys237,

Lys239, Gln244 and Lys238. The complex exhibited 7 hydrogen bonds formed at Ile241, Lys238, Asp205, Asn262, Cys261, Lys239 and Cys237. However, no catalytic triad of PL was observed bound to corilagin which derives that corilagin might bind far from the active site of PL.

In Figure 1(c), Epicatechin-1lpb showed that it contacted 7 amino acids which are Pro180, Ser152, Tyr114, Phe77, Phe215, His263, Asp79 with H-bond formed at Ser152. epicatechin-1lpb interacts with Ser152, which is one of the pockets of the catalytic triad, assumed to be responsible for lipolysis activity (Zhao & Huang, 2011). While it formed hydrophobic interactions with other proteins.

Figure 1(d) depicted the visualization of Orlistat-1lpb. The ligand contacted 12 amino acids of PL which are Leu264, Gly76, His151, Ser152, Phe77, His263, Ile78, Phe215, Tyr114, Pro180, Leu213 and Ala259. This positive control succeeded to form hydrophobic interaction with two active sites which are His263 and Ser152 and hydrogen bond at Phe77 with a distance of 3.17 Å.

Focus docking: Free binding energy, ΔG

Table 3.0 presents the result of average free binding energy for each complex using focus docking. All the free binding energy showed negative values. The lowest average free binding energy recorded was the epicatechin-1lpb complex. This depicted that epicatechin had the highest affinity towards 1lpb, thus, showing the best potential as a PL inhibitor. While orlistat- 1lpb complex stated the highest average free binding energy compared to other complexes. The SD recorded for all compounds is <0.05 which is statistically significant.

Focus docking: Inhibition constant, K_i

K_i values for each complex were tabulated in Table 4.0. The lowest K_i value recorded was epicatechin-1lpb (0.45 M). The other complexes were observed to obtain a lower K_i value than the orlistat-1lpb complex. The SD for all compounds is <0.05 which is acceptable except for orlistat.

Focus docking: Analysis of protein-ligand complexes

The 2D analysis from focus docking was depicted in Figure 2. This could be shown in Figure 2 (a), quercetin contacted 15 amino acids of PL which were Pro180, Alal78, Phe77, Ile78, Phe215, His263, Asp176, Leu264, Arg256, Asp79, Tyr267, Gly76, His151, and Trp85. Eight H-bonds formed at His263, Phe215, Asp176, Asp79, His151, Arg256, Gly76, Trp85 with one H-bond formed at Asp176, the active site of PL.

Next, epicatechin-1lpb in Figure (2b) contacted with 12 amino acids included Phe215, His263, Asp176, Ser125, Gly76, Pro180, Tyr267, His151, Arg256, Leu264, Asp79, Tyr114 and Ala178. Nine H-bonds formed, including all the catalytic triad, Ser152, His263 and Asp176. Ser125 is the primary residue that is vital in a lipolytic activity, indicating the potential of quercetin as a PL inhibitor. This result also denotes that this complex has high stability as it manages to form many hydrophobic interactions.

The result of corilagin-1lpb observed in Figure 2(c) indicated interactions with 12 amino acids involving Ala260, Ala259, Phe258, Phe215, Gln244, Leu213, Lys239, Glu233, Asn262, Lys238, Asn212 and Asp205. Six H-bonds formed at Lys239, Lys238, Glu233, Asn212, Asn262 and Asp205. However, this compound does not bound at the active sites of 1lpb. While, In Figure 2(d) Orlistat-1lpb showed interactions with 14 amino acids involving His263, Ser152, Pro180, Tyr11, Ala178, Ile209, Phe215, Leu153, Gly76, Phe7, Als260, Asp79, Ile78 and Leu264. Three H-bond formed with Phe77, Leu153 and Ser152. It is noted that orlistat interacted with Ser125, one of the 1lpb active sites.

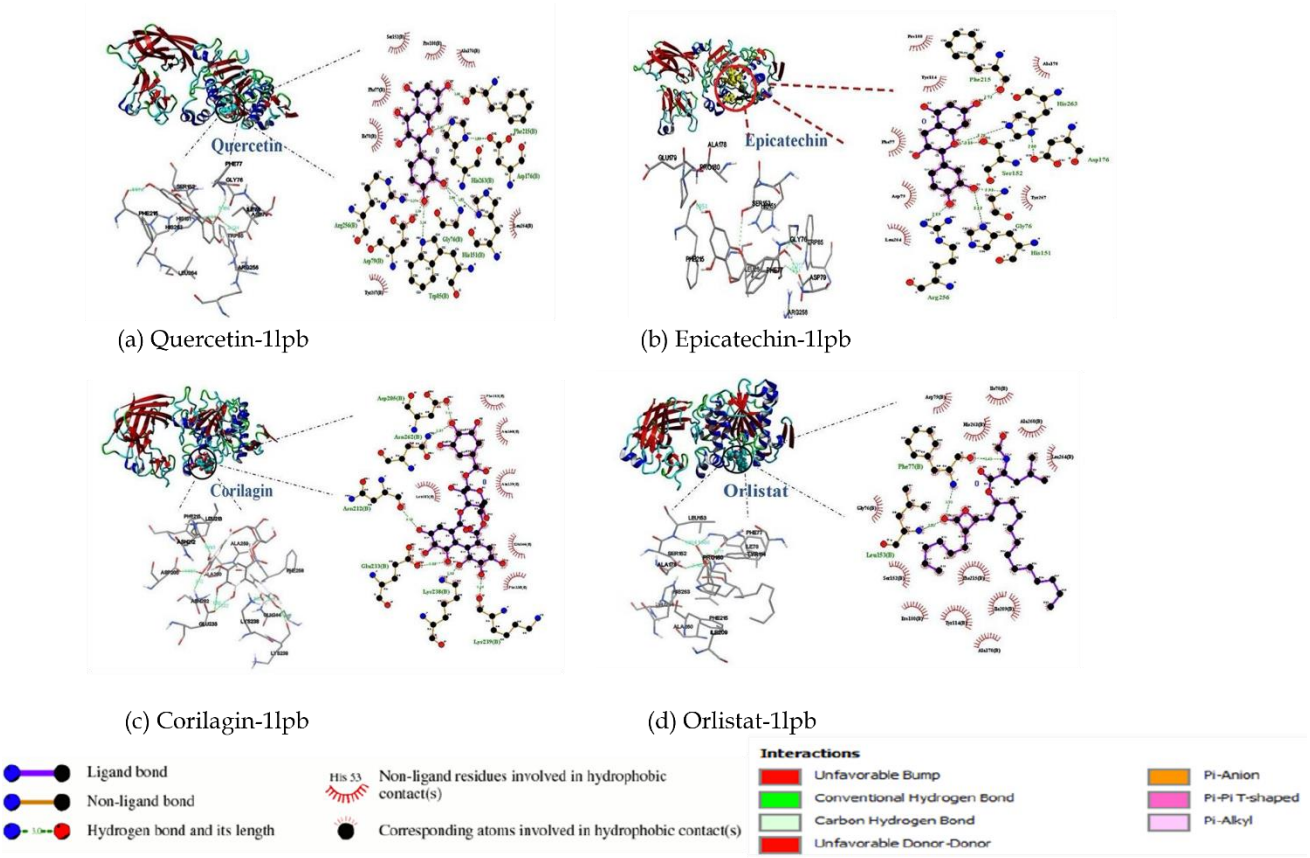
Table 4: K_i value of each complexes using focus docking.

Complex	1st Trial	2nd Trial	3rd Trial	Average K_i value (M)	Standard Deviation
Quercetin-1lpb	4.15	4.17	4.16	4.16	0.01
Epicatechin -1lpb	0.44	0.46	0.45	0.45	0.01
Corilagin - 1lpb	1.06	1.08	1.05	1.06	0.01
Orlistat-1lpb	163.01	163.65	157.11	161.26	3.6

Comparison between Blind and Focus Docking

Binding energy

The lower the free binding energy, the greater the affinity of the ligand (Zhao & Huang, 2011). Based on the result of both dockings, epicatechin marked the lowest binding energy, indicating the highest affinity. Figure 3 showed the comparison between blind and focus docking for all the compounds selected along with orlistat, the positive control. As shown, the free



(a) Quercetin-1lpb (b) Epicatechin-1lpb

(c) Corilagin-1lpb (d) Orlistat-1lpb

Figure 2: The 2D analysis of all complexes for focus docking using LigPlot+

binding energy depicted an almost similar pattern in both approaches, as the compound with the lowest free binding energy was epicatechin-1lpb then followed by quercetin, corilagin and orlistat in blind docking. While corilagin recorded lower energy than quercetin and orlistat in focus docking. This might be due to improve and smaller coverage docking sites during focus docking as corilagin shows a decrease of binding energy compared to previous blind docking.

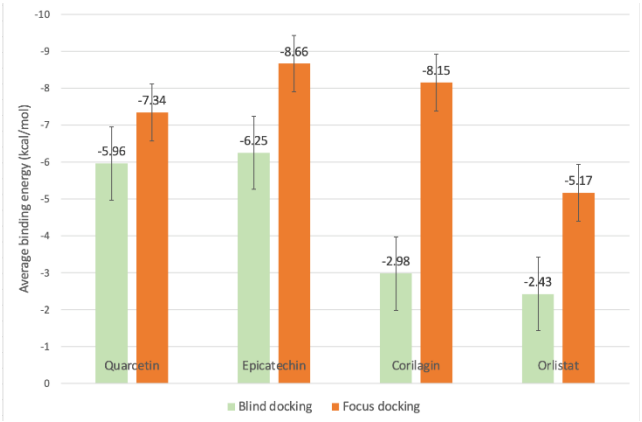


Figure 3: Free binding energy of ligands resulted from blind and focus docking.

Inhibition constant, K_i

Figure 4 shows a stark difference in K_i value between blind and focus docking. However, the trend of K_i value between the dockings was similar and the result does not show any contrast gaps between the compounds in blind docking, but huge difference of K_i value of orlistat complex compared to the other complexes was observed. The lowest K_i value recorded was quercetin for blind docking where else, epicatechin-1lpb was noted as the lowest in focus docking.

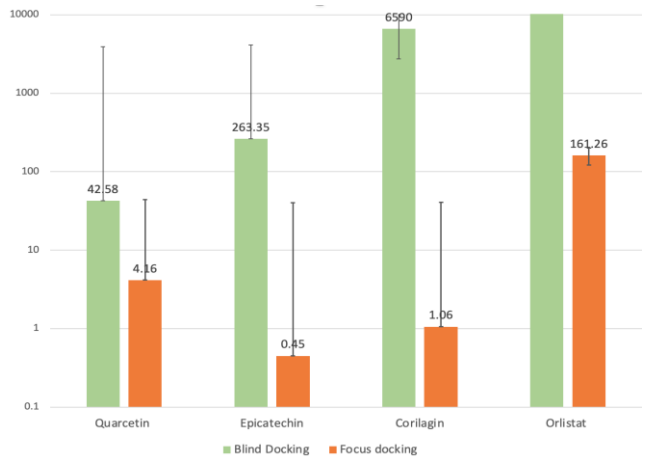


Figure 4: Average inhibition constant of ligands resulted from blind and focus docking.

Table 5: The important residues bind with the ligand by hydrogen bonds. Highlighted yellow residue are the catalytic triad.

Complex	Blind docking	Focus docking
Quercetin-1LPB	Asp79, His151, Gly76	His263, Phe215, Asp176, Asp79, His151, Arg256, Gly76, Trp85
Corilagin-1LPB	Asp205, Ile241, Cys 261	Lys239, Glu233, Asn262, Lys238, Asn21, Asp205
Epicatechin-1LPB	Ser125	Phe215, His263, Asp176, Ser152, Gly76, His151, Arg256
Orlistat-1LPB	Phe77	Leu153, Phe77, Ser152

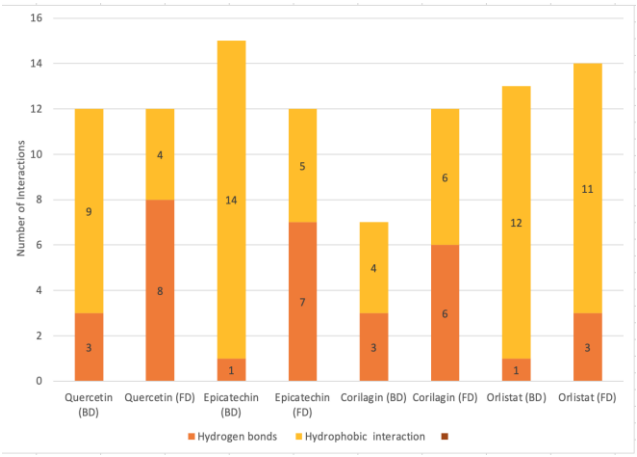


Figure 5: The analysis of weak interaction of hydrogen bonds and hydrophobic interaction for each complex in focus and blind docking.

Number of Hydrogen Bond and Hydrophobic Interaction

Hydrogen bonding plays a pivotal role in the determination of protein structure and binding specificity (Hamid et al., 2013). Figure 5 summarizes the hydrogen bond formation between the ligands and PL. Epicatechin formed abundant of hydrogen bonds and relatively strong hydrophobic interaction in focus docking. It also formed H-bond at Ser152 while quercetin recorded the highest number of hydrogen bonds compared to the other complexes compared to all complexes. It is also noted that all the bonds are conserved in triplicates and produced a consistent result each time.

The result in Table 5 shows epicatechin and orlistat bind to Ser152 which is a significant amino acid in hydrolysis activity. The inhibitors would bind to the triad pocket binding at Ser125, resulting to a chemical shift of the atoms (Miyake, 2001) which then stop the lipolysis activity. Epicatechin had the best potential as PL inhibitor as it has the lowest binding energy, the low inhibitor constant and stable intermolecular interaction. Though corilagin also showed low readings in both free energy binding and inhibition constant, however no binding occurred at the active site of PL. These findings were found similar to a study by Sudeep & Shyam Prasad, 2014 in which corilagin showed formation of hydrogen bonds at other amino acid but not Ser125. (Sudeep & Shyam Prasad, 2014). This might be due to the formation of a salt bridge between two amino acids which disrupt the interactions within the active-site residues (Tomar & Aggarwal, 2017). Therefore, it can be derived that epicatechin possesses the best potential as PL inhibitor.

Conclusion:

In conclusion, the ligand-protein interaction between PL and the selected phytochemicals, quercetin, epicatechin and corilagin were successfully performed via molecular docking. From this study, epicatechin displayed the best potential as a PL inhibitor. Epicatechin shows the best binding affinity towards PL and successfully bound to all three recognized active sites, Ser152, His263, and Asp176. This can be supported by the low free binding energy and inhibition constant (K_i). Epicatechin also have high number of hydrogen bonds and hydrophobic reactions. All results obtained proposed that epicatechin have the best potential as a PL inhibitor replacing Orlistat in the future. Further studies are prompt to further explore the potential of epicatechin since epicatechin is a regular flavonoid that is widely available.

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