1. Introduction

The number of people with T2DM is increasing globally. The use of synthetic antidiabetic drugs has side effects accompanying the current epidemiological burden of T2DM. Alternatively, numerous herbal materials can decrease blood sugar levels by inhibiting diabetes-related multi-proteins. However, the putative antidiabetic properties of herbal materials remain scattered [1-2].

*Peperomia* is the second largest species of the Piperaceae family. Nevertheless, only a few species of *Peperomia* have been investigated for their bioactive compounds. *Peperomia blanda* (Fig. 1) is a native shrub to tropical sites like Asia, Africa, Australasia, and Polynesia. Its habitat is on damp rocks, crevices, and...
steep stream banks, creeping and crawling around river ledges and tree trunks. As an arid-land shrub, it is drought and wind-tolerant. It grows as a perennial, succulent herb to 30-60 cm in height. Peperomia species have been recorded to have numerous uses as traditional medicine to treat skin diseases, burns, eye infections, asthma and antibiotics. In addition, Peperomia blanda has various uses in traditional remedies. It has been reported to be used for treating cancer, inflammation, and infection. As far as our knowledge, no report is available for its antidiabetic potential. But reports on its bioactive compounds, particularly C-glycosylated flavone of Peperomia blanda indicate its antidiabetic potential. Five C-glycosyl flavones (shown in Fig. 2), vitexin, isovitexin, schaftoside, vicenin 2, and vicenin 3, have been detected from polar or aqueous extracts of Peperomia blanda leaves.

Figure 2. Chemical structure of vitexin, isovitexin, schaftoside, vicenin 2, and vicenin 3 (PubChem)

They possess significant bioactivities, including antimicrobial activity (isoorientin, vitexin), hepatoprotective activity (isoorientin), and antioxidant activity (isovitexin). In addition, they may become new and more efficacious antidiabetic herbal drugs and nutraceuticals to lessen the global burden of diabetes. Bioactive compounds such as C-glycosyl flavones may potentially inhibit the activity of multi-diabetic proteins. These proteins may become targets of C-glycosyl flavone. This study selects five diabetic-associated proteins: α-amylase, α-glucosidase, GLUT1, DPP4, and PTP1B. T2DM is associated with multiple genes (polygenic), but modern pharmacological interventions mainly apply a monogenic approach, "single drug for single target." Applying this principle requires a high drug dose that increases the probability of adverse events. Therefore, as an alternative principle, an antidiabetic formula generally found in traditional pharmacological interventions can target multiple diabetic proteins and possesses antidiabetic activity at a low dose with minimal side effects. Polygenic diseases, like T2DM, can be targeted via multiple pathways by herbal formula. Lead bioactive compounds in antidiabetic herbal formulas can modulate multiple proteins involved in T2DM. Segregating phytoconstituents from the herbal material can target multiple proteins for better therapeutic outcomes.

The present study aimed to compile in silico studies between several ligands of C-glucosyl flavone from Peperomia blanda with multiple proteins associated with T2DM. Thus, this study aimed to predict an in-silico molecular docking approach to screen and support the antidiabetic property of Peperomia blanda. Their binding affinities of C-glucosyl flavone ligands with target proteins associated with T2DM were evaluated, as well as the hydrogen bond residues and drug-likeness scores.

1.1 Short overview of multi-diabetic-related proteins
1.1.1 α-amylase and α-glucosidase
α-Amylase and α-glucosidase are responsible for the breakdown of polysaccharides into glucose for further absorption. Their inhibition can help to control blood glucose levels. In this case, acarbose is often used for T2DM therapy to inhibit these enzymes.

1.1.2 Glucose transporter 1 (GLUT1)
Glucose enters or leaves cells principally with the assistance of two membrane-integrated transporters belonging either to the facilitative glucose transporters (GLUTs) or to the sodium-glucose cotransporters (SGLTs). Several glucose transporters, such as GLUT1, GLUT2, GLUT3, GLUT4,
and GLUT5, mediate the facilitative uptake of glucose into cells. GLUT1 is a Na and insulin-independent, unipor facultative transporter. It has an essential role in several cells, such as in cells in the blood, blood-brain barrier, cornea, intestinal glucose absorption and transport from epithelial cells into the bloodstream [9], in glomeruli of the kidney [10-11], in heart muscle cells and muscle tissue [12], and in the retina [13]. Human beta cells of the pancreas express mainly GLUT1. GLUT1 overexpression is associated with obesity and non-insulin-dependent diabetes, although the correlation is still unknown [14]. Therefore, GLUT1 is considered a promising therapeutic target for T2DM [13].

GLUT1 inhibitors, like metformin, forskolin, and genistein, may reduce glucose level transport [12]. Forskolin or genistein can bind GLUT1, inhibit glucose transport, and significantly reduce retinal glucose. In contrast, brain glucose levels are not increased in people with diabetes or reduced by forskolin [13]. Particular natural products can function as GLUT1 inhibitors, like rubusoside and curcumin. In silico analysis of rubusoside pinpoint a tryptophan residue in GLUT1 [15]. Curcumin has an immediate inhibitory effect on basal glucose uptake. It binds directly to GLUT1 at a site that overlaps with the cytochalasin B binding site [16].

1.1.3 Protein tyrosine phosphatase 1B (PTP1B)

Insulin resistance caused by the overexpression of PTP1B is one of the leading causes of T2DM. PTP1B has fundamental regulatory roles and is a critical negative and positive regulator of several signaling cascades. PTP1B's direct regulation of the insulin and the leptin receptors makes it an ideal therapeutic target for T2DM [17].

The role of PTP1B in the pathogenesis of T2DM is related to insulin resistance, which is caused mainly by impairment in the insulin receptor (IR) signal transduction pathway. PTP1B is one of the main negative regulators of the IR signaling pathway, which is broadly expressed in various cells and tissues. PTP1B decreases the phosphorylation of the IR, resulting in insulin resistance in various tissues. Recently, targeting PTP1B using PTP1B inhibitors has been considered an attractive target for treating T2DM. PTP1B inhibitors improve the sensitivity of the insulin receptor and can cure insulin resistance-related diseases [18-19].

1.1.4 Dipeptidyl Peptidase 4 (DPP4)

The incretin system is a potential source of therapies for T2DM. Administered glucose can stimulate a substantial release of insulin. Two incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) trigger the gastrointestinal tract to release insulin whenever glucose is consumed. These incretin hormones bind to receptors on beta cells of the pancreas, thereby stimulating insulin secretion in response to glucose absorption. Nevertheless, their secretion is decreased in patients with T2DM. Increasing GLP-1 levels decreases hyperglycaemia. GIP is not active in patients with T2DM. In addition to glucose-dependent insulin secretion, GLP-1 regulates glucose homeostasis via inhibition of glucagon secretion (thereby reducing liver glucose output) and gastric emptying [20].

GLP-1 is an essential molecular target in light of T2DM. In T2DM, resolving insulin resistance and impairing insulin secretion is crucial. Incretin is a collective peptide hormone released from the digestive tract with glucose or food intake and acts on the β cells of the pancreas to stimulate insulin release. GIP and GLP-1 are two incretins known so far. Because the sensitivity of GLP-1 in the β cells of the pancreas is not reduced in T2DM patients, it is considered for the prevention and treatment of T2DM. GLP-1 enhances insulin secretion depending on the blood glucose concentration. GLP-1 is a potent endogenous therapeutic agent for the treatment of T2DM and has been proven to protect pancreatic β-cells from glucotoxicity; however, its mode of action is not clearly understood. Probably, GLP-1 may protect β-cells from glucotoxicity by promoting autophagy through the modulation of 5’AMP-activated protein kinase (AMPK) [21].

GIP and GLP1 are potential in glucose-induced insulin and suppression of glucagon secretion. However, they are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4). DPP4 destroys the incretins that are responsible for improving pancreatic beta cell function. DPP-4 inhibition is essential in treating T2DM [20, 22]. Therefore, the dipeptidyl peptidase-4 inhibitors, exenatide, sitagliptin, vildagliptin, and liraglutide, are important
1.2. C-glycosyl flavone of Peperomia blanda: vitexin, isovitexin, schaftoside, vicenin 2 and vicenin 3

The polar extracts of Peperomia blanda are rich in two C-glycosyl flavones, namely vitexin, and vicenin 2 (Fig. 2). They exist in the methanol extract from aerial parts of the P. blanda. Vitexin and vicenin 2 have numerous biological activities, like antioxidant and antidiabetic activity and antiosteoporosis [26]. Moreover, vicenin 2 is a potential agent for treating bone-related disorders [27] and has a beneficial effect on neurological and cognitive function and in treating liver diseases. It can protect LPS-induced liver damage by inhibiting the TLR-mediated inflammatory pathway [28].

*In vitro* and *in vivo* studies have been performed with vitexin and isovitexin derivatives relating to T2DM. Vitexin and isovitexin have multitargeted mechanistic actions in controlling T2DM. Vitexin and isovitexin can target diverse pathophysiological and metabolic pathways and molecular drug points involved in the clinical manifestations of T2DM [29]. Vitexin can be given as oral medicament to reduce the postprandial blood glucose level. Oral administration of vitexin can decrease the postprandial blood glucose level. Moreover, vitexin does not show toxicity. Its capacity is probably due to the ability of vitexin to inhibit *in vivo* α-glucosidase [26].

Schaftoside is found also in Peperomia blanda. However, it is not yet used in traditional medicine for treating T2DM. The α-glucosidase (AGI) activities were lower than the antidiabetic drug acarbose. Schaftoside is responsible for the AGI activity [30].

As vitexin, vicenin-2 is also known as an antidiabetic flavonoid. Even though the antidiabetic-related experiment using vicenin 2 from *Peperomia blanda* is not yet investigated, even vicenin 2 is found in Peperomia blanda. The presence of vicenin 2 indicates that *Peperomia blanda* might have antidiabetic potential. Its antidiabetic potential is due to the inhibitory activity against α-glucosidase, advanced glycation end products (AGE) formation, PTP1B, and rat lens aldose reductase (RLAR). Therefore, vicenin-2-rich extract might be valuable in treating T2DM and its associated complications [31]. In addition, topical application of vicenin 2 may improve diabetic wound healing due to its antibacterial activity [32-33].

### 2. Materials and methods

#### 2.1 Method of Docking studies

Five C-glycosyl flavones were retrieved from the published literature [26-28]. Their sdf 3D format was obtained from the PubChem database along with its canonical SMILES, molecular weight, and molecular formula. All ligands were converted to pdbqt format using Pyrx 0.8.

Five different target diabetic proteins were retrieved from the RCSB Protein Data Bank, namely α-amylase (PDB ID: 1B2Y), α-glucosidase (PDB ID: 3WY1), GLUT1 (PDB ID: 5EQ1), PTP1B (PDB ID2: NT7), and DPP4 (PDB ID: 4J3J). Protein preparation was performed using BIOVIA Discovery Studio 2021 Client 21.1. Water molecules and native ligands on the protein were removed, and then hydrogen was added. The prepared protein was then converted to pdbqt format using Pyrx 0.8. The precise placement of the active site during the docking process in Pyrx 0.8 was enabled by establishing the coordinates (X, Y, and Z) of the active site using Discovery Studio. The active site position was set as follows: α-amylase (x: 18.9; y: 5.7; z:47), α-glucosidase (x: -3.6; y: -15.5; z: 21.3), GLUT1 (x: -39.4; y: 11.3; z:12.1), PTP1B (x: 48.1; y: 10.1; z:2.9), DPP4 (x: 5.3; y: 16.3 ; z:-23). Subsequently, docking was performed using Pyrx 0.8. Ligand-protein interactions were analyzed using BIOVIA Discovery Studio 2021 Client 21.1.

#### 2.2 Drug-likeness scoring

MolSoft and Swiss-ADME were used to evaluate the drug-likeness scoring of the five C-glycosyl flavones.

### 3. Results

T2DM is a polygenic metabolic disorder that involves many functional proteins. Many antidiabetic drugs are inhibitors, like α-amylase, α-glucosidase inhibitors, DPP4 inhibitors, etc. *Peperomia blanda*, as a traditional medicine plant, possesses C-glycosyl flavones that prove their antidiabetic potential by inhibiting multi-diabetic-associated proteins. The bioinformatics tools are essential in the current pharmacology for the screening the bioactive compounds from traditional plants. Molecular docking is an *in-silico* approach for finding the lead hit, which gives information about the binding affinity of the bioactive compounds to a particular receptor. Binding affinity is inversely proportional to the
Table 1. Binding affinity (kcal/mol) of ligands from *Peperomia blanda* with targets multi proteins associated with T2DM.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Vitexin</th>
<th>Isovitexin</th>
<th>Scaftoside</th>
<th>Vitexin-2</th>
<th>Vitexin-3</th>
<th>Controls</th>
<th>Quercetin (this study)</th>
<th>Quercetin [8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase</td>
<td>-6.8</td>
<td>-8.9</td>
<td>-9.5</td>
<td>-8.7</td>
<td>-8.8</td>
<td>-8.3 (acarbose)</td>
<td>-8.0</td>
<td>-7.8</td>
</tr>
<tr>
<td>GLUT-1</td>
<td>-10.3</td>
<td>-10.1</td>
<td>-11.0</td>
<td>-10.4</td>
<td>-11.0</td>
<td>-4.7 (metformin)</td>
<td>-8.7</td>
<td>-7.2</td>
</tr>
<tr>
<td>DPP4</td>
<td>-8.2</td>
<td>-8.3</td>
<td>-8.5</td>
<td>-8.3</td>
<td>-8.6</td>
<td>-6.8 (Saxagliptin)</td>
<td>-7.8</td>
<td>-8.2</td>
</tr>
<tr>
<td>PTP1B</td>
<td>-7.2</td>
<td>-7.8</td>
<td>-7.2</td>
<td>-7.3</td>
<td>-7.3</td>
<td>-0.7 (Sitagliptin)</td>
<td>-8.2</td>
<td>-8.7</td>
</tr>
</tbody>
</table>

binding energy of the phytoconstituents with targeted protein. Our study outlined the possible binding energies for five C-glucosyl flavones from *P. blanda* with five different targets (α-amylase, α-glucosidase, GLUT1, PTP1B, DPP4), which can be targeted for the management of diabetes.

Molecular docking is an *in-silico* approach for finding the lead hit, which gives information about the binding affinity of the bioactive compounds to a particular receptor. Binding affinity is inversely proportional to the binding energy of the phytoconstituents with targeted protein. Our study outlined the possible binding energies for five different C-glucosyl flavones from *Peperomia blanda* with five different targets (α-amylase, α-glucosidase, GLUT1, PTP1B, and DPP4), which can be targeted for the management of diabetes. In our study, C-glucosyl flavone from *P. blanda* was found to bind with these proteins with binding energies comparable to or more vital than controls (Table 1). All C-glucosyl flavones tested possessed the highest binding affinity towards GLUT1. All C-glucosyl flavones tested involved hydrogen and Pi-Stalked, and Pi-alkyl bond residues for binding with the five proteins: α-amylase, α-glucosidase, GLUT1, DPP4, and PTP1B, as represented in Table 2. As a representative model, a picture of the interactions between vitexin and GLUT1 is presented in Fig.3.

Oral bioavailability for the absorption of a drugable molecule affects the pharmacokinetics and pharmacodynamics properties associated with biological spectra. Hence, in the present study, we attempt to investigate the C-glucosyl flavones for their oral biostability using the “Rule of Five” model using MolSoft as explained by Lipinski in which vitexin scored the highest drug-likeness score, i.e., 0.60 with the molecular weight of 304.25 Da, seven hydrogen bond acceptors, five hydrogen bond donors, and 0.71 molLogP contributing to the antidiabetic effect. Similarly, isovitexin scored 0.59. Both scored slightly higher than quercetin as control (0.52). The other C-glucosyl flavones, such as schaftoside, vicenin 2, and vicenin 3 scored 0.32, 0.20, and 0.32, respectively, as depicted in Table 3.

4. Discussion
Flavones belong to the group of flavonoids, present in
the form of aglycone glycosylated with sugar moieties. Because of their higher stability and reactivity, Flavone-C-glycosides have better therapeutic properties than O-glycosylated flavones. The therapeutic potential of flavones and their C-glycosylated flavones is specifically on antidiabetic properties. The discussion focuses on the relationship between the physicochemical properties of C-glycosylated flavones and the biochemical pathway of diabetic syndrome [7].

All ligands tested in this study significantly inhibit five proteins, α-amylase, α-glucosidase, GLUT1, DPP4, and PPT1B. The inhibitory potential lies in their bonding energy and interactions with the amino acid residues.

Vitexin and isovitexin are available in diverse bioresources. They have been studied to explore their pharmacological relevance in T2DM. Data collected hint that vitexin and isovitexin work by targeting diverse pathophysiological and metabolic pathways and molecular drug points involved in the clinical manifestations of T2DM. They are expected to
Table 4. Antidiabetic plants with C-glycosyl flavones

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Part</th>
<th>C-glycosyl flavone</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajuga iva</td>
<td>Herb ivy</td>
<td>Aerial part</td>
<td>Vicenin 2</td>
<td>[57]</td>
</tr>
<tr>
<td>Artemisia capillaris</td>
<td>Wormwood-fragrant wormwood</td>
<td>Whole plant</td>
<td>Vicenin 2</td>
<td>[31]</td>
</tr>
<tr>
<td>Artemisia campestris</td>
<td>Field wormwood</td>
<td>Aerial part</td>
<td>Vicenin 2</td>
<td>[58]</td>
</tr>
<tr>
<td>Artemisia herba-alba</td>
<td>Desert or white wormwood</td>
<td>Aerial part</td>
<td>Vicenin 2</td>
<td>[57]</td>
</tr>
<tr>
<td>Aspalathus linearis</td>
<td>Rooibos</td>
<td>Leave</td>
<td>Vitexin, isovitexin</td>
<td>[59]</td>
</tr>
<tr>
<td>Beta vulgaris</td>
<td>Beet</td>
<td>Beetroot</td>
<td>Vitexin, isovitexin</td>
<td>[60]</td>
</tr>
<tr>
<td>Bombax ceiba</td>
<td>Red silk-cotton tree</td>
<td>Leave</td>
<td>Vitexin, isovitexin</td>
<td>[61]</td>
</tr>
<tr>
<td>Clinacanthus nutans</td>
<td>Dandang gendis</td>
<td>Leave</td>
<td>Vitexin, isovitexin,schaftoside</td>
<td>[62-63]</td>
</tr>
<tr>
<td>Costus spiralis</td>
<td>Spiral ginger</td>
<td>Leave</td>
<td>schaftoside</td>
<td>[30]</td>
</tr>
<tr>
<td>Cyclopia subternata</td>
<td>Valley tea</td>
<td>Leaf</td>
<td>Vicenin 2</td>
<td>[64-65]</td>
</tr>
<tr>
<td>Ficus deltoidea</td>
<td>Golden Mistletoe Fig</td>
<td>Leaf</td>
<td>Vitexin, isovitexin</td>
<td>[35]</td>
</tr>
<tr>
<td>Gmelina philippensis</td>
<td>Parrot’s Beak</td>
<td>Aerial part</td>
<td>Vicenin 2</td>
<td>[34]</td>
</tr>
<tr>
<td>Gymnocarpos decandrus</td>
<td>Nachtfrucht</td>
<td>Flowering aerial part</td>
<td>Vitexin, isovitexin</td>
<td>[36]</td>
</tr>
<tr>
<td>Hylocereus polyrhizus</td>
<td>Dragon fruit or red pitaya</td>
<td>Fruit</td>
<td>Vicenin 2</td>
<td>[40]</td>
</tr>
<tr>
<td>Ocimum gratissimum</td>
<td>Basil</td>
<td>Leave</td>
<td>Vicenin 2</td>
<td>[47]</td>
</tr>
<tr>
<td>Peperomia blanda</td>
<td>The arid-plant peperomia</td>
<td>Leave</td>
<td>Vitexin, isovitexin,schaftoside, vicenin2, vicenin 3</td>
<td>This study</td>
</tr>
<tr>
<td>Peperomia pellucida</td>
<td>Pepper elder</td>
<td>Leave</td>
<td>Vitexin, isovitexin</td>
<td>[66]</td>
</tr>
<tr>
<td>Pereskia bleo</td>
<td>Rose cactus</td>
<td>Leave</td>
<td>Vitexin, isovitexin</td>
<td>[67]</td>
</tr>
<tr>
<td>Prosopis spp.</td>
<td>Aerial part</td>
<td>Schaftoside, vicenin 2</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>Saccharum officinarum</td>
<td>Sugarcane</td>
<td>Leave</td>
<td>Schaftoside</td>
<td>[41]</td>
</tr>
<tr>
<td>Sauromatum guttatum</td>
<td>Voodoo Lilly or Snake Plant</td>
<td>Tuberc</td>
<td>Schaftoside, vicenin 2</td>
<td>[38]</td>
</tr>
<tr>
<td>Trigonella foenum-graecum</td>
<td>Fenugreek</td>
<td>Seed</td>
<td>Vitexin, isovitexin,schaftoside, vicenin 2</td>
<td>[69-71]</td>
</tr>
<tr>
<td>Vigna radiata</td>
<td>Mung bean</td>
<td>Seed coat</td>
<td>Vitexin, isovitexin</td>
<td>[45,72]</td>
</tr>
</tbody>
</table>

provide a deeper understanding of its actions and serve as a catapult for clinical trials and application research [29].

Several antidiabetic plants are rich in C-glycosyl flavone, particularly vitexin, isovitexin, Schaftoside, vicenin2, and vicenin 3 (Table 4). They have been proven for their potential to inhibit α-amylase and α-glucosidase. Several crude extracts from Artemisia capillaris, Costus spiralis, Ficus deltoidea, Gmelina philippensis, Gymnocarpos decandrus, Hylocereus polyrhizus Sauromatum guttatum, and Vigna radiata have inhibitory activities against α-amylase and α-glucosidase activities [30-31, 34-40].

All compounds have strong binding affinity to α-amylase compared to acarbose as control. Among all compounds, Schaftoside has the strongest binding affinity (-9.1 kcal/mol). Isovitexin has a higher binding affinity (-8.8 kcal/mol) towards α-amylase than vitexin (-8.3 kcal/mol) [35]. Schaftoside, Isovitexin and Vitexin are potent and stable against the α-amylase enzyme. The molecular dynamics findings showed that the vitexin-α-amylase complex is more stable during the simulation of 20 ns than the isovitexin-α-amylase complex. Thus, Vitexin could be developed as a therapeutic drug for treating diabetes [35]. The residues Asp197, Glu233, and Asp300 are crucial in inhibiting α-amylase. This finding followed by Kan et al. which multiple hydrogen bonds and electrostatic interactions exist between C-glycosyl flavone and α-amylase. C glycosyl flavone strongly binds with the
catalytic triad (Asp197, Glu233, and Asp300) of α-
amylose [41]. The results of this study are in
accordance with the opinion of Kawaga et al. who
explained that the α-Amylase has two aspartic
residues and one glutamic acid residue as the catalytic
residues [42]. The docked view of our ligands showed
varied binding residues on Glu and Asp. The number
of amino acids does not follow the previous reports
that stated the Glu276 and Asp214 as catalytic triad
[43], or Asp412, Glu304, Glu276, and Asp349 residues
which are catalytic residues [44]. This difference is
probably due to the different types of the enzyme.
All tested ligands showed predictive inhibitory
activities against the α-glucosidase enzyme compared
to control (acarbose). Schaftoside has the highest
binding affinity (-9.5 kcal/mol) (Table 1). Asp333,
Glu377, Val380, Glu396 residues are critical roles in
binding α-glucosidase. All ligands exhibited good
binding interactions within the active site of the
enzyme. Nevertheless, our findings are not in
accordance with the opinion of Halim et al. who
reported that the docked view was at Glu276 and
Asp214 residues [43].
All compounds have strong binding affinity with
GLUT-1, with their strength significantly greater
compared to metformin (Table 1). However, it
appears that Schaftoside has the highest binding
affinity at -11 kcal/mol. C-glycosyl flavone, like vitexin,
can protect the cells against high glucose
toxicity. Mungbean seed coat extract can increase
glucose uptake [45] and improve insulin sensitivity
[39]. Vitexin can improve glucose transporter-2
(GLUT-2), and glucose-stimulated insulin secretion
[46-47] C-glycosyl flavone, like schaftoside and vicenin 2, can also inhibit advanced glycation end
products (AGE) [31, 40]. Our ligands showed binding
with residues Thr30, Thr137, Gln282, Gln283, and
Asn411 playing critical roles in ligand binding to
GLUT [48-49].
In this study, C glycosyl flavone tested showed
significant DPP4 inhibitory effects. The results show
that all the compounds have higher binding affinity
compared to saxagliptin as control. Vicenin-3 has the
highest binding score (-8.6 kcal/mol) (Table 1). The
Arg125, Asp545, Asn710, Asp739, and Gly741
residues may play a crucial role in inhibiting DPP4 by
schaftoside, vicenin 2, and vicenin 3. According to
Kan et al., the residues Arg125 and Tyr662 of DPP4
may play crucial roles in inhibiting the activity of
DPP4 [41]. Residues that are important in the catalytic
activity of DPP4 are residues Ser630, Asp708 and
His740 (the catalytic triad), Tyr547 in the hydrolase
domain, and substrate binding sites with saline
bridging residues, such as Glu205, Glu206 and Tyr662
that are located in the β-propeller domain [50-51].
Vitexin, isovitexin, schaftoside, and vicenin 2 have
been proven for their potent PTP1B inhibitory activity
[31, 52], an enzyme overexpressed in T2DM [53]. The
lack of clinically approved PTP1B inhibitors has
continued to prompt research in plant-derived
therapeutics, possibly due to their relatively lesser
toxicity profiles. The five C-glycosyl flavones docked
against the enzyme have an affinity with a binding
score from -7.2 to -7.8 kcal/mol. These results follow
the previous study by Rampadarath et al. which
stated that reported a binding score of -7.3 kcal/mol
for the interaction between vitexin and PTP1B (Table
1) [54]. The active site of PTP1B is Cysteine215 with
a surrounding catalytic loop and an allosteric site
surrounded by α3 helix, α6 helix, and α7 helix. An
allosteric transition in PTP1B accompanying its
catalysis, which is situated about 20Å away from the
catalytic domain, including active site Cys215 and
catalytic loop consisting of His214, Ser216, Ala217,
Gly218, Ile219, Gly220, and Arg221 [55]. From the
description above, it is evident that the ligands are
bound to the catalytic site of the protein. This indicates
that these ligands are capable of inhibiting the
protein’s function by binding to its active site.
The Lipinski rule outlines certain thresholds for
molecular weight (MW), the number of hydrogen bond
acceptors and donors (HBA and HBD), and the
water/octanol partition coefficient (log P). It was
determined that compounds not meeting two or more
of these criteria are probably candidates for exclusion
from further development. Adequate drug absorption
and penetration within the body, as per Lipinski’s rule,
involve the following criteria: a maximum of 5 HBD,
a molecular weight not exceeding 500 Da, a log P
value no greater than 5, and a maximum of 10 HBA
(Lipinski, 1997).56 The highest drug likeness score is
found for vitexin and isovitexin. The other
compounds were scoreless due to their higher
molecular weight than 500 KD. Lipinski’s rule of five,
vitexin and isovitexin are the best drug-likeness rather than schaftoside, vicenin 2, and vicenin 3.

5. Conclusions
T2DM is a polygenic metabolic disorder that is associated with several proteins. *Peperomia blanda* is a non-toxic plant with multiple bioactivities that can target various pathogenic pathways and synergistically exert antidiabetic effects. Our study screened the antidiabetic potential of five C-glycosylated flavonoids towards five different diabetic proteins: α–amylase, α–glucosidase, GLUT1, DPP4, and PTP1B, using *in silico* molecular docking approach where C-glycosylated flavonoids, vitexin, isovitexin, schaftoside, vicenin 2, and vicenin 3 were found to possess higher affinity towards the five first protein targets. Thus, the C-glycosylated flavone-rich extract of *Peperomia blanda* has antidiabetic potential and can be used to investigate and develop novel antidiabetic preparations.

**Abbreviations**
DPP4: Dipeptidyl peptidase; GLUT1Glucose transporter 1; PTP1B: Protein tyrosine phosphatase 1-B; T2DM: type 2 diabetes mellitus;

**Authors’ contributions**

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All data will be made available on request according to the journal policy.

**Conflicts of interest**
The authors declare no conflict of interest.

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