PROCEEDING
3rd PLANAR : International Pharmacy Ulul Albab Conference & Seminar
Malang, Oct 25-26th, 2023

IN SILICO ANALYSIS OF SECONDARY METABOLITES OF *Clerodendrum Inerme* AS A POTENTIAL ANTIDIABETES COMPOUNDS

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Abstract

Clerodendrum inerme can potentially alleviate diabetes, but little is known about its molecular mechanisms. This study aimed to investigate the chemical compound of *C. inerme* and its molecular mechanism to treat diabetes. The KNApSAcK was used to find secondary metabolite of *C. inerme*. A screening was done to find compounds by estimating Absorption, Distribution, Metabolism, and Excretion (ADME) on the SwissADME. The SwissTargetPrediction tool connects predictions of target proteins from compounds that pass screening to various probable proteins and utilizing the StringDB to show the network between target proteins and associated diseases. After finding the target protein, continue docking the chemical compound to the target protein using PyRx with AutoDock 4.2.6. The result from StringDB found four chemical compounds ((Z)-3-Hexenyl beta-D-glucopyranoside, Rhodioloside, Sammangaoside B, Clerodermic acid) that can connected to 4 target proteins (DPP4, IL1B, PPARA, PPARG). According to the docking results, clerodermic acid has good protein binding properties with DPP4, IL1B, PPARA, PPARG, rammangaoside B with PPARG, and rhodioloside with DPP4. *C. inerme* contains clerodermic acid, rammangaoside B, and rhodioloside compounds, which can potentially treat diabetes mellitus.

***Kata Kunci**: Clerodendrum inerme, diabetes, network pharmacology, molecular docking, diabetes mellitus*

Background

Diabetes mellitus is a collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is insulin secretion disorder, insulin disorder effect, or usually both (Petersmann *et al.*, 2019). Diabetes mellitus is classified into three types. Type 1 diabetes causes cell damage, resulting in the body's inability to produce insulin. Insulin resistance, a condition in which cells fail to respond to insulin properly, is the starting point of type 2 diabetes (Tanase *et al.*, 2020). experienced by pregnant women is due to a decrease in the body's ability to produce insulin to control blood sugar levels during pregnancy (Setiabudy, Nafriadi and Instiaty, 2016). The goal of diabetes mellitus treatment is to achieve normal levels of insulin in the plasma (Ferguson and Finck, 2021).
According to IDF data, by 2021, 537 million adults aged 20-79 years are diagnosed with diabetes. The number is expected to rise to 643 million by 2030 and 783 by 2045. In Southeast Asia, 90 million adults have diabetes, which causes 747,000 deaths (Webber, 2021). New therapies must be invented to meet the needs of health care, including promotion, prevention, treatment, and rehabilitation, so that the prevalence of diabetes mellitus can be decreased. Dozens or even hundreds of new drugs are released to the market every year after going through a time-consuming and expensive development process (Hairunnisa, 2019).
Clerodendrum inerme, commonly known as gambir laut in Indonesia, belongs to the Verbenaceae family. It is commonly found in Australia, Asia, Malaysia, and the Pacific Islands. *C. inerme* is traditionally used to treat malaria. It is also used as a thermal suppressant, uterine stimulant, pest control agent, and antiseptic (Kar *et al.*, 2019). Although it is not clear that this plant has antidiabetic effects, it is a good plant to study the contents of secondary metabolite compounds with antidiabetic effects. This research aims to find new drugs with antidiabetic effects through network pharmacology and molecular docking. The method is used as an early stage of research before further in-vivo research.

Research Methods

Tools

This study was conducted using several online databases and software. Online database used were KNApSAcK (https://www.knapsackfamily.com/KNApSAcK), Pubchem (https://pubchem.ncbi.nlm.nih.gov/), SwissADME (http://www.swissadme.ch/), SwissTargetPrediction (http://www.swisstargetprediction.ch/), GeneCards (https /www.genecards.org/), Venny 2.1.0. (https://bioinfogp.cnb.csic.es/tools/venny/), StringDB (https://string-db.org/), Protein Data Bank (https://www.rcsb.org/), and Proteins.Plus (https://proteins.plus/). The software applications used were Avogadro, BIOVIA Discovery Studio Visualizer version 4.5, and PyRx version 0.8.

Secondary Metabolite of C. inerme Identification and Network Pharmacology Analysis

The secondary metabolite of *C. inerme* was obtained from KNApSAcK, and PubChem was used to search for the canonical SMILE compounds (Kim, 2021). Screening of compounds using the SwissADME website that will predict the bioavailability of the compound using the BOILED-egg method. Furthermore, SwissTargetPrediction was used to predict proteins that can interact with secondary metabolite compounds (Lena *et al.*, 2023). Search for protein targets that interact with diabetes was carried out using the GeneCards (Stelzer *et al.*, 2016). followed by looking for protein intersections predicted to have ties to compounds from the plant using the Venny database (Oliver, 2015). Furthermore, the intersection results are entered into the StringDB for network pharmacology analysis (Szklarczyk *et al.*, 2021).

Molecular Docking Analysis

Docking molecules using 3D files obtained from PubChem and prepared using Avogadro by MMF94s Method. Separation of 3D files between the target protein of diabetes mellitus and its ligand was done using BIOVIA Discovery Studio. Molecular docking was done using PyRx 0.8 with autodock 4. The results of the docking are then visualized using the Proteins.Plus webserver.

Result and Discussions

Identification, Bioavailabity Prediction, and Network Pharmacology Analysis of Secondary Metabolite of C. Inerme

The secondary metabolite of *C. inerme* was obtained using the KNApSAcK database. There were found 24 metabolite compounds contained in the *C. inerme*. SMILES canonical code was searched using PubChem, but six of these compounds were not found in its SMILES code, so they were not included in the study (**Table 1**).

Table 1. List of metabolite compounds contained in *C. inerme* taken from the KNApSAcK website.

NO	Metabolite	Compound Code
1	Acteoside	Mol 1
2	Scutellarein 4'-methyl ether	Mol 2
3	Monomelittoside	Mol 3
4	Melittoside	Mol 4
5	Rehmannioside D	Mol 5
6	(Z)-3-Hexenyl beta-D-glucopyranoside	Mol 6
7	Decaffeoylacteoside	Mol 7
8	Isoacteoside	Mol 8
9	Leucosceptoside A	Mol 9
10	Rhodioloside	Mol 10
11	Sammangaoside A	Mol 11
12	Sammangaoside B	Mol 12
13	Benzyl beta-primeveroside	Mol 13
14	14,15-Dihydro-15beta-methoxy-3-epicaryoptin	Mol 14
15	14,15-Dihydro-15-hydroxy-3-epicaryoptin	Mol 15
16	(7S,8R)-Dehydrodiconiferyl alcohol 4-O-beta-glucopyranoside	Mol 16
17	(7S,8R)-Dehydrodiconiferyl alcohol 9-O-beta-glucopyranoside	Mol 17
18	11-Pentacosanone	Mol 18
19	Leonuriside A	Mol 19
20	6-Nonacosanone	Mol 20
21	Clerodermic acid	Mol 21
22	Darendoside B	Mol 22
23	Sammangaoside C	Mol 23
24	Seguinoside K	Mol 24

Then, the next stage is carried out, namely, selecting compounds based on ADME. The term used is Lipinski's Rules of Five (RoF), which says that in compounds which have a molecular weight lower than 500 Da, the number of hydrogen bond donors is less than 5, the number of hydrogen bond acceptors is less than 10, and x log P is lower than 5 have high bioavailability (Nogara *et al.*, 2015). From Lipinski's RoF, it was obtained four compounds: Mol 6, Mol 10, Mol 12, and Mol 21. Besides that, the compounds were also examined using the Brain Or Intestinal EstimateD (BOILED-Egg) method. For this purpose, BOILED-Egg is proposed as an accurate prediction model that calculates the lipophilicity and polarity of small molecules (Daina and Zoete, 2016).

From these results, one compound (Mol 21) penetrated the blood-brain barrier, marked in the yellow section. The other three compounds (Mol 6, Mol 10, and Mol 12) are in the white part, meaning these compounds cannot penetrate the blood-brain barrier but can be absorbed in the digestive tract.

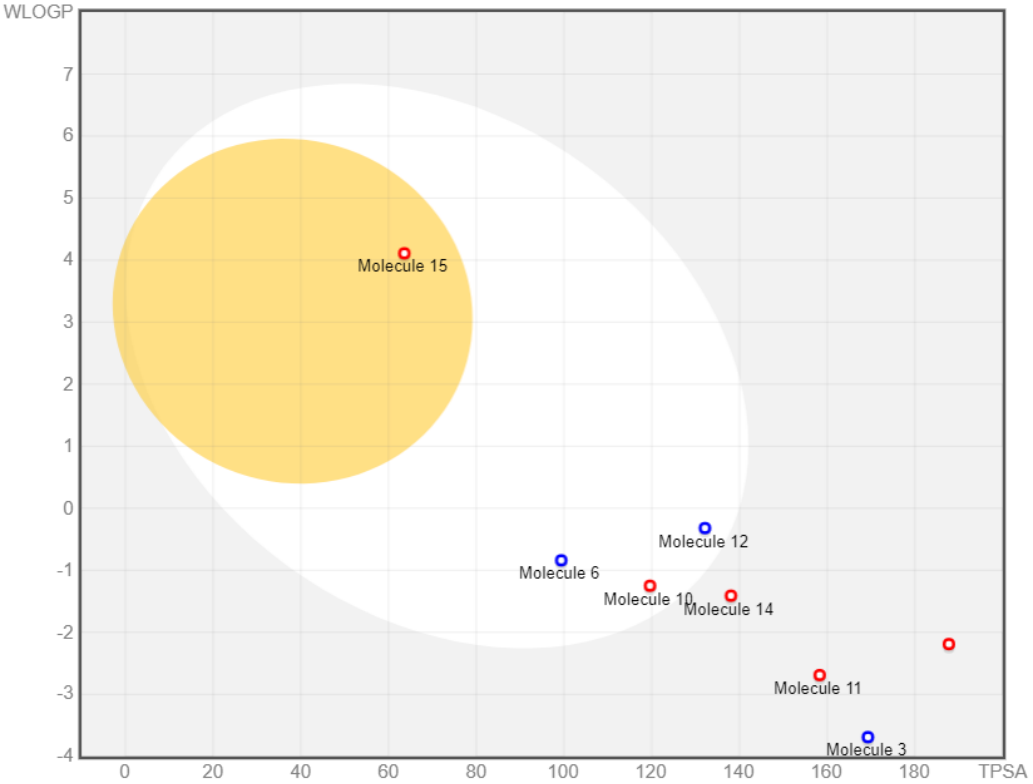

Figure 1. Bioavailability prediction with BOILED-Egg Method

The four compounds that pass ADME will be checked to see whether they can bind to proteins using SwissTargetPrediction. The prediction results are a percentage probability of the target protein binding. After knowing the target protein of the plant compound, continue comparing it with the Diabetes Mellitus-related protein obtained from GeneCards. 178 proteins were predicted to interact with secondary metabolite compounds, and there are found 15,390 diabetes mellitus-related proteins. Intersection with Venny found that only 159 proteins from SwissTargetPrediction were related to Diabetes Mellitus.

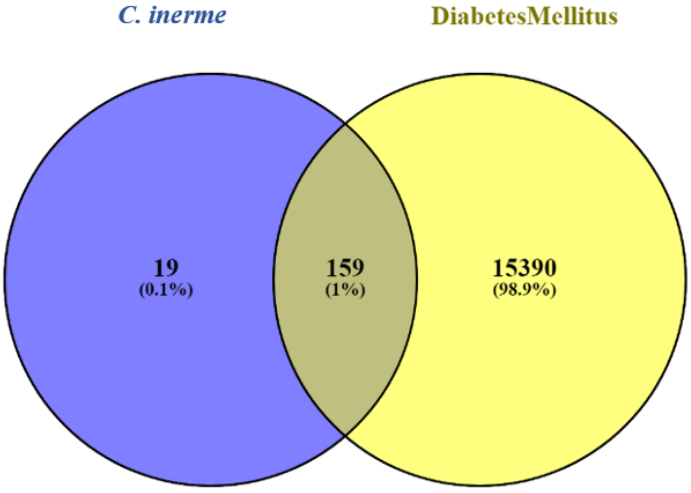

Figure 2. Results of target protein selection between selected compound target protein from *C. inerme* and target proteins from Diabetes Mellitus

Table 2. A list of target proteins after selection using Venny

Code Protein
ACE, ADA, ADAM17, ADK, ADORA1, ADORA2A, ADORA2B, ADORA3, ADRA1A, ADRA1D, ADRA2A, ADRA2B, ADRA2C, AGTR1, AGTR2, AHCY, AKR1B1, AKR1C3, ALOX5, AMPD3, AR, ATP12A, BCL2, BCL2L1, CA1, CA2, CA3, CA4, CA6, CA7, CA9, CCKBR, CDA, CDC25A, CDC25B, CDC25C, CDK1, CES2, CMA1, CSNK2A1, CTRC, CTSG, CXCR2, CYP17A1, CYP19A1, CYP26A1, CYP2C19, CYP2D6, DAO, DHODH, DPP4, ECE1, EDNRA, EGFR, EPAS1, EPHX2, ESR2, F2, F2RL1, FGF1, FGF2, FLT1, FOLH1, FUCA1, GAA, GAPDH, GRK1, GRM2, GSK3B, HCAR2, HDAC1, HK1, HK2, HMGCR, HPSE, HRAS, HSD11B1, HSD11B2, HSP90AA1, HSP90AB1, HSPA5, HSPA8, HTR2B, IDO1, IGFBP3, IL1B, IL2, KDM4C, KIT, LGALS3, LGALS4, MAOA, MAP3K7, MAPK1, MCL1, MDM2, MME, MMP1, MMP12, MMP13, MMP2, MMP3, MMP7, MMP8, NR3C1, NR3C2, OGA, OXER1, PDE7A, PGR, PIN1, PLA2G2A, PLA2G4A, PNP, POLA1, PPARA, PPARD, PPARG, PPP1CC, PPP2CA, PREP, PRKCA, PRSS1, PTGDR2, PTGES, PTGES2, PTGS2, PTPN1, PTPN2, PYGL, RARA, RARB, RARG, RBP4, RORC, RXRA, RXRB, RXRG, SERPINA6, SHBG, SLC22A12, SLC29A1, SLC5A1, SLC5A2, SLC5A4, SORT1, SRD5A2, STAT3, TBXA2R, TBXAS1, THRA, THRB, TK1, TP53, TRPA1, TYMP, TYMS, TYR, VEGFA

The next step is to look for functions and correlations between protein targets by using network pharmacology. This correlation includes direct (physical) and indirect (functional) associations, which can be done via the StringDB. that aims to place its focus on coverage (application of thousands of genome-sequenced organisms), on rich sources of evidence (e.g. including automated text mining) and usability features (such as customization, enrichment detection and programmatic access) (Szklarczyk *et al.,* 2021). The target prediction results from 159 target proteins showed that there were five proteins associated with Diabetes Mellitus: Dipeptidyl peptidase-4 (DPP4), Interleukin-1 beta (IL1B), Peroxisome Proliferator-Activated Receptor Alpha (PPARA), Peroxisome Proliferator-Activated Receptor Gamma (PPARG), Solute Carrier Family 5 Member 2 (SLC5A2). When searching for 3D proteins from Protein Data Bank, no SLC5A2 protein was found, so this protein was eliminated.

Inhibition of DPP4 has the effect of inhibiting GLP-1, GLP-2 and GIP which will reduce blood sugar (Nistala and Savin, 2017). IL1B, a cytokine that promotes inflammation and influences crucial metabolic functions such as insulin production and β-cell apoptosis (Alfadul, Sabico and Al-Daghri, 2022). PPARA agonist affects glucose homeostasis through pancreatic function so that PPARA agonists can maintain B cells (Lin, Wang and Li, 2022). Controlling adipogenesis in white adipose tissue is one of the main roles of PPARG. Many endogenous ligands that serve as an indication of the metabolic state of the cell can be obtained with PPARG (Frkic, Richter and Bruning, 2021).

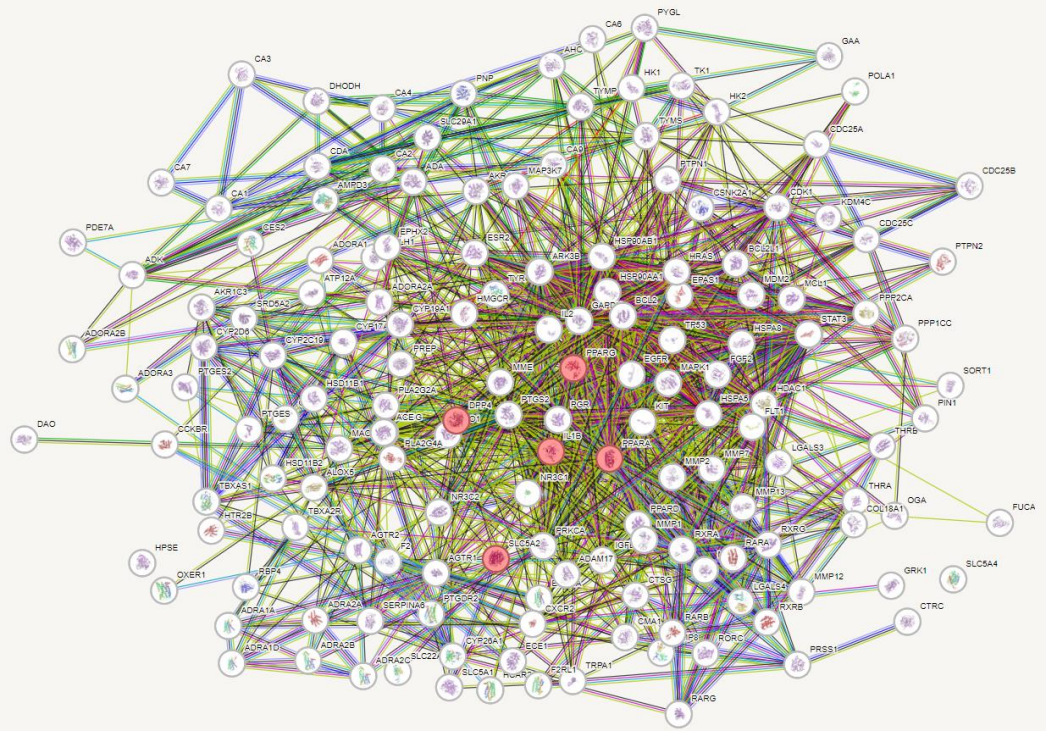

Figure 3. Network Pharmacology prediction results using String-db. The red color shows the target protein associated with Diabetes Mellitus.

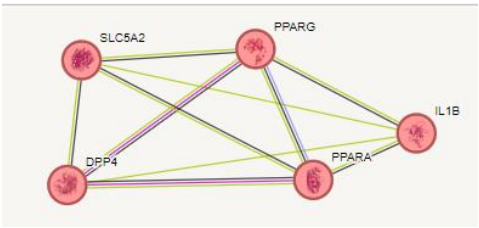

Figure 4. Network pharmacology of target prediction protein for Diabetes Mellitus

Molecular Docking Analysis

Molecular docking was carried out between the four secondary metabolite that pass Lipinski's RoF and BOILED-Egg, with the proteins DPP4 (PDB ID = 6EOR), IL1B (PDB ID = 6Y8I), PPARA (PDB ID = 3KDT), and PPARG ((PDB ID = 8HUP). More complete docking results can be seen in **table 4**. The docking results obtained only a few have the highest potential as anti-diabetics. A compound can be said to be good if the energy binding value and the inhibition constant value were low. The lower the value of the energy binding and inhibition constant, the better the compound binds to protein (Muchlisin *et al.*, 2022). Docking result that have good potential only Mol 10 to DPP4 (-8.16 kcal/mol, 1.04 µM), Mol 12 to PPARG (-6.98 kcal/mol, 7.6 µM), and Mol 21 to DPP4 (-7.79 kcal/mol, 1.94 µM), IL1B (-7.63 kcal/mol, 2.56 µM), PPARA (-9.07 kcal/mol, 225.97 ηM), and PPARG (8.09 kcal/mol, 1.17 µM) which has high potential as an anti-diabetic drug candidate. The bonds formed between the compound and the target protein are in the form of hydrogen and hydrophilic bonds which can be seen in **table 5**. In the docking results of Sammangaoside B to PPARA, no hydrogen or hydrophilic bonds occurred.

Table 3. Docking results of secondary metabolite compounds of *C. inerme* to the target proteins DPP4, IL1B, PPARA, PPARG.

Compound Code	PPARG		IL1B		PPARA		DPP4	
	Protein Binding	Inhibition Constant	Protein Binding	Inhibition Constant	Protein Binding	Inhibition Constant	Protein Binding	Inhibition Constant
Mol 6	-5.06 kcal/mol	196.85 µM	-4.93 kcal/mol	245.20 µM	-5.51 kcal/mol	91.58 µM	-5.01 kcal/mol	288.67 µM
Mol 10	-6.47 kcal/mol	18.05 µM	-6.18 kcal/mol	29.65 µM	-6.52 kcal/mol	26.37 µM	-8.16 kcal/mol	1.04 µM
Mol 12	-6.98 kcal/mol	7.6 µM	-3.62 kcal/mol	3.56 µM	-5.99 kcal/mol	40.76 µM	-6.65 kcal/mol	13.33 µM
Mol 21	-8.09 kcal/mol	1.17 µM	-7.63 kcal/mol	2.56 µM	-9.07 kcal/mol	225.97 nM	-7.79 kcal/mol	1.94 µM
Ligand	-7.63 kcal/mol	2.54 µM	-6.82 kcal/mol	10.02 µM	-9.73 kcal/mol	73.62 nM	-12.40 kcal/mol	811.94 pM

Table 4. Hydrogen bond and hydrophilic interaction of secondary metabolite of *C. inerme*

Compound Code	PPARG		IL1B		PPARA		DPP4	
	Hydrogen	Hydrophilic	Hydrogen	Hydrophilic	Hydrogen	Hydrophilic	Hydrogen	Hydrophilic
Mol 6	CYS285, LEU340, SER342, GLU343	ARG288	THR147, GLN149	GLN15, THR147	PHE273, GIN277, SER280, TYR314	SER280, ILE317	GLN648, GLN650, ASN653, LYS660	
Mol 10	CYS285, ARG288, GLU291, LEU340, GLU343	PHE264, ARG288	ASP12, MET148	GLN15, THR147	PHE273, GLN277, SER280, THR283, TYR314	THR279	GLU248, GLU249, GLN648, SER730, VAL756 TYR762,	TYR731, VAL756 TYR766
Mol 12	HIS266, ARG288, ILE326, SER342	ARG288, ALA292, ILE326, LEU330, LEU333	ASN108, MET148	MET148, PHE150			GLN648, GLN650, LYS660, TYR661, TRP729	PHE642, TYR644, LEU651
Mol 21	TYR327, GLU343, LYS367,	ARG288, LEU330, LEU333, VAL339, ILE341	MET148	MET148, GLN149, PHE150	THR279, TYR314, HIS440, TYR464	CYS276, SER280, PHE318, MET355, HIS440	LYS660, TRP729	PHE642, TYR644, TRP729
Ligand	PHE264, ARG288, SER342	PHE264, HIS266, GLY284, ARG288	GLN39, THR9		CYS276, SER280, TYR314, HIS440	CYS275, THR279, SER280	GLU249, TYR762, ASN810	HIS500, GLN648, VAL649, SER730, TYR731, VAL756, TYR766

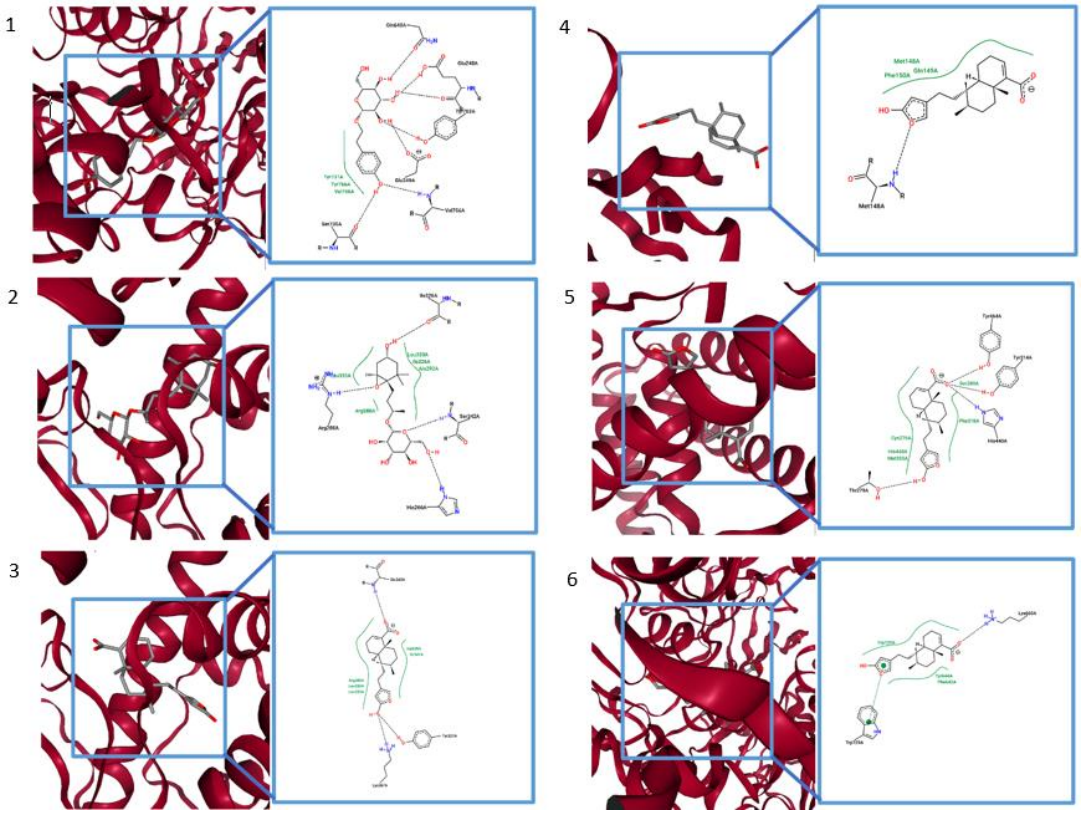

Figure 5. 2D and 3D images of 1. Rhodioloside to DPP4, 2. Sammangaoside B to PPARG, 3. Clerodermic acid to DPP4, 4. Clerodermic acid to IL1B, 5. Clerodermic acid to PPARA, 6. Clerodermic acid to PPARG.

Conclusion

The compounds Rhodioloside, Sammangaoside B, Clerodermic Acid have potential as new anti-diabetic drugs by binding to the proteins DPP4, IL1B, PPARA, PPARG.

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