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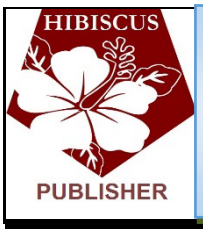

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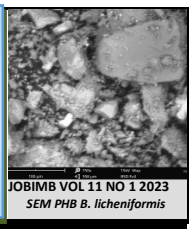

Identification of Insulin-Mimetic Phytochemicals from Mas Cotek (*Ficus deltoidea*) for Treatment of Type 2 Diabetes via LC-MS/MS and Molecular Docking Analyses

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ABSTRACT

Type 2 diabetes mellitus, a metabolic syndrome, has become increasingly prevalent in recent years. In the treatment of chronic T2DM, patients are required to take insulin daily, commonly through injections, as the hormone can easily be degraded by the digestive system if taken orally. This can be an uncomfortable experience for the patient. Thus, finding an alternative to insulin, especially from natural compounds, would be beneficial. *Ficus deltoidea*, which belongs to the Moraceae family, is a medicinal plant known for its anti-diabetic properties. Therefore, this study aimed to identify the phytochemicals from *F. deltoidea* that mimic insulin by studying their ability to bind to insulin receptors using in silico analysis. A total of 36 phytochemicals were identified in the methanolic extract of *F. deltoidea* through LC-MS/MS analysis. They were then subjected to molecular docking to determine their binding free energy with the insulin receptor (IR). The results revealed seven phytochemicals with the lowest binding free energy, with 2,3-dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino] butanediamide exhibiting the lowest binding free energy at -10.0 kcal/mol. Hence, these phytochemicals demonstrate potential as insulin-mimetic compounds that can be used in the treatment of T2DM.

INTRODUCTION

Diabetes mellitus has emerged as one of the most prevalent chronic non-communicable diseases (NCDs) worldwide. According to the World Health Organization (WHO), the number of diabetes mellitus cases surged from nearly 30 million people worldwide in 1985 to a staggering 135 million in 1995 [1]. Projections suggest that by the year 2025, approximately 300 million people will be affected. Diabetes mellitus is defined as a heterogeneous metabolic disorder characterized by chronic hyperglycemia, caused by impairment in insulin production, faulty insulin action, or both. Pathologically, diabetes mellitus is classified into two major types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM)[2]. Type 1 diabetes mellitus constitutes about 5-10% of all diabetes cases. Its pathophysiology involves absolute insulin insufficiency, caused by the autoimmune destruction of insulin-secreting pancreatic β-cells by T cells and B cells. Meanwhile, approximately 90-95% of total diabetes cases are classified as type 2 diabetes mellitus. The main pathophysiological features of T2DM are prominent insulin resistance combined with impaired insulin secretion, resulting in a predominant failure in insulin production alongside insulin resistance [2].

The insulin-signaling pathway of glucose uptake begins with the binding of insulin hormone to the insulin receptor (IR). The binding of insulin to the extracellular subunits of the receptor causes activation of catalytic domains of tyrosine kinases in the cytoplasm as the dimerization of IR allows the binding of ATP to the β-subunits. The receptor then undergoes autophosphorylation upon activation and catalyze the phosphorylation of several intracellular substrates, including insulin receptor substrate (IRS) proteins. The phosphorylated IRS proteins trigger the activation of the phosphatidylinositol-3-kinase (PI3K) pathway by binding

IRS proteins to the PI3 kinase regulatory subunits through SH2 domains resulting in the formation of phosphatidylinositol (3,4,5)-triphosphate (PIP3). The PIP3 will subsequently activate PIP3-dependent kinases (PDK-1 and PDK-2) and eventually lead to the activation of protein kinase B (AKT/PKB kinase) and atypical PKC. Thus, this signalling results in the translocation of GLUT4 from the cytoplasmic vesicles onto the cell membrane surface as a result of increasing the insulin-dependent glucose transport into the cell [3,4].

Insulin resistance occurs when the insulin receptor (IR) is altered, resulting in decreased IRS-1 tyrosine phosphorylation and, as a result, limiting the ability of peripheral tissues to take in glucose. In the presence of circulating insulin, skeletal muscles typically utilize more than 80% of the circulating glucose, however in the case of T2DM, this effect is diminished [5]. In the pharmaceutical field, various synthetic antidiabetic drugs such as biguanides, thiazolidinediones, sulfonylureas, dipeptidyl peptidase-4 inhibitors, and alpha-glucosidase inhibitors have been developed for the treatment of T2DM. However, when patients diagnosed with type 2 diabetes cannot achieve sufficient glycemic control through antidiabetic drugs, insulin administration becomes necessary. Over time, a significant number of patients diagnosed with type 2 diabetes may require the implementation of multiple daily injection therapy to achieve the most favourable diabetes management[6]. However, this therapy may lead to an unpleasant experience for the patient, in addition to its drawbacks, such as hypoglycemia and weight gain[7]. Thus, finding an alternative that can mimic insulin action and can be administered orally would be desirable. Natural products can serve as excellent alternatives to insulin due to the perception that they are safe and have fewer side effects. Compound binding to protein targets [8–12] and understanding the molecular mechanics of numerous physiological systems in the cell, such as discovering innovative alternatives to insulin, notably from natural compounds, have dominated the field of computer-aided drug design (CADD) since its debut [13–18].

Ficus deltoidea, an indigenous plant in Malaysia commonly known as 'Mas Cotek' by locals in Peninsular Malaysia, is also found in several Southeast Asian countries, including Thailand and Indonesia. Its name is derived from the golden spotting on its leaves. In Malaysia, *F. deltoidea* has been traditionally used as an ancient Ayurvedic medicine to treat various ailments such as wounds, sores, and rheumatism. However, modern pharmacological studies have also extensively explored this plant for a range of medicinal applications, including antidiabetic, anti-inflammatory, anticancer, and antioxidant properties, owing to its bioactive constituents with diverse pharmacological potentials [19]. Although several studies have shown that *F. deltoidea* has the potential to exhibit antidiabetic properties, there is still limited research on identifying its phytochemicals which can mimic insulin action. Thus, the study aimed to elucidate the insulin-mimetics properties of phytochemicals from *F. deltoidea* using *in silico* analysis.

METHODOLOGY

Ficus deltoidea Methanolic Extract Preparation

Methanolic extract of *Ficus deltoidea* was prepared by maceration method which 50 g of commercialized dried powdered form *F. deltoidea* leaves were soaked in 500 mL of methanol for three days at room temperature by changing the solvent daily. The combined suspension was filtered using Whatman filter paper no.1. The filtrate was evaporated to generate a paste product under pressure at 30 °C using a rotary evaporator to yield methanolic extract of *F. deltoidea*. The weight of the rotary flask without the sample was weighed before and after evaporation together with the methanolic extract of *F. deltoidea* to calculate the yield of extraction. The methanolic extract was then collected into a 25 mL Scott bottle.

Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) analysis

The phytochemical compounds identification of *F. deltoidea* methanolic extract was determined by using Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) analysis. About two mg sample extract of *F. deltoidea* used for phytochemical compounds identification was diluted in 1 mL of methanol with a concentration of 2 mg/mL. The soluble extract was filtered using a 0.45 µm sterile nylon syringe filter before analysis and was then sent to Monash University Malaysia for LC-MS/MS analysis. The phytochemical compounds of *F. deltoidea* methanolic extract were determined by using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source. A 3.0 µL volume of sample was injected by an autosampler with the temperature of 4 °C into a C-18 column (Agilent Eclipse XDB-C18 Narrow-bore, 150 mm x 2.1 mm, 3.5-micron) with the temperature of 25 °C.

The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a flow rate of 0.5 mL/min. The mass spectrometry was operated in positive ionization mode with the parameters set as follows where the fragmentor voltage was set at 125 V with the temperature of the drying gas (N_2) at 300 °C, at a gas flow rate of 10 L/min and a nebulizing pressure (N_2) of 45 psig. The mass spectra were recorded by scanning the mass range from *m/z* 100 to 3200 and 50 to 3200 in MS and MS/MS modes respectively. The data obtained were then processed by using Agilent MassHunter Qualitative Analysis B.07.00 which provided a list of possible molecular formulas. The molecular formula of compounds proposed by the MassHunter was then identified by searching with Metlin_AM_PCDL-N-170502.cdb.

In Silico Analysis

Protein Preparation

The three-dimensional X-ray crystallographic structure of the protein insulin receptor (PBD ID: 1IR3) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank and saved in PDB format (.pdb). The protein was prepared and optimized by using AutoDock Tool (ADT), bundled with the MGL Tools package (version 1.5.7) [20]. The crystallographic structure chain A was used structures for the docking process. The ligands, water molecules and heteroatoms were removed, while polar hydrogens and charges were added in the preparation process. Energy minimization of protein was done with Gromos96 43B1 using Swiss-PbdViewer (v.4.1.0) software. The minimized protein was then converted and saved in PDBQT format (.pdbqt) using AutoDock Tool (ADT).

Screening of LC-MS/MS Result

The screening of results was based on the small molecule QTOF basic guidelines sent by Monash University Malaysia. The compounds were narrowed down from the compound list by filtering the compounds with Score Db or Score MFG close to 100. From the compounds obtained above, only compounds with Diff (Db.ppm) or Diff (MFG.ppm) values within -2 and +2 were chosen.

Ligand Preparation
Chemical 3D structure and SMILES of the screened ligands were retrieved from the PubChem compound database. The two-dimensional (2D) and three-dimensional (3D) chemical structures of the ligands were downloaded in SDF format. The ligands in SDF format were converted and saved in PDB format (.pdb) using Open Babel v.2.3.2. Energy minimization of ligands was done with the MMFF94 force field using Avogadro v.1.2.0 software. The minimized ligands were then converted and saved in PDBQT format (.pdbqt) by using AutoDock Tool (ADT).

Protein-Ligand Docking
The molecular docking between protein and ligands was performed using Autodock Vina v1.2.3 [21]. The active binding site of protein was determined based on a published journal [22] as the grid centre and obtained by removing the ligand. The center grid box dimensions were chosen to include all active binding sites for ligands. The grid box was created with sizes 46, 50 and 40 (for x, y and z points), grid center of x, y and z dimensions of -24.489, 28.737 and 7.931 respectively and grid spacing of 0.375Å. A configuration text file (config.txt) was created to run AutoDock Vina 1.2.3. The configuration file consists of the receptor and ligand in PDBQT format (.pdbqt), the size of the grid box and the centre grid box coordinates. The docking scores resulted in the generated log files (log.txt) and the output docking results were defined as affinity binding (kcal/mol).

Visualization of Protein-Ligand Interaction
The interactions between the ligands and the target protein receptor were visualized and analyzed using PyMol v2.5.4 Software. The bond energies such as van der Waals interaction, hydrogen bond and electrostatic energy between receptor and ligands and their interactions were ranked according to their binding affinity.

RESULTS AND DISCUSSION

LC-MS/MS Analysis of *F. deltoidea* Methanolic Extract
An LC-MS/MS phytochemical analysis was performed in the positive ion mode to identify the phytochemical compounds present in the methanolic extract of *F. deltoidea*. In the positive ion mode of electrospray ionization mass spectrometry (ESI-MS), the small molecules were typically charged positively via protonation. According to their mass-to-charge (*m/z*) ratio, the ions subsequently pass through the mass analyzer and usable signals generated will be detected by a detector. The signals detected were then graphically shown by the computer as a mass spectrum, which portrays the relative abundance of the signals based on their *m/z* ratio [23]. The chromatogram shows all the phytochemicals present in the methanolic extract of *F. deltoidea* of their retention time and mass-to-charge ratio by the mass relative abundance. There was a total of 36 phytochemical compounds identified through LC-MS/MS analysis of *F. deltoidea* methanolic extract. The data from the LC-MS/MS analysis were summarized in **Table 1**.

According to the results obtained in **Table 1**, various phytochemicals were identified from the positive ion mode for the methanolic extract of *F. deltoidea*. The compounds identified were phenolic compounds, amino acids, alkaloids, terpenoids, flavonoids, organic acids and their derivatives.

Various literature had actually proven that different parts of *F. deltoidea* plant consist of multiple types of phytochemicals. A report published by [24] verified that leaf, stem and fruit extract of *F. deltoidea* contain phenols, flavonoids, tannins and saponins. Besides, the leaf extract of *F. deltoidea* has also been proven to contain proteins, polysaccharides, flavonoids, tannins, glycosaponins and phenolics [25]. The presence of all these various phytochemicals in *F. deltoidea* introduces it as a medicinal plant that exhibits multiple pharmacological properties such as antidiabetic, anti-inflammatory, anticancer and antioxidant properties [26].

Various phytochemicals identifications on Ficus species ~~has~~ have been carried out and the most commonly identified phytochemicals with various pharmacological properties are vitexin, isovitexin, caffeic acid, catechin, rutin, quercetin and naringenin. [27] reviewed that both vitexin and isovitexin exhibit various pharmacological properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic and others [27]. It has been proven by Inamdar *et al.* [28] that treatment of vitexin in non-alcoholic fatty liver disease (NAFLD) mice enhanced insulin signalling by upregulating the insulin receptor substrate-1 (IRS-1) and downstream target AKT . Current scientific research has shown that vitexin inhibits apoptosis and protects pancreatic β-cells from damage [29]. Besides, Sadikan *et al.* [30] reported that the presence of phytochemicals such as flavonoids and phenolic compounds in *F. deltoidea* enable the plant to exhibit antioxidant and anti-angiogenic properties in become a potential agent for the therapy of diabetic retinopathy.

Even though there were many phytochemicals profiling have been done by many researchers, there were still some phytochemicals that were first discovered in this research such as asperphenamate, biemamide A and dibutyl phthalate. Asperphenamate is a linear amino acid ester that is well-known for its antitumor properties. According to Yuan *et al.* [31] and Li *et al.* [32], their research has proven that asperphenamate showed a great inhibitory effect on human breast carcinoma MCF-7 cells by induction of autophagy. Besides, biemamide A, which is one of the pyrimidinedione derivatives, has been reported to exhibit TGF-β inhibitors based on the *in vitro* experiment by suppressing the TGF-β signaling in NMuMG cells [33]. Furthermore, the dibutyl phthalate identified in this research has been reported by previous researchers that it could worsen T2DM by compromising the insulin signalling pathway and reducing the secretion of insulin using a mouse model [34].

There was also a first identified phytochemical known as tofogliflozin that has been proven as a highly effective oral hypoglycemic drug along with administration with other antidiabetic medications such as metformin, sulfonylureas and dipeptidyl peptidase-4 [35]. Besides, it is classified as a highly selective SGLT2 inhibitor that aids in lowering blood sugar levels by inhibiting the kidney from reabsorbing glucose [36]. Other phytochemicals identified in this research were not discussed due to a lack of previous studies on the properties that they exhibit. The phytochemicals obtained were then used as ligands for the *in silico* molecular docking analysis against insulin receptors (PDB: ID 1IR3) to determine the possibility of the insulin-mimetic properties.

Table 1. Phytochemical profile of *Ficus deltoidea* methanolic extract analyzed by LC-MS/MS QTOF in the positive ion mode and the binding free energy of identified phytochemicals in molecular docking analysis.

No	Compound Name	Chemical Formula	m/z	Mass	Binding free energy (kcal/mol)	Retention Time (min)
1.	N-Acetyl-L-glutamic acid	$C_7H_{11}NO_5$	190.0706	189.0634	-5.5	0.98
2.	Ethyl maltol (2-Ethyl-3-hydroxy-4-pyrone)	$C_7H_8O_3$	141.0546	140.0472	-4.9	3.924
3.	3,4-Dihydroxybenzaldehyde	$C_7H_6O_3$	139.0386	138.0316	-5.2	5.583
4.	Ethyl 4-(4-carbamoylphenyl)-6-(4-nitrophenyl)-2-[2-[(5-nitropyridin-2-yl)amino]ethylamino]pyrimidine-5-carboxylate	$C_{27}H_{24}N_8O_7$	595.1667	572.1764	- 7.8	7.683
5.	Biemamide A	$C_{24}H_{44}N_4O_4$	453.3411	452.3356	-6.9	8.46
6.	2,3-dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino]butanediamide	$C_{28}H_{26}N_4O_{10}$	579.1744	578.1648	-10.0	8.686
7.	N,N-bis(4-acetamidobutyl)-17-methyloctadec-6-enamide	$C_{31}H_{59}N_3O_3$	283.7173	521.4549	-5.6	8.922
8.	N-[[1-[4-amino-6-[4-(2-morpholin-4-ylethyl)piperazin-1-yl]pyrimidin-2-yl]piperidin-4-yl]methyl]-N'-cyclohexylpropane-1,3-diamine	$C_{29}H_{53}N_9O$	566.4293	543.4374	-8.0	8.941
9.	(2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one	$C_{34}H_{30}O_{12}$	631.1797	630.1746	-8.5	10.391
10.	9-(2,3-dihydroxy-3-methylbutoxy)furo[3,2-g]chromen-7-one)	$C_{16}H_{16}O_6$	305.1027	304.0949	-7.2	10.431
11.	1-[4-(dimethylamino)phenyl]-2-hydroxy-2-phenylethanone	$C_{16}H_{17}NO_2$	256.1329	255.1264	-6.5	10.87
12.	4-hydroxy-2-oxo-1-phenyl-N-(pyridin-3-ylmethyl)-1,8-naphthyridine-3-carboxamide	$C_{21}H_{16}N_4O_3$	373.1286	372.1226	-8.6	11.103
13.	(3E)-3-(1-methoxyethenyl)-2-N,2-N'-dimethyl-2-N,2-N'-bis[2-(methylamino)ethyl]hexa-3,5-diene-2,2-diamine	$C_{17}H_{34}N_4O$	333.2641	310.2731	-5.8	11.29
14.	Bergapten	$C_{12}H_8O_4$	217.0495	216.042	-6.8	12.155
15.	Aurantiamide	$C_{25}H_{26}N_2O_3$	403.2027	402.1947	-7.1	12.529
16.	1-Benzyl-1,4,8,11-tetraazacyclotetradecane	$C_{17}H_{30}N_4$	313.2368	290.2475	-6.8	12.823
17.	3a,4,7,7a-Tetrahydro-2-[4-[4-(2-pyrimidinyl)piperazino]butyl]-4,7-methano-1H-isoindole-1,3(2H)-dione	$C_{21}H_{27}N_5O_2$	404.2091	381.2171	-7.6	12.89
18.	Tofogliflozin	$C_{22}H_{26}O_6$	387.182	386.1733	-7.9	12.93
19.	Gancaonin L (5,7,3',4'-Tetrahydroxy-8-prenylisoflavone)	$C_{20}H_{18}O_6$	355.1182	354.1108	-8.5	13.539
20.	Demethoxycurcumin	$C_{20}H_{18}O_5$	339.1231	338.1153	-7.3	13.854
21.	2,5-Dimethyl-2,4-hexadiene	C_8H_{14}	111.1166	110.1095	-4.2	14.087
22.	4-[4-[2-[2-Aminoethyl(2-aminoprop-2-enyl)amino]ethylamino]-2-methylbutan-2-yl]oxy-2-methylbutan-2-ol	$C_{17}H_{38}N_4O_2$	369.2643	330.2998	-5.6	14.463
23.	2-(1-Phenylethyl)-p-xylene	$C_{16}H_{18}$	211.1482	210.1409	-7.2	14.969
24.	Asperphenamate	$C_{32}H_{30}N_2O_4$	507.2304	506.2206	-8.3	15.154
25.	Clavatustide C	$C_{30}H_{55}N_5O_5$	566.4293	565.421	-7.0	15.452
26.	Linolenic acid	$C_{18}H_{30}O_2$	279.2312	278.2242	-5.4	15.637
27.	Ricinoleic acid	$C_{18}H_{34}O_3$	299.2581	298.2508	-5.1	15.83
28.	Linoleic acid	$C_{18}H_{32}O_2$	281.2468	280.2398	-5.0	15.87
29.	4-(4-(6-(4-Phenylpiperazin-1-yl)hexyl)piperazin-1-yl)quinoline	$C_{29}H_{39}N_5$	480.3096	457.3208	-7.8	16.208
30.	9-OxoODE (9-Oxo-10(E),12(Z)-octadecadienoic acid)	$C_{18}H_{30}O_3$	295.2263	294.2194	-5.1	16.332
31.	Dibutyl phthalate	$C_{16}H_{22}O_4$	279.1656	278.1522	-5.7	16.859
32.	Ivospemin	$C_{16}H_{38}N_4O_2$	341.2892	318.3	-4.7	17.612
33.	1,4-Bis[2-(1-piperazinyl)-ethyl]-piperazine	$C_{16}H_{34}N_6$	311.2902	310.2849	-6.4	18.452
34.	N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide	$C_{31}H_{40}N_8O_7$	637.3114	636.3023	-8.5	18.83
35.	Fmoc-L-Hgn(Trt)-OH ((S)-Fmoc-2-amino-5-(trityl-carbamoyl)pentanoic acid)	$C_{40}H_{36}N_2O_5$	625.271	624.2628	-8.8	19.105
36.	3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide	$C_{36}H_{32}N_8O_2$	609.2762	608.265	-9.9	19.332

In silico Molecular Docking Analysis

Molecular docking is a structure-based modeling technique that analyzes the interaction between targeted proteins and ligands. It is a technique that uses to evaluate the binding site for a particular protein receptor as well as the affinity of the protein-ligand interaction based on a scoring parameter by utilizing computational tools as one of the comprehensive approaches for discovering novel ligands with pharmacological potential. By incorporating and optimizing factors like hydrophobic, steric, and electrostatic complementarily, molecular docking techniques aim to fit a ligand into the binding site of a target protein and consequently predict its binding affinities. [37,38].

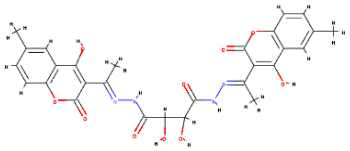
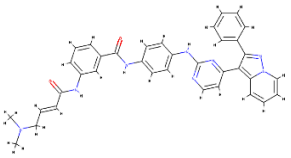
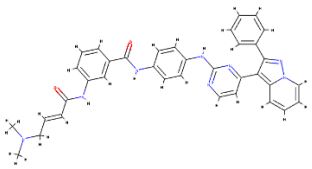
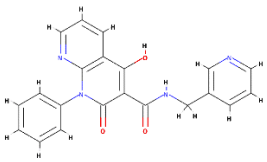
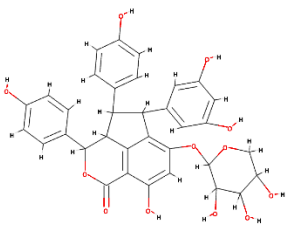
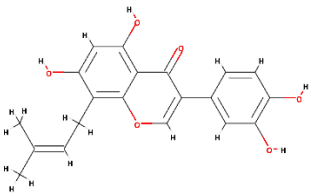
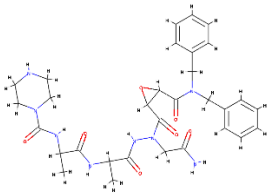
The efficiency of pharmaceutical drugs and the affinity of biomolecular interactions are frequently evaluated via binding free energy. Hence, the computed binding free energy of phytochemicals determined that the compound with the highest binding affinity towards insulin receptors was assumed to have the lowest binding free energy [39]. According to the results in **Table 2**, the interaction between insulin receptor and 2,3-

dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino]butanediamide had the highest binding affinity, followed by 3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide, (S)-Fmoc-2-amino-5-(trityl-carbamoyl)pentanoic acid, 4-hydroxy-2-oxo-1-phenyl-N-(pyridin-3-ylmethyl)-1,8-naphthyridine-3-carboxamide, (2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one, Gancaonin L and N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide with the binding free energy of -10.0, -9.9, -8.8, -8.6, -8.5, -8.5 and -8.5 kcal/mol respectively.

PyMol v2.5.4 Software was used to visualize and analyze the interaction between the 7 phytochemicals with the lowest binding free energy and insulin receptor. Ganugapati *et al.* [22] discovered that the active sites of insulin receptor were SER 1006, LYS 1030, GLU 1077, ASP 1083, ASN 1137, ASP 1150, and MET 1079. Their research found out that banana flower which contain flavonoid hesperetin had the lowest binding free energy with insulin receptor which was -8.4 kcal/mol that interact with amino acid residues ASN 1137, ASP 1150, LYS 1030 and SER 1006 by hydrogen bonds. Hesperetin triacetate had the second lowest binding free energy which was -8.2 kcal/mol that interacts with SER 1006, ASN 1137 and LYS 1030. The other banana major flavonoids compound which they identified to have interaction with the insulin receptor were cyanidin, pelargonidin, peonidin, malvidin, naringenin, naringenin pelargonidin and naringenin flavonone. According to their finding, they concluded that banana flower contains flavonoids that were able to activate the insulin receptor tyrosine kinase activity as a potential diabetes mellitus treatment.

Apart from the previously discovered active sites by Ganugapati *et al*. [22], amino acid residues such as ASP1132, ARG1136, LEU1170, GLU1047 and GLU1043 was first found to have interaction with insulin receptor in this research and can be suggested that these amino acid residues had the potential to be the new active sites for insulin receptor. The molecular interaction of the 7 phytochemicals with the lowest binding energy against insulin receptor and the amino acid residues that they were interact with were shown in **Fig. 1**. According to **Fig. 1**, the amino acid residues of phytochemicals also formed hydrogen bonds with the insulin receptor. It was found that phytochemicals such as 3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide, (S)-Fmoc-2-amino-5-(trityl-carbamoyl)pentanoic acid, 4-hydroxy-2-oxo-1-phenyl-N-(pyridin-3-ylmethyl)-1,8-naphthyridine-3-carboxamide, (2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one and N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide were having interaction with the first identified amino acid residues ASP1132, ARG1136, LEU1170, GLU1047 and GLU1043. Hence, the results obtained in this research suggested that the phytochemicals in **Table 2** have the ability to exhibit insulin-mimetic activity by binding to the insulin active sites. Thus, this provides an opportunity for the discovery of new antidiabetic drugs.

Table 2. Ligands with the 7 lowest binding free energy.

Ligand	2D Structures	Binding free energy (kcal/mol)
2,3-dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino]butanediamide		-10.0
3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide		-9.9
Fmoc-L-Hgn(Trt)-OH ((S)-Fmoc-2-amino-5-(trityl-carbamoyl)pentanoic acid)		-8.8
4-hydroxy-2-oxo-1-phenyl-N-(pyridin-3-ylmethyl)-1,8-naphthyridine-3-carboxamide		-8.6
(2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one		-8.5
Gancaonin L (5,7,3',4'-Tetrahydroxy-8-prenylisoflavone)		-8.5
N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide		-8.5

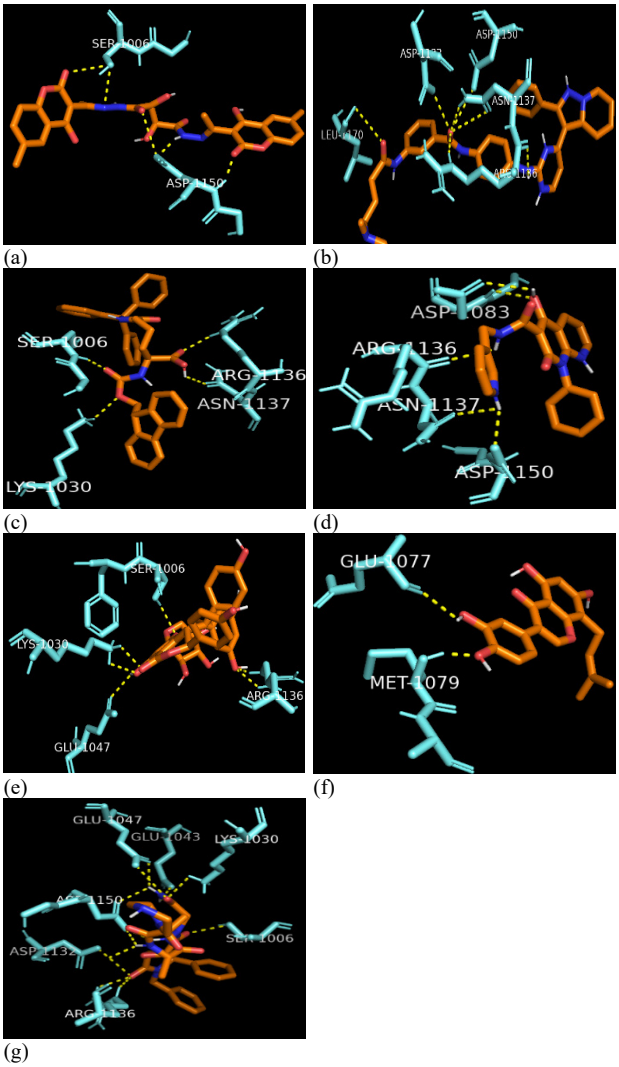


Fig. 1. Molecular interactions of 7 phytochemicals with lowest binding affinity against insulin receptor. **(a)** 2,3-dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino]butanediamide **(b)** 3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide **(c)** Fmoc-L-Hgn(Trt)-OH ((S)-Fmoc-2-amino-5-(trityl-carbamoyl)pentanoic acid) **(d)** 1,8-Naphthyridine-3-carboxamide, 1,2-dihydro-4-hydroxy-2-oxo-1-phenyl-N-(3-pyridinylmethyl)- **(e)** (2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one **(f)** Gancaonin L (5,7,3',4'-Tetrahydroxy-8-prenylisoflavone) **(g)** N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide.

CONCLUSION

In conclusion, several compounds that were detected in the methanol extract of *F. deltoidea such as* asperphenamate, biemamide A, dibutyl phthalate and tofogliflozin found to exhibit pharmacological properties including antitumor and antidiabetic properties. Besides, in silico molecular docking analysis found that 2,3-dihydroxy-N,N'-bis[(E)-1-(4-hydroxy-6-methyl-2-oxochromen-3-yl)ethylideneamino]butanediamide, 3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide, Fmoc-L-Hgn(Trt)-OH, 4-hydroxy-2-oxo-1-phenyl-N-(pyridin-3-ylmethyl)-1,8-naphthyridine-3-carboxamide, (2S,3R,4S,5S)-2-(3,5-dihydroxyphenyl)-9-hydroxy-3,5-bis(4-hydroxyphenyl)-11-[(2R,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-6-oxatricyclo[6.3.1.04,12]dodeca-1(11),8(12),9-trien-7-one, Gancaonin L and N-[(2R)-1-[[(2S)-1-[2-(2-amino-2-oxoethyl)-2-[(2S,3S)-3-(dibenzylcarbamoyl)oxirane-2-carbonyl]hydrazinyl]-1-oxopropan-2-yl]amino]-1-oxopropan-2-yl]piperazine-1-carboxamide were the potential phytochemicals that exhibit insulin-mimetic activities that responsible for the anti-diabetic properties. Moreover, the first identified amino acid residues that interact with insulin receptors including ASP1132, ARG1136, LEU1170, GLU1047 and GLU1043 can be suggested as the new active site for insulin receptors.

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