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Research Article

Potential activity of *Stevia rebaudiana* Bert. in Inhibiting Cyclooxygenase and Lipooxygenase Enzymes as Anti-inflammatory Candidates: A Molecular Docking Study and ADMET Prediction

Meida Adelia[1], Arif Setiawansyah[1]*, Rina S.E Sitindaon[2]

[1] *Faculty of Pharmacy, Kader Bangsa University, Palembang, South Sumatera, 30253, Indonesia*

[2] *Faculty of Health, Kader Bangsa University, Palembang, South Sumatera, 30253, Indonesia*

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*Address for Correspondence:

Arif Setiawansyah, Faculty of Pharmacy, Universitas Kader Bangsa, Palembang, South Sumatera, 30253, Indonesia

Abstract

Stevia rebaudiana Bert. is one of the plants that has been traditionally used to treat inflammation. Numerous articles have reported scientific evidence of the anti-inflammatory activity of *Stevia rebaudiana* Bert. However, research regarding the bioactive contributing to its anti-inflammatory activity has never been discovered. This study was implemented to identify the molecules regulating the anti-inflammatory activity of *Stevia rebaudiana* Bert. and assess their pharmacokinetics and toxicological profile. The potent phytochemical derived from *Stevia rebaudiana* Bert. was screened via a molecular docking approach using autodock 4.2 assisted by ADT interface to COX-1, COX-2, and 5-LOX. The ADMET assessment was undertaken via biosig pkCSM web server. Molecular docking results revealed that β-sitosterol, campesterol, and stigmasterol exhibited high affinity to COX-1, COX-2, and 5-LOX with free energy of binding value of -11.12, -11.43, and -10.62 kcal/mol for COX-1, -11.45, -11.34, and -11.84 kcal/mol for COX-2, and -5.95, -11.34, and -9.08 kcal/mol for 5-LOX, respectively. The ADMET prediction also indicated those bioactive have an excellent pharmacokinetics and toxicity profile. Therefore, β-sitosterol, campesterol, and stigmasterol can be considered to be developed as anti-inflammation agents. However, further research regarding their anti-inflammatory activities including in vitro and in vivo assays is necessary to be implemented.

Keywords: anti-inflammation, molecular docking, *Stevia rebaudiana* Bert.

1. INTRODUCTION

Inflammation is a local protective response caused by damaging chemicals, microbiological substances, and physical trauma caused by tissue damage [1]. In cases of severe inflammation such as joint damage, arachidonic acid is overproduced and will be converted through cyclooxygenase (COX) and lipooxygenase (LOX) pathways into potent inflammatory substances such as prostaglandins, leukotrienes, and thromboxane A2. COX and 5-LOX are enzymes involved in the formation of inflammatory mediators [2].

In general, treatment of inflammation can use synthetic drugs, namely anti-inflammatory drugs, steroids which are corticosteroid class, and non-steroidal anti-inflammatory drugs (NSAIDs). AINS class drugs are more often used because corticosteroid groups in the long term cause side effects such as moon face, stomach irritation, suppressing immunity, and bone loss [3]. However, AINS drugs that inhibit cyclooxygenase and lipooxygenase enzymes are also not free from the risk of side effects because some inhibitors of these enzymes have been reported to be associated with cardiovascular problems [4]. Besides being able to cause cardiovascular problems, side effects often occur such as duodenal ulcers and impaired platelet function due to inhibition of thromboxane A2 biosynthesis [5].

One plant that has the potential to be developed as an anti-inflammatory agent is the sweet plant (*Stevia rebaudiana* Bert.) which also contains active compounds but there has been no research related to its use as an anti-inflammatory. The leaves are widely used as a traditional medicine for diabetes [6]. In addition to diabetes, the plant *Stevia rebaudiana* Bert. It has pharmacological effects for several diseases such as cancer, hypertension, anti-inflammatory, cystic fibrosis, obesity, and tooth decay. This is because of the plant *Stevia rebaudiana* Bert. has glycoside compounds such as steviol, stevioside, rebaudioside A, and rebaudioside C [7].

One way that can be done to find out compounds that have the potential as anti-inflammatories can be done through molecular docking which can provide the results of compounds that have the potential as drug candidates. Molecular docking is carried out to avoid the synthesis of a compound that requires time and expensive costs, but the new compound does not necessarily have the expected activity [8].

Therefore, this study was conducted to evaluate the potential of *Stevia rebaudiana* Bert. plants as anti-inflammatory candidates by understanding their molecular interactions

against cyclooxygenase and lipooxygenase with the help of molecular docking.

2. MATERIALS AND METHODS

2.1. Research Tools and Materials

The tools used in this study consisted of hardware and software. The hardware used is a Laptop Hp DESKTOP-UFDJ08O with specifications AMD E2-9000e RADEON R2, RAM 4 GB, and *hard disk* 500 GB. The software used is in the form of Windows 10 Pro operating system, ChemDraw Ultra 12.0, Chem3D Pro 12.0, BIOVIA Discovery Studio Visualizer, Autodock4, Notepad+++, and Microsoft Excel 2010. Lipinski's Rule of Five Screening uses the SCFBio website. ADMET predictions using the pkCSM website.

The materials used in the study were COX-1 enzymes downloaded from https://www.rcsb.org/ with PDBid: 1EQG, COX-2 enzymes with PDBid: 6COX, 5-LOX enzymes with PDBid: 6NCF. The structure of the ligand is derived from the plant compound *Stevia rebaudiana* Bert. including austroinulin, dulcosides, campesterol, sterebin A-H, stigmasterol, b-sitosterol, steviosida, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, steviolbioside A, and dulcoside A, as well as a comparison compound, licofelone downloaded from http://PubChem.nbi.nlh.nih.gov.

2.2. Prediction of Physiocochemical Properties

Prediction of physicochemical properties carried out in this study using the SCFBio website with the method Lipinski's Rule of Five. The parameters consist of Molecular Weight, Log P, Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and Molar Refractivity. The optimized 2D structure is inserted into the columns that are already available through the SCFBio website. The results of the prediction of physicochemical properties are presented in the following table 1.

2.3. Prediction ADMET

Prediction of absorption, distribution, metabolism, elimination, and toxicology (ADMET) was performed to analyze the pharmacokinetic properties of the plant *Stevia rebaudiana* Bert. ADMET prediction using the pkCSM web (http/biosig.unimelb.edu.au/pkcsm/prediction) using the SMILES code of each test compound. The parameters analyzed were Human Intestinal Absorption (HIA) and Caco-2 cells for absorption, Plasma Protein Binding (PPB) and Blood Brain Barrier (BBB) for distribution, CYP inhibitors for metabolism, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 as aspects of metabolism and excretion, and Ames Toxicity for mutagenicity and carcinogenicity and hepatoxicity for liver damage reactions.

2.4. Ligan Preparation

Stevia rebaudiana Bert. plant test compounds in this study there were 14 compounds. The comparison compound used is licofelone. These compounds are depicted in 2D form and then converted into 3D molecular models using the Chem3D Pro 12.0 application. Each compound structure is energy minimized and then saved in .pdb file format.

2.5. Protein Preparation

Cyclooxygenase-1 enzyme with PDBid code: 1EQG, COX-2 enzyme with PDBid: 6COX, 5-LOX enzyme with PDBid: 6NCF downloaded via https://www.rcsb.org/ website with .pdb format. Protein structures are separated from solvents, native ligands or residues using the BIOVIA Discovery Studio Visualizer application by clicking the file then open, searching for the receptor file folder that will be separated so that the protein structure display will appear in a three-dimensional model. Then click water/ligands/protein chains then click remove and save the receptor file in .pdb format.

Native ligands from the protein structure are separated for receptor validation using BIOVIA Discovery Studio Visualizer by clicking the file then open, searching for the folder where the receptor file will be separated. Solvents and proteins (amino acids) are removed by clicking water/protein chains then clicking remove/press delete on the keyboard. Native ligand files are saved in .pdb format. Proteins that have been removed from unneeded residues and native ligands are prepared are then stored in .pdbqt format [9].

2.6. Methode Validation

The prepared 1EQG, 6COX, 6NCF and native ligand enzymes are opened on the AutoDock tools worksheet to parameterize the gridbox. The gridbox is set up with steps click grid, then click grid box, click center, click center on ligand. Save the grid option by clicking File then clicking Close Saving Current. The grid box parameter file is saved by clicking the grid, clicking Output, and then clicking save GPF. Click analyze and run the autogrid4 command according to the saved file.

The docking parameters are set using the Genetic Algorithm, while the number of GA runs is made 100 times the ligand-receptor interaction, the settings for other parameters are set by default. Saved according to the Lamarckin GA settings and parameter files are saved in a working folder in .dpf format. The docking simulation is run, click analyze and run the autodock4 command, fill the folder with files that have been saved in .dpf format. The results of the docking simulation will automatically be saved to a folder with .dlg format [9]. Docking in test compounds there are differences in the grid parameters section adjusted to the validation of the method.

3. RESULTS AND DISCUSSION

3.1. Method Validation or Redocking

Method validation in this study was carried out by redocking the native ligand on the three enzymes, namely 1EQG, 6COX, and 6NCF downloaded from the Protein Data Bank website. The validation results to be analyzed are by looking at the RMSD value of each enzyme. RMSD values < 2.0 Å are used as success criteria for molecular docking methods. The docking method is said to be valid if the RMSD value is less than 2 Å so that it can be used for test compounds [10]. Based on table 3, the RMSD value of the three enzymes < 2.0 Å. This indicates that the docking method is valid so that it can be continued for use against the test compound.

Table 1: RMSD Value of Validation Results

PDB Code	RMSD Value (Å)
1EQG	0,953
6COX	0,778
6NCF	1,736

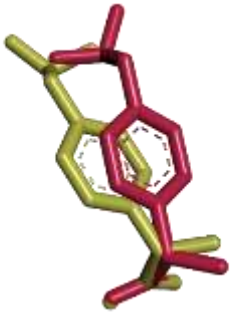


Figure 1: Overlapping visualization 1EQG native ligand (pink) and re-docking ligand (yellow) with 0.953 Å RMSD value

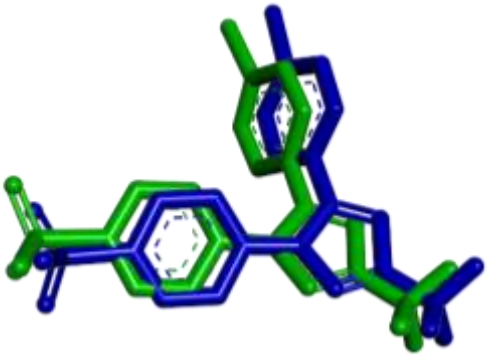


Figure 2: Overlapping visualization 6COX native ligand (blue) and re-docking ligand (green) with 0,778 Å RMSD value

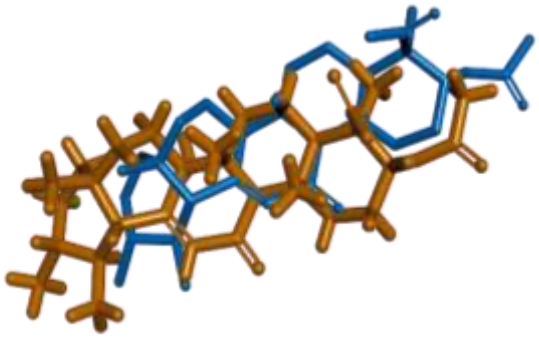


Figure 3: Overlapping visualization 6NCF native ligand (orange) and re-docking ligand (light blue) with 1,736 Å RMSD value

3.2. Lipinski's Rule of Five Predictions

Lipinski's Rule of Five predictions to analyze the physicochemical properties of a compound consisting of 5 parameters, namely the molecular weight of a compound ≤ 500 Da, Hydrogen Bond Donors (HBD) expressed by the number of O-H and N-H groups ≤ 5, Hydrogen Bond Acceptors (HBA) expressed by the number of O and N atoms ≤ 10, the logarithmic value of the octanol partition coefficient (Log P) ≤ 5, and molar refractivity 40-130 [11]. The test compound must meet Lipinski's rule and a maximum of 1 parameter does not meet. Test compounds that meet Lipinski's rule there are only 2 compounds, namely austroinulin and sterebin A that can be administered orally.

Table 2: Prediction of Physicochemical Properties

No.	Test Compounds	Molecular Weight (BM)	Log P	HBA	HBD	*Molar Refractivity*
1.	Austroinulin	322	3,44	3	3	94
2.	b-sitostesol	414	8,02	1	1	128,22
3.	campesterol	400	7,63	1	1	123,60
4.	dulkosida-A	788	-1,91	17	10	184,99
5.	Rebaudiosida-A	966	-5,11	23	14	219,13
6.	Rebaudiosida-B	804	-2,37	18	11	186,91
7.	Rebaudiosida-C	950	-4,09	22	13	217,62
8.	Rebaudiosida-D	1128	-7,29	28	17	251,66
9.	Rebaudiosida-E	966	-5,11	23	14	219,01
10.	Rebaudiosida-F	936	-4,47	22	13	213,02
11.	Sterebin A	310	2.07	4	3	85,18
12.	Steviolbiosida	642	-0,20	13	8	154,17
13.	Steviosida	804	-2,94	18	11	186,40
14.	Stigmasterol	412	7,80	1	1	128,12

Note: (HBA) Hydrogen Bond Acceptors, **(**HBD) Hydrogen Bond Donors

3.3. ADMET Prediction

ADMET's predictions are based on drug design failure rates and costs in the manufacturing process. ADMET prediction to predict the pharmacokinetic and toxicity properties of a compound from its chemical structure. ADMET predictions via the pkCSM Online Tool website. The parameters analyzed were Human Intestinal Absorption (HIA) and Caco-2 cells for absorption, Plasma Protein Binding (PPB) and Blood Brain Barrier (BBB) for distribution, CYP inhibitors for metabolism, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 as aspects of metabolism and excretion, as well as Ames Toxicity for mutagenicity and carcinogenicity and hepatoxicity for liver damage reactions.

Table 3: Results of Prediction Absorption, Distribution and Toxicity of Plant Compounds *Stevia rebaudiana* Bert.

Test Compounds	Absorption		Distribution		Toxicity	
	HIA (%)	Caco-2 (10^{-6} cm/s)	PPB (%)	BBB	Hepatoxicity	AMES Test
Austroinulin	94,226	1,44	84,439	0,348	No	No
b-sitosterol	94,464	1,201	31,397	0,781	No	No
campesterol	94,543	1,223	98,676	0,774	No	No
dulkosida-A	7,91	-0,756	85,625	-1543	No	No
Rebaudiosida-A	0	-1,391	98,671	-2,616	No	No
Rebaudiosida-B	0	-0,857	98,314	-2,137	No	No
Rebaudiosida-C	0	-1,136	41,586	-2,192	No	No
Rebaudiosida-D	0	-1,734	28,646	-3,246	No	No
Rebaudiosida-E	0	-1,43	23,991	-2,659	No	No
Rebaudiosida-F	0	-1,376	29,215	-2,448	No	No
Sterebin A	96,024	0,867	26,271	-0,708	No	No
Steviolbiosida	1,74	-0,688	19,552	-1,55	No	No
Steviosida	0	-1,087	23,946	-2,029	No	No
Stigmasterol	94,97	1,213	24,858	0,771	No	No

Table 4: Metabolic Prediction Profile of Plant Compounds *Stevia rebaudiana* Bert.

Test Compounds	CYP Inhibitors				
	1A2	2C19	2C9	2D6	3A4
Austroinulin	No	No	No	No	Yes
b-sitosterol	No	No	No	No	Yes
Campesterol	No	No	No	No	Yes
Dulkosida-A	No	No	No	No	No
Rebaudiosida-A	No	No	No	No	No
Rebaudiosida-B	No	No	No	No	No
Rebaudiosida-C	No	No	No	No	No
Rebaudiosida-D	No	No	No	No	No
Rebaudiosida-E	No	No	No	No	No
Rebaudiosida-F	No	No	No	No	No
Sterebin A	No	No	No	No	No
Steviolbiosida	No	No	No	No	No
Steviosida	No	No	No	No	No
Stigmasterol	No	No	No	No	Yes

The predicted results of absorption, distribution, metabolism, and toxicity can be seen in Table 3 and Table 4. A compound can be absorbed well if the HIA value is 70-100%, sufficient in the range of 20-70%, and bad in the range of 0-20%. Based on the results of ADMET prediction, there are 5 compounds that have good HIA values. Modeling of Caco-2 cells to predict absorption of active substances via oral route in vitro. High permeability of Caco-2 cells has value > 0,9 $\times 10^{-6}$ cm/s. Austroinulin, β-sitosterol, campesterol, sterebine A, and stigmasterol compounds show high permeability results meaning that these compounds can penetrate cell membranes. The value of Plasma Protein Binding (PPB) can affect the pharmacokinetic and pharmacodynamic properties of the drug. PPB values > 90% indicate that the drug is strongly bound to plasma proteins while PPB< 90% of drugs are weakly bound to plasma proteins. Based on Table 3, campesterol, rebaudioside A, and rebaudioside B compounds have PPB values > 90%.

The next parameter is the Blood Brain Barrier (BBB). This parameter is to determine the ability of the drug to penetrate the blood-brain barrier. BBB values > 0.3 are considered easy to cross the blood-brain barrier and < -1 values are not well distributed to the brain. Test compounds that have a value of > 0.3 are austroinulin, β-sitosterol, campesterol, and stigmasterol.

The toxicity profile is seen through the Hepatoxicity parameter and AMES assay. Hepatoxicity for damage reactions in the liver while Ames Toxicity for mutagenicity and carcinogenicity. Based on the results of ADMET predictions, all plant test compounds *Stevia rebaudiana* Bert. Not potentially mutagen and damage to the liver.

The metabolic profile can be determined by testing the inhibitory power of these compounds against cytochrome enzymes. The five main isoforms are CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Inhibition of these five isoenzymes can cause drug interactions related to pharmacokinetics resulting in toxic side effects or unwanted drug reactions due to lower clearance and drug accumulation [12]. ADMET prediction results show austroinulin, β-sitosterol, campesterol, and stigmasterol compounds inhibit 1 main isoform of cytochrome P450 enzyme. Docking of Test Compounds against COX-1, COX-2, and 5-LOX Enzymes.

3.4. Docking of Test Compounds Against COX-1, COX-2, and 5-LOX Enzymes

PDB codes to represent the COX-1, COX-2, and 5-LOX enzymes used in this study are 1EQG, 6COX, and 6NCF. The docking process begins with the preparation of test ligands from Stevia rebaudiana plant compounds and comparison ligands, namely licofelone using Chem3D Pro 12.0. The prepared ligands are then docked on validated proteins. The docking results of test ligands, comparison ligands, and native ligands can be seen in the following table.

Table 5: Results of Docking Ligands with COX-1, COX-2, and 5-LOX Enzymes

No.	Test Compounds	Docking Score		
		COX-1	COX-2	5-LOX
1.	Austroinulin	-8,18	-8,23	-6,37
2.	β-sitosterol	-11,12	-11,45	-5,95
3.	campesterol	-11,43	-11,34	-11,34
4.	dulkosida-A	+34,15	+33,02	-7,30
5.	Rebaudiosida-A	+83,31	+149,84	-4,41
6.	Rebaudiosida-B	+33,48	+51,37	-5,95
7.	Rebaudiosida-C	+104,44	+122,92	-4,42
8.	Rebaudiosida-D	+434,29	+514,90	-0,88
9.	Rebaudiosida-E	+95,92	+182,44	-4,17
10.	Rebaudiosida-F	+90,11	+83,47	-5,71
11.	Sterebin A	-8,40	-7,89	-6,35
12.	Steviolbiosida	+5,82	-0,72	-6,99
13.	Steviosida	+31,63	+23,05	-6,61
14.	Stigmasterol	-10,62	-11,84	-9,08
15.	Licofelone	-7,88	-10,04	-6,92
16.	Native Ligand	-8,55	-10,72	-10,65

Table 6: Value of Inhibition Constant with COX-1, COX-2, and 5-LOX Enzymes

No.	Test Compounds	Inhibition Constant (IC)		
		COX-1	COX-2	5-LOX
1.	Austroinulin	1,00 uM	926,00 nM	21,59 nM
2.	β-sitostesol	7,11 nM	4,01 nM	43,74 uM
3.	campesterol	4,21 nM	4,85 nM	4,85 nM
4.	dulkosida-A	-	-	4,46 uM
5.	Rebaudiosida-A	-	-	585,77 uM
6.	Rebaudiosida-B	-	-	43,74 uM
7.	Rebaudiosida-C	-	-	573,90 uM
8.	Rebaudiosida-D	-	-	225,96 uM
9.	Rebaudiosida-E	-	-	873,15 Um
10.	Rebaudiosida-F	-	-	64,75 uM
11.	Sterebin A	692,64 nM	1,65 uM	22,12 uM
12.	Steviolbiosida	-	294,53 mM	7,49 uM
13.	Steviosida	-	-	14,18 uM
14.	Stigmasterol	16,43 nM	2,10 nM	222,05 nM
15.	Licofelone	1,67 uM	43,48 nM	8,46 uM
16.	Native Ligand	541,13 nM	13,87 nM	15,74 nM

From the docking results that predict the interaction of *Stevia rebaudiana* Bert. plant compounds with COX-1 enzymes from proteins with the PDB code 1EQG, it can be seen that the docking score β-sitosterol compounds, campesterol, and stigmasterol is lower than that of its native ligand. This suggests that the ligand's ability to interact with proteins is more likely due to the large number of hydrogen bonds formed. Unlike the case with comparison ligands, the value is smaller than that of native ligands. When viewed from the value of the inhibition constant, the activity of test ligands, namely β-sitosterol, campesterol, and stigmasterol is better than the original ligand. The bond between the test ligand and the protein is more stable when bonded. This shows that the protein bond complex with the active plant compound Stevia rebaudiana is more stable than the complex between proteins and native ligands. The visualization results show that the test compounds (dulcoside A, rebaudioside C, rebaudioside D, rebaudioside F, and steviolbiocide) can interact with amino acid residues Arg120 and Tyr355 from the COX-1 enzyme with different bond distances which are likely interactions in the form of hydrogen bonds or van der Waals forces. The bond distance between amino acid residues of the test compound is closer when compared to the native ligand. This shows that the bond strength between the protein complex and the test compound is stronger and more stable than the protein-native ligand complex or with licofelone. By looking at the results of docking and visual interaction, some test compounds have the potential to be further developed as anti-inflammatory agents.

From the docking results that predict the interaction of *Stevia rebaudiana* Bert. plant compounds with COX-2 enzymes from proteins with PDB code 6COX, it can be seen that the docking score β-sitosterol, campesterol, and stigmasterol compounds and the comparison compound, licofelone, is lower than that of its native ligand. This suggests that the ability of test ligands and comparison ligands to interact with proteins is more likely due to the large number of hydrogen bonds formed. Unlike the case with comparison ligands, the value is smaller than that of native ligands. When viewed from the value of the inhibition constant, the activity of test ligands, namely β-sitosterol, campesterol, and stigmasterol is better than the native ligand and comparison compound. The bond between the test ligand and the protein is more stable when bonded. This shows that the protein bond complex with the active plant compound Stevia rebaudiana is more stable than the protein complex with native ligands. The visualization results show that test compounds (rebaudioside C, rebaudioside D, steviolbiocides) and comparison compounds (licofelone) can interact with amino acid residues Arg120, Phe517, Gln192, and Sr353 from the COX-2 enzyme with different bond distances which are likely interactions in the form of hydrogen bonds or van der Waals forces. The binding distance between the amino acid residues of the test compound and the comparison compound is closer when compared to the native ligand. This shows that the bond strength between the protein complex and the test compound is stronger and more stable than the protein-native ligand complex or with licofelone. By looking at the results of docking and visual interaction, some test compounds have the potential to be further developed as anti-inflammatory agents.

From the docking results that predict the interaction of the plant compound *Stevia rebaudiana* Bert. with the 5-LOX enzyme from a protein with the PDB code 6NCF, it can be seen that the docking score of only campesterol compounds is lower than that of its native ligand. This suggests that the ligand's ability to interact with proteins is more likely due to the large number of hydrogen bonds formed. Unlike the case with comparison ligands, the value is higher than that of native ligands. When viewed from the value of the inhibition constant, the activity of the test ligand, campesterol, is better than the original ligand and comparison compound. The bond between the test ligand and the protein is more stable when bonded. This shows that the bond strength between the protein complex and the test compound is stronger and more stable than the protein-native ligand complex or with licofelone. The visualization results showed that test compounds (dulcosides, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, steviolbiocides, and steviosides) and comparison compounds (licofelone) can interact with amino acid residues Arg101, Thr137, Arg138, Val109, His130 from the 5-LOX enzyme with different bond distances which are likely to be hydrogen bonds or van der Waals forces. The binding distance between amino acid residues of the test compound (rebaudioside A, rebaudioside C, rebaudioside E, and stevioside) is closer when compared to the native ligand and comparison compound, licofelone. This shows that only a few test compounds have stronger and more stable bond strength between protein complexes than either protein-native ligand complexes or licofelone. By looking at the results of docking and visual interactions, some test compounds have the potential to be further developed as anti-inflammatory agents.

Table 7: Interaction of Amino Acid Residues from Docking Ligands with COX-1 Enzyme

		Hydrogen Bonding			
No.	**Compounds**	**Amino Acid Residues**	**Distance**	**Hydrophobic Interactions**	**Electrostatic Interaction**
1.	Austroinulin	Ser350	2,98	Val349, Leu352, Ile523, Ala527, Tyr355, Trp387, Phe518, Tyr385	-
2.	β-sitosterol	Tyr385	2,17	Val116, Val349, Leu359, Ala527, Leu531, Tyr355	-
3.	Campesterol	-	-	Val119, Val349, Leu352, Leu359, Ile523, Ala527, Leu531, Tyr355, Tyr385	-
4.	Dulkosida-A	Arg120, Tyr 385, Gly526, Met522, Ser530	3,39; 2,58; 2,29; 2,42; 3,73	-	-
5.	Rebaudiosida-A	Ser530	2,38	-	-
6.	Rebaudiosida-B	Leu531, Ser353, Met522, Ile523, Gly526	2.90; 2,34; 2,16; 2,93; 2,04	Val116	-

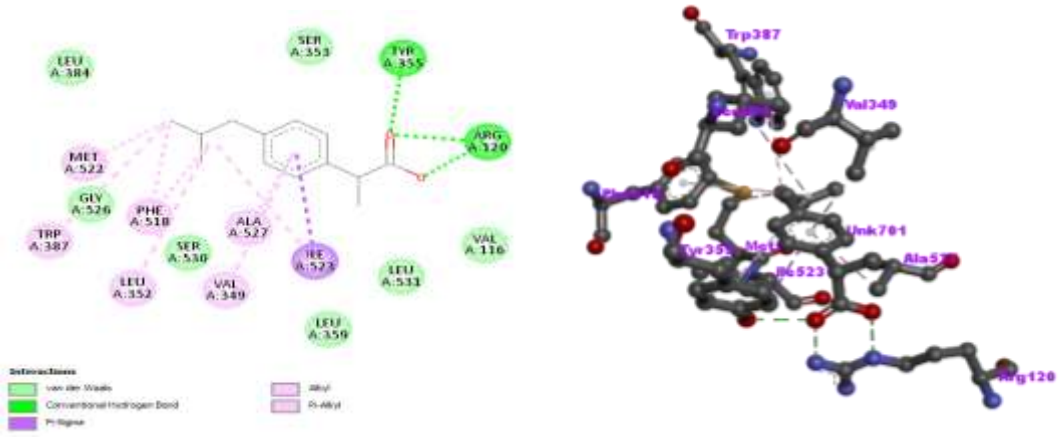
7.	Rebaudiosida-C	Tyr355, Tyr385, Met522, Val116, Ser353, Gly526	2,85; 2,47; 1,67; 2,69; 3,34; 2,42	-	-
8.	Rebaudiosida-D	Tyr355, Val349, Ser530	2,44; 1,99; 1,82	-	-
9.	Rebaudiosida-E	Val116, Ser530, Gly526, Met522	2,52; 2,51; 2,08; 1,87	-	-
10.	Rebaudiosida-F	Tyr355, Ile523, Met522, Ser530	2,02; 1,84; 2,01; 2,53	-	-
11.	Sterebin A	Met522, Ser530	1,81; 3,68	Tyr385, Val349, Leu352	-
12.	Steviolbiosida	Tyr355, Val116	2,44; 2,94	Val349, Ile523, Ala527	-
13.	Steviosida	-	-	-	-
14.	Stigmasterol	Tyr385	2,14	Val116, Val349, Leu352, Leu359 Ala527, Leu351, Ile89, Val116, Val119, Arg120	-
15.	Licofelone	Ser530	1,99	Ile523, Val116, Leu359, Tyr355, Val349, Ile523, Ala527, Val349, Leu359, Ala527, Leu352,	-
16.	Native Ligand	Arg120, Tyr355	2,90; 2,77 2,77	Ile523, Leu352, Met522, Trp387, Phe518, Val349, Ala527	-

Table 8: Interaction of Amino Acid Residues from Docking Ligands with COX-2 Enzymes

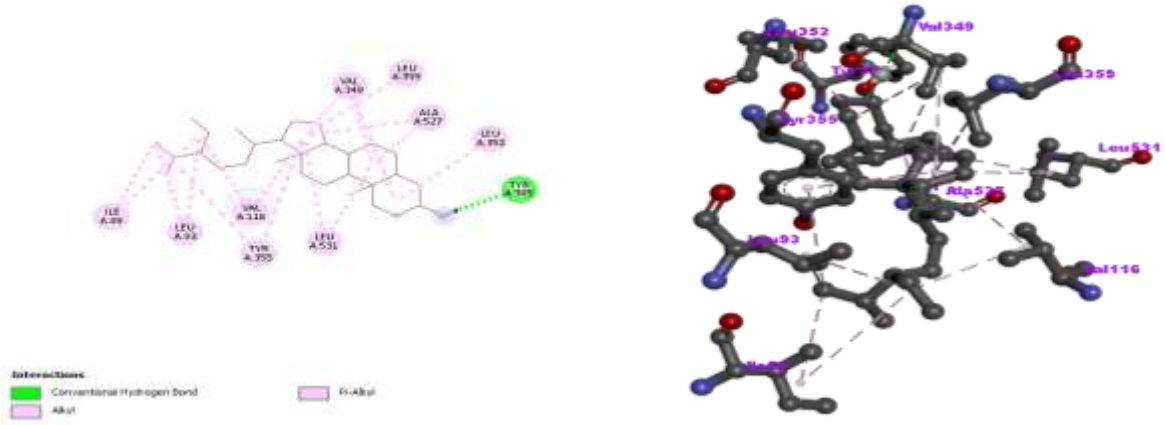
		Hydrogen Bonding			
No.	**Compounds**	**Amino Acid Residues**	**Distance**	**Hydrophobic Interactions**	**Electrostatic Interaction**
1.	Austroinulin	Leu352	1,94	Val349, Ala527, Val116, Leu359, Leu531	-
2.	β-sitosterol	Met522	2,73	Phe518, Val116, Leu352, Val523, Ala527, Tyr355	-
3.	Campesterol	Met522	2,13	Val16, Tyr355, Phe518	-
4.	Dulkosida-A	Ser119, Val349, Tyr355	2,47; 2,46; 3,57	Ala527, Tyr385	-
5.	Rebaudiosida-A	Val523, Ser530, Leu384, Phe518	2,42; 2,30; 3,66; 2,04	-	-
6.	Rebaudiosida-B	Ser530, Gly526, Val344, Ala527, Tyr385	2,39; 1,80; 2,49; 3,06; 2,98		-
7.	Rebaudiosida-C	Arg120, Gly526, His90, Ser530	2,57; 2,83; 2,03; 3,19	Phe518, Val116, Tyr355	-
8.	Rebaudiosida-D	Arg120, Val344, Ile345, Val523, Gly526, Ser530, Thr521	2,38; 2,95; 2,78; 1,59; 2,46; 3,04; 3,79	Tyr385	Tyr385, Phe518
9.	Rebaudiosida-E	Val349, Tyr385, Tyr355, Ser119, Val116, Leu352	2,19; 3,04; 2,00; 2,63; 2,23; 2,94	-	-
10.	Rebaudiosida-F	Val116, Ser119, Met522, Ala527, Ser530	3,04; 2,97; 2,57; 1,81; 2,48	-	-
11.	Sterebin A	Leu352	1,99	Val349, Ala527	-
12.	Steviolbiosida	Arg120, Leu352, Gln192, Leu352, His90, Tyr355, Met522	2,63; 2,37; 2,73; 1,99; 2,09; 2,04; 1,95	-	-
13.	Steviosida	Tyr385, Gly526, Val116, Ser119	2,31; 1,88, 2,11; 2,68	-	-
14.	Stigmasterol	Met522	2,06	Val116, Ala527, Tyr355, Phe518	-
15.	Licofelone	Arg120, Tyr355	2,49; 1,83	Ser353, Val523, Phe518, Ala516, His90, Val349, Ala527, Leu352	-
16.	Native Ligand	Arg120, Phe518, Gln192, Ser353	3,03; 3,07; 2,94; 3,61	Ser353, Val523, Leu384, Met522, Val349, Leu359, Tyr355, Tyr385, Tyr387, Ala527, Leu352	Arg120

Table 9: Interaction of Amino Acid Residues from Docking Ligands with 5-LOX Enzyme

		Hydrogen Bonding			
No.	**Compounds**	**Amino Acid Residues**	**Distance**	**Hydrophobic Interactions**	**Electrostatic Interaction**
1.	Austroinulin	Arg68	3,19	Val110, Leu66, His130	-
2.	β-sitosterol	Tyr385	2,17	Val116, Val349, Leu359, Ala527, Leu531, Tyr355	-
3.	Campesterol	Asp166	1,86	Val107, Val110, Lys133, His130	-
4.	Dulkosida-A	Arg101, Arg138, Glu134, Val110, Glu108, His130	2,82; 3,07; 1,89; 2,83; 2,23; 5,34	-	-
5.	Rebaudiosida-A	Arg101, Arg138, Glu134, Glu108, Thr137	3,18; 2,87; 2,38; 2,01; 3,78	-	-
6.	Rebaudiosida-B	Arg68, Arg101	2,77; 3,02	-	-
7.	Rebaudiosida-C	Arg101, Glu108, Trp102, Asp166, Thr137	3,38; 3,03; 2,83; 2,13; 2,61	Val110, His130	-
8.	Rebaudiosida-D	Arg68, Arg101, Glu108, Thr104, Trp102, Thr137, Glu134	2,86; 2,90; 2,01; 2,04; 2,09; 2,95; 2,87	-	-
9.	Rebaudiosida-E	Arg101, Val110, Thr137, Val110, Glu108, His125, Ile126, His130	3,05; 2,84; 2,20; 2,21; 1,80; 2,69; 3,25; 3,02	-	-
10.	Rebaudiosida-F	Arg68, Arg101, Val110, Glu108, Thr137, Trp102	2,53; 2,81; 2,94; 1,96; 2,81; 2,12	-	-
11.	Sterebin A	Arg68, Glu108	3,18; 2,16	Val110, Lys133, His130	-
12.	Steviolbiosida	Arg101, Thr137, Glu108, Glu134, Asp166	3,32; 2,20; 2,21; 3,62; 3,76	-	-
13.	Steviosida	His130, Thr137, Glu108, Ile126, Thr137, Asp166	2,85; 2,63; 2,08; 2,03; 3,52; 3,15	-	-
14.	Stigmasterol	Arg68 3,09		His130, Val107, Val110	-
15.	Licofelone	His130, Arg138, Arg101, Thr137	3,07; 2,65; 4,05; 4,56	Val107, Lys133, His130, Val107	-
16.	Native Ligand	Arg101, Thr137, Arg138, Val109, His130	2,59; 2,69; 2,85; 3,71; 2,89	Ile523, Leu352, Met522, Trp387, Phe518, Val349, Ala527	Arg101



a)



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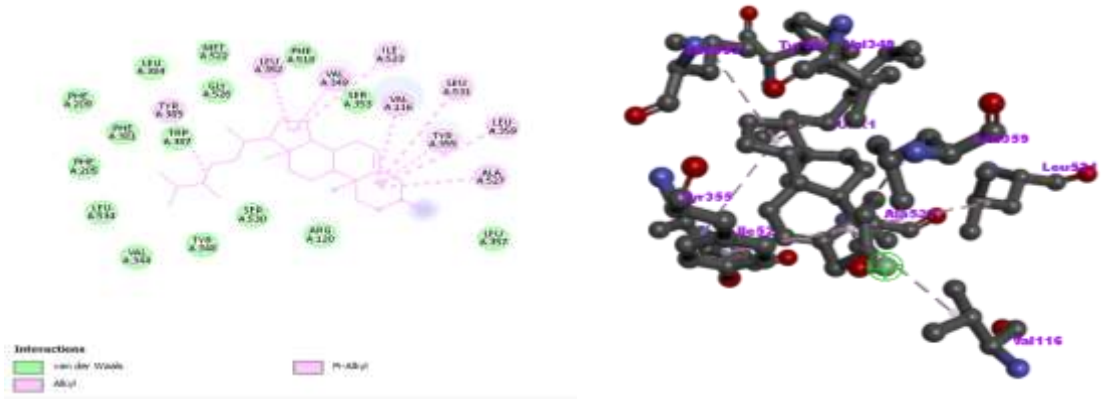

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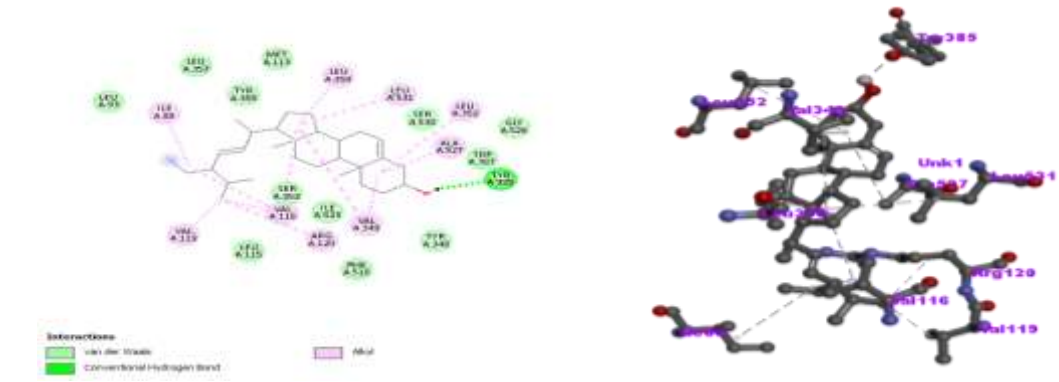

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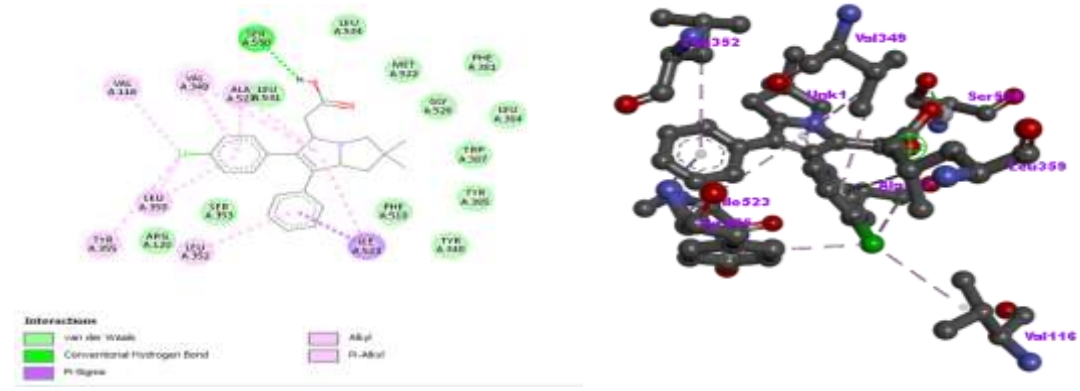
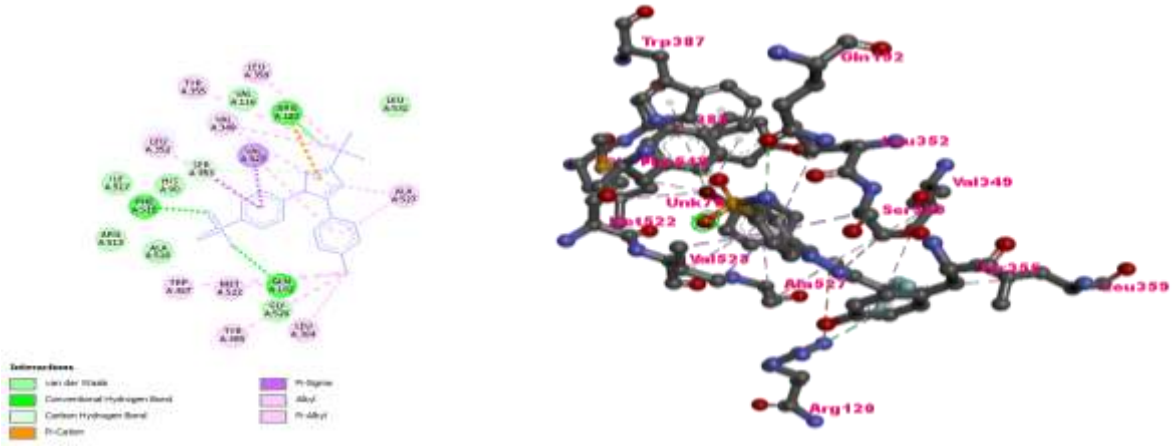
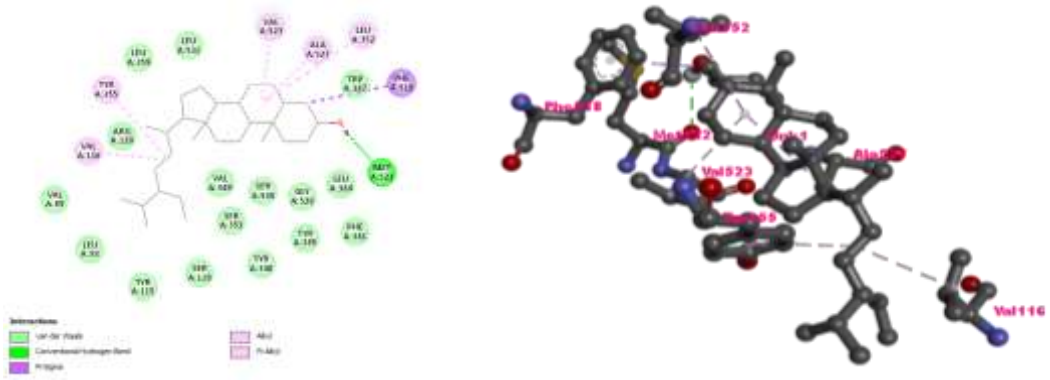


e)

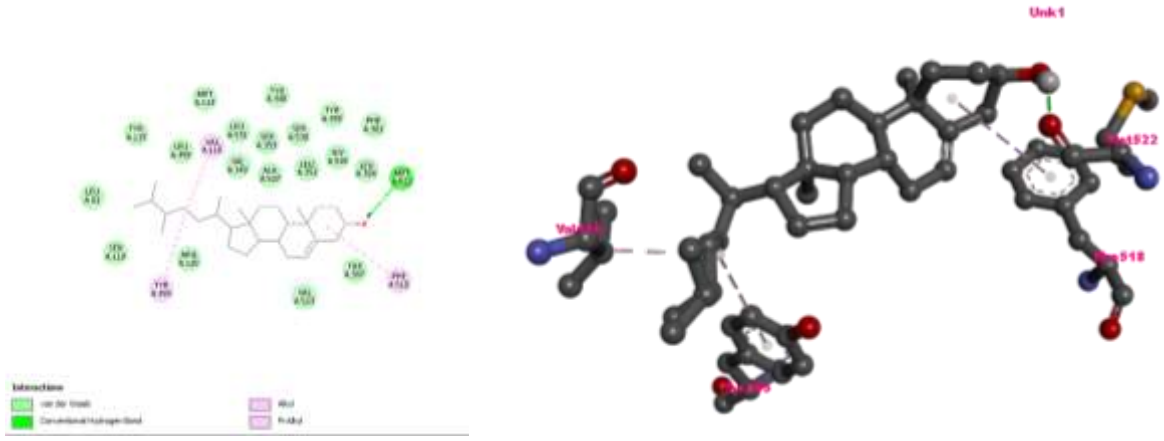
Figure 4: 2D and 3D Visualization with COX-1 Enzymes a) Native Ligand b) β-sitosterol c) Campesterol d) Stigmasterol e) Licofelone


Trp387
Val349
Val523
Ala527
Arg120

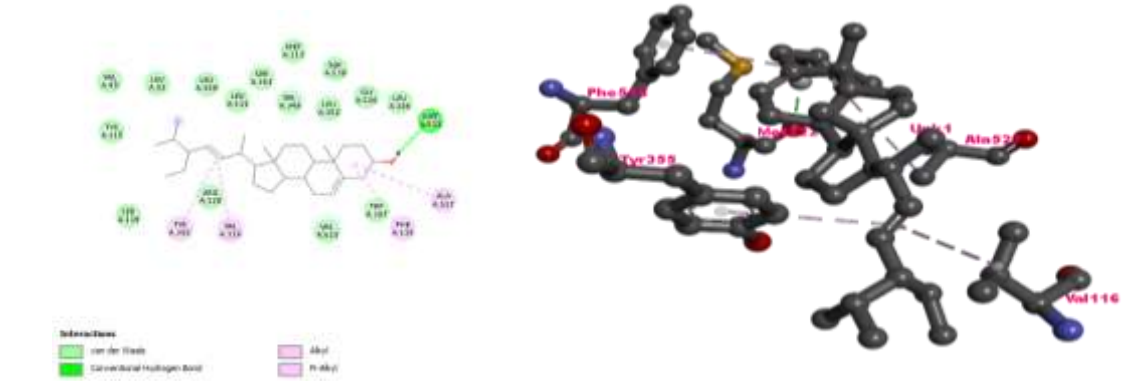
a)


Val523
Val116

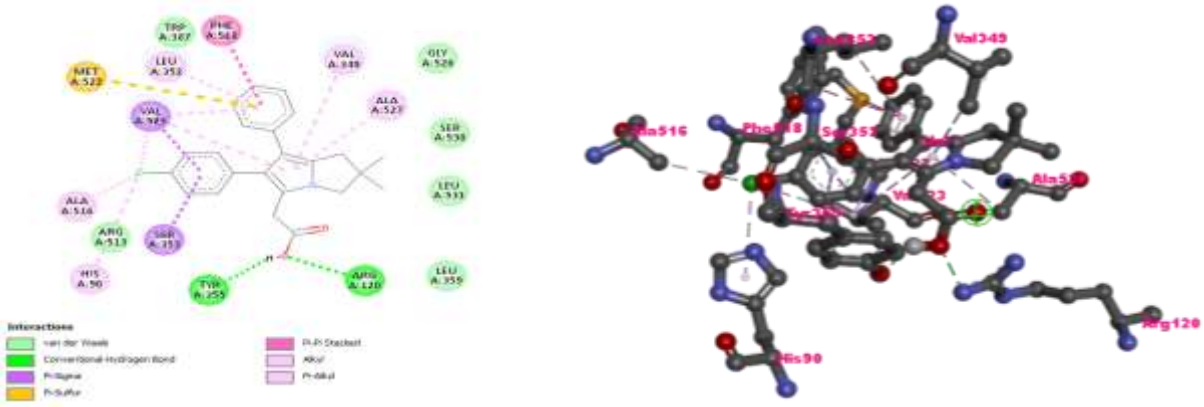
b)


Unk1

c)

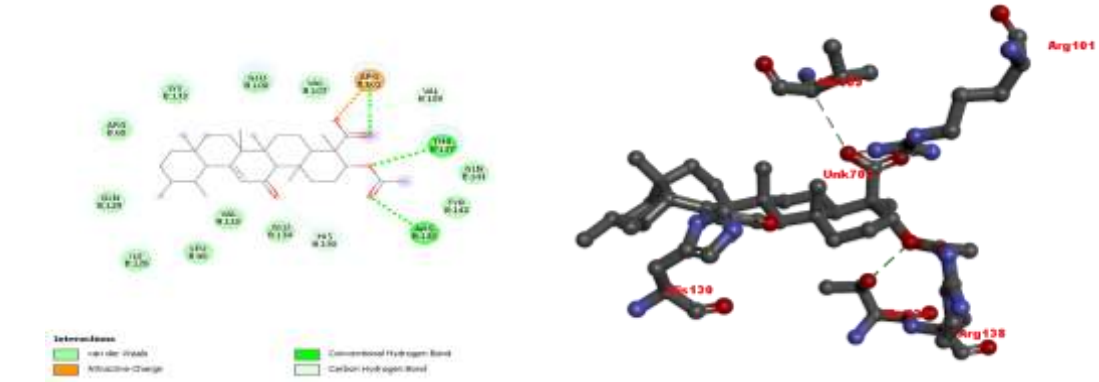

Unk1
Ala527
Val116

d)

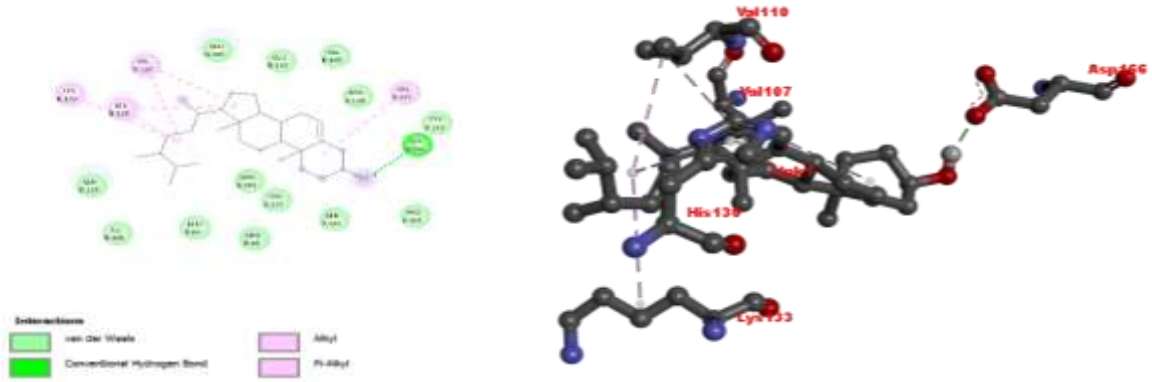


e)

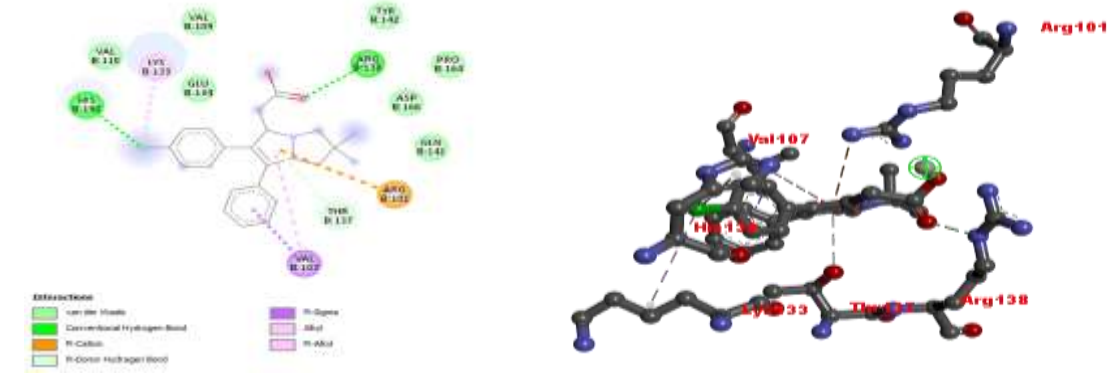
Figure 5: 2D and 3D Visualization with COX-2 Enzymes a) Native Ligand b) β-sitosterol c) Campesterol d) Stigmasterol e) Licofelone



a)



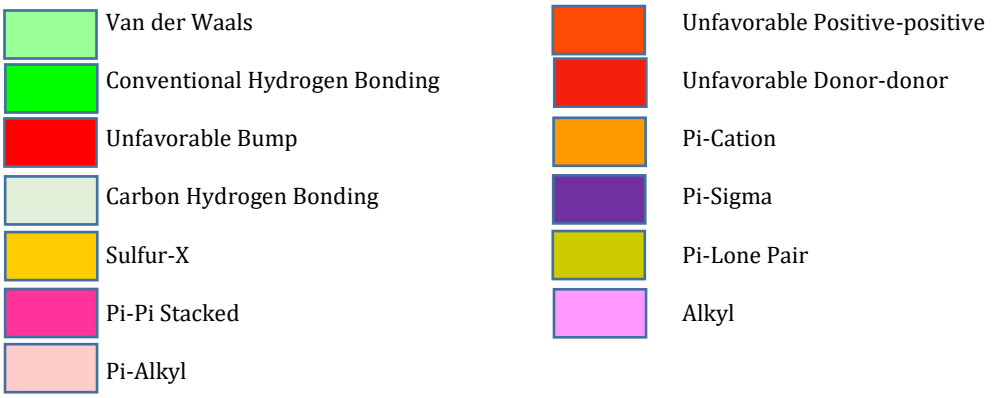
b)



c)

Figure 6: 2D and 3D Visualization with 5-LOX Enzymes a) Native Ligand b) Campesterol c) Licofelone

Note:



4. CONCLUSION

Based on the results of Lipinski's Rule of Five analysis of 14 *Stevia rebaudiana* Bert. plant compounds show that austroinulin and sterebin A compounds are predicted to be able to be used as oral drugs while other compounds are predicted to be less good used as oral drugs. The β-sitosterol and stigmasterol compounds of the plant *Stevia rebaudiana* Bert. interact better with COX-1 and COX-2 receptors than comparison compounds and native ligands indicated by binding affinity values and inhibition constants. Both compounds work non-selectively to inhibit COX-1 and COX-2 enzymes at once. Amino acid residues thought to be involved in ligand interactions with COX-1 enzymes are Arg120 and Tyr355. Amino acid residues thought to be involved in ligand interactions with COX-2 enzymes are Arg120, Phe517, Gln192, and Ser353.

In contrast to the plant's campesterol compound, Stevia rebaudiana interacts better with 5-LOX receptors than comparison compounds and native ligands indicated by binding affinity values and inhibition constants. Campesterol compounds are also non-selective because they can inhibit COX-1, COX-2, and 5-LOX enzymes. Amino acid residues thought to be involved in protein-ligand interactions are Arg101, Thr137, Arg138, Val109, and His130. Based on its pharmacokinetic profile, the plant campesterol compound Stevia rebaudiana Bert. It has a good pharmacokinetic profile.

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