



***In silico* ADME-T and molecular docking study of phytoconstituents from *Tithonia diversifolia* (Hemsl.) A. Gray on various targets of diabetic nephropathy**

[ADME-T *in silico* y estudio de acoplamiento molecular de fitoconstituyentes de *Tithonia diversifolia* (Hemsl.) A. Gray en varias dianas de nefropatía diabética]

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Abstract

Context: *Tithonia diversifolia* (Hemsl.) A. Gray has been known for treatment of diabetes mellitus, yet its mechanism as anti-diabetic has not been defined. There are various therapeutic targets for diabetes and its complication such as diabetic nephropathy.

Aims: To investigate the mechanism of phytoconstituents in *Tithonia diversifolia* to various targets of diabetic nephropathy and predict the pharmacokinetic profile such as absorption, distribution, metabolism, elimination, and toxicity (ADME-T).

Methods: Eighteen phytoconstituents in *Tithonia diversifolia* were analyzed for drug-likeness. The molecular docking of molecules was performed to protein targets, then its molecular interaction was determined. ADME-T properties were predicted using three different web servers.

Results: Drug-likeness analysis showed that all the phytoconstituents were within the range set by Lipinski's rule of five. This study showed that *Tithonia diversifolia* have potential as a candidate for diabetic nephropathy therapy agent with various mechanisms by inhibiting α -glucosidase, ACE, ALR, DPP-4, LMW-PTP, RAGE, SGLT2, SUR1, and an analog of PPAR- γ . 5-Caffeoylquinic acid, catechin, diversifolin, hispidulin, tagitin A, tagitin C, tagitin F, tithonine, and tirotundin were phytoconstituents with a high binding affinity to several proteins. β -gurjunene, tagitin A, tagitin C, tagitin F, and tirotundin were predicted to have a better ADME-T properties than other compounds. In summary, tagitin A, tagitin C, tagitin F, and tirotundin showed the high binding affinity to various diabetes-related proteins and have a good ADME-T profile.

Conclusions: This study suggests that *Tithonia diversifolia* constituents have potential properties as an anti-diabetic nephropathy agent, and further studies to analyze its potency are required.

Keywords: diabetes; insulin leaf; nephropathy; *Tithonia diversifolia*.

Resumen

Contexto: *Tithonia diversifolia* (Hemsl.) A. Gray ha sido conocida para el tratamiento de la diabetes mellitus, pero su mecanismo como antidiabético no ha sido definido. Existen varios objetivos terapéuticos para la diabetes y sus complicaciones, como la nefropatía diabética.

Objetivos: Investigar el mecanismo de los fitoconstituyentes en *Tithonia diversifolia* para varias dianas de la nefropatía diabética y predecir el perfil farmacocinético como la absorción, distribución, metabolismo, eliminación y toxicidad (ADME-T).

Métodos: Se analizaron 18 fitoconstituyentes en *Tithonia diversifolia* para determinar su similitud con las drogas. Se realizó el acoplamiento molecular de moléculas a dianas proteicas, luego se determinó su interacción molecular. Las propiedades de ADME-T se predijeron utilizando tres servidores web diferentes.

Resultados: El análisis de semejanza con las drogas mostró que todos los fitoconstituyentes estaban dentro del rango establecido por la regla de cinco de Lipinski. Este estudio mostró que *Tithonia diversifolia* tiene potencial como candidato para el tratamiento de la nefropatía diabética con varios mecanismos mediante la inhibición de la α -glucosidasa, ACE, ALR, DPP-4, LMW-PTP, RAGE, SGLT2, SUR1 y un análogo de PPAR- γ . Ácido 5-cafeoilquinico, catequina, diversifolina, hispidulina, tagitina A, tagitina C, tagitina F, titionina y tirotundina fueron fitoconstituyentes con una alta afinidad de unión a varias proteínas. Se predijo que β -gurjunene, tagitina A, tagitina C, tagitina F y tirotundina tenían mejores propiedades ADME-T que otros compuestos. En resumen, tagitina A, tagitina C, tagitina F y tirotundina mostraron una alta afinidad de unión a varias proteínas relacionadas con la diabetes y tienen un buen perfil ADME-T.

Conclusiones: Este estudio sugiere que los componentes de *Tithonia diversifolia* tienen propiedades potenciales como agente anti-nefropatía diabética, y se requieren más estudios para analizar su potencia.

Palabras Clave: diabetes; hoja de insulina; nefropatía; *Tithonia diversifolia*.

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INTRODUCTION

Diabetes mellitus is an emerging disease with a prevalence of people with diabetes in 2019 was 463.0 million adults aged 20-79 years (9.3%) worldwide and estimated that 79.4% live in low-and middle-income countries. This number is projected to increase by 700.2 million (10.9%) in 2045, respectively (IDF, 2019). Uncontrolled hyperglycemia leads to diabetic complications, such as diabetic nephropathy or diabetic kidney disease (Hussain et al., 2021). Diabetic nephropathy developed in 40% of type 2 diabetes mellitus patients and became the leading cause of chronic kidney disease (CKD) worldwide (Alicic et al., 2017). Prevention of type 2 diabetes mellitus is the most effective strategy to the delayed onset or preventing the progression of nephropathy.

Eight main mechanisms, described as the ominous octet, contribute to the pathophysiology of hyperglycemia in diabetes. These pathophysiologies comprise of decreased insulin secretion, increased glucagon secretion, decreased glucose uptake by muscle cells, decreased incretin effects, increased renal glucose reuptake, increased hepatic glucose production, increased lipolysis, and dysfunction of neurotransmitters that regulate the physiology of insulin secretion and sensitivity (DeFronzo, 2009). Inadequate control of blood sugar levels needed the use of multiple oral hypoglycemic agents and insulin therapy in diabetic patients with certain conditions (Kelwade et al., 2017). However, prolonged use of multiple hypoglycemic agents results in various inconvenient side effects, including increased body weight and hypoglycemia. A previous study revealed that people with impaired glucose tolerance (IGT) had lost over 80% of their β -cell function (DeFronzo, 2009). This, in turn, emphasize that the treatment of diabetes mellitus must be initiated from the prediabetes level to prevent further beta cell dysfunction (Ferrannini et al., 2015). It is stated by De Fronzo (2009) that the treatment for diabetes is not simply reducing A1C but also reversing its known pathogenic abnormality.

Tithonia diversifolia (Hemsl.) A. Gray, known as *insulin leaf* in Indonesia, has been widely used in many countries and is believed to treat diabetes and other inflammatory diseases. But the study regarding this fact is very limited and still has low-grade recommendations. In a previous study conducted by Miura et al. (2005), treatment with *Tithonia diversifolia* extract improved blood glucose tolerance in the KK-Ay mouse model. Based on Yazid et al. (2021) study, the most effective dose of *Tithonia diversifolia* leaf extracts as an anti-diabetic agent was 600 mg/kg. In this study, *Tithonia diversifolia* extract decreased the level

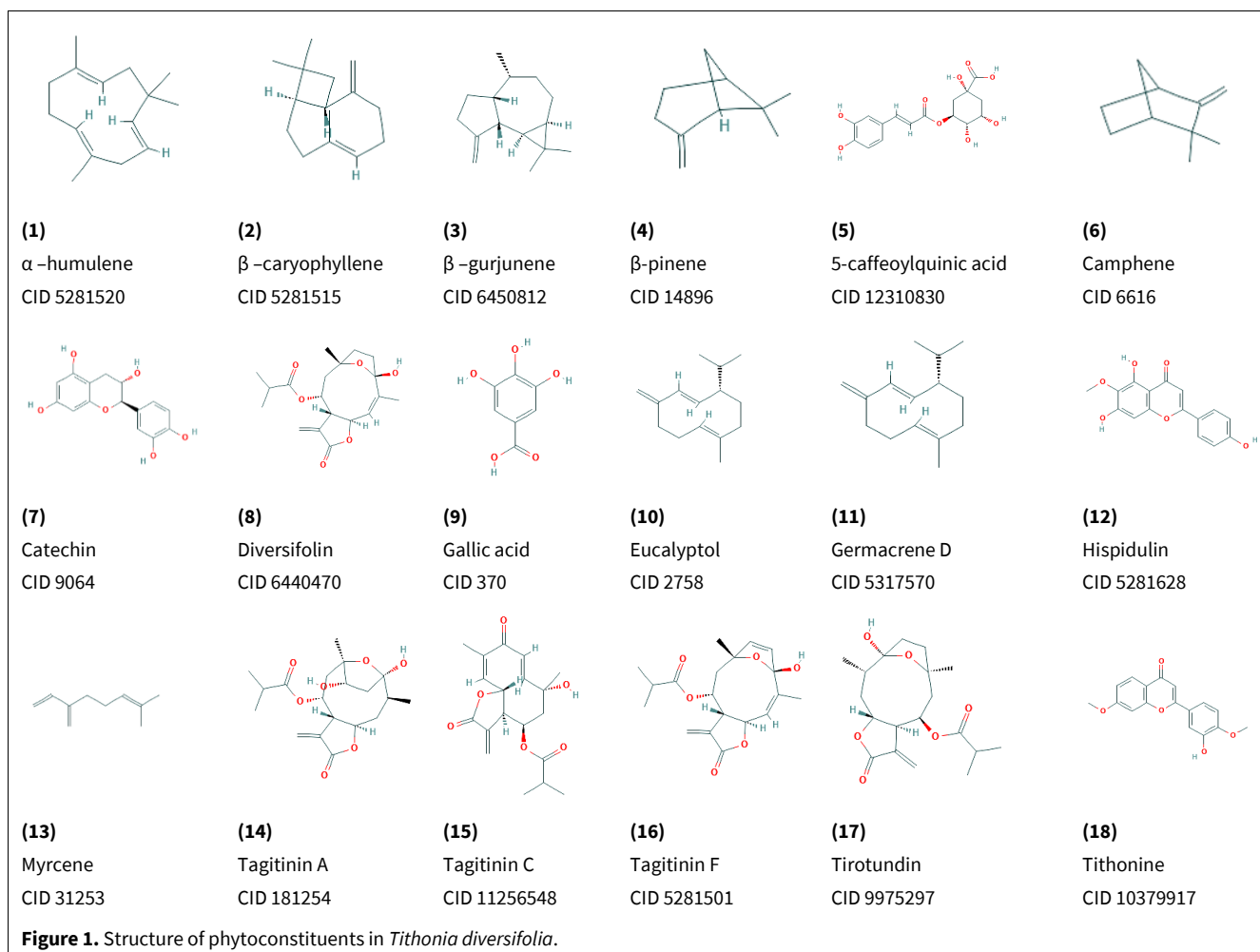
of fasting blood glucose and improved the histology structure of the pancreas in diabetic rats induced by alloxan. However, the molecular mechanism and protein target of *Tithonia diversifolia* in diabetes inhibition are remain unclear. The existing research indicating its mechanism to treat diabetes and its complication are limited.

Computational molecular docking of small molecules into the structures of macromolecular targets and score its potential complementarity to the binding sites are widely used in hit identification and lead optimization (Kitchen et al., 2004). Using this approach, this study is aimed to investigate the molecular interaction of *Tithonia diversifolia* constituents in various targets that play an important role in the pathogenesis of diabetic nephropathy, such as α -glucosidase, angiotensin-converting enzyme (ACE), aldose reductase (ALR), dipeptidyl peptidase-VI (DPP-4), low molecular weight protein tyrosine phosphatase (LMW-PTP), peroxisome proliferator-activated receptor- γ (PPAR- γ), receptor for advanced glycation end products (RAGE), sodium/glucose cotransporter 2 (SGLT-2), and sulfonylurea receptor 1 (SUR1). Furthermore, the pharmacokinetic properties and toxicity predictions of these phytoconstituents were performed in this study to support the development and discovery of drugs taken orally, where the achievement of therapeutic effects and therapeutic safety were influenced by absorption, distribution, metabolism, excretion, and toxicity profiles (ADME-T) related to the physicochemical properties of the drug when it enters the body.

MATERIAL AND METHODS

Ligand preparation and drug-likeness analysis

A total of 18 metabolite compounds of *Tithonia diversifolia* were selected for this docking analysis, including α -humulene, β -caryophyllene, β -gurjunene, β -pinene, 5-caffeoylquinic acid, camphene, catechin, diversifolin, eucalyptol, gallic acid, germacrene D, hispidulin, myrcene, tagitinin A, tagitinin C, tagitinin F, tirotundin, and tithonine. They were selected based on compounds identified from the previous study (Rüngeler et al., 1998; Adebayo et al., 2009; Lin, 2012; Zhao et al., 2012; Amanatie, 2015; Rinawati et al., 2019; Wang et al., 2020; Chunudom et al., 2020). The reference compounds used for this study were acarbose as an α -glucosidase inhibitor (Rahman et al., 2019), lisinopril as ACE inhibitor (Ramanjaneyulu et al., 2013), epalrestat as ALR inhibitor (Antony and Vijayan, 2015), sitagliptin as DPP-4 inhibitor (Meduru et al., 2016), ethyl-3,4-dephostatin as LMW-PTP inhibitor (Ren et al., 2017), tesaglitazar as PPAR- γ agonist

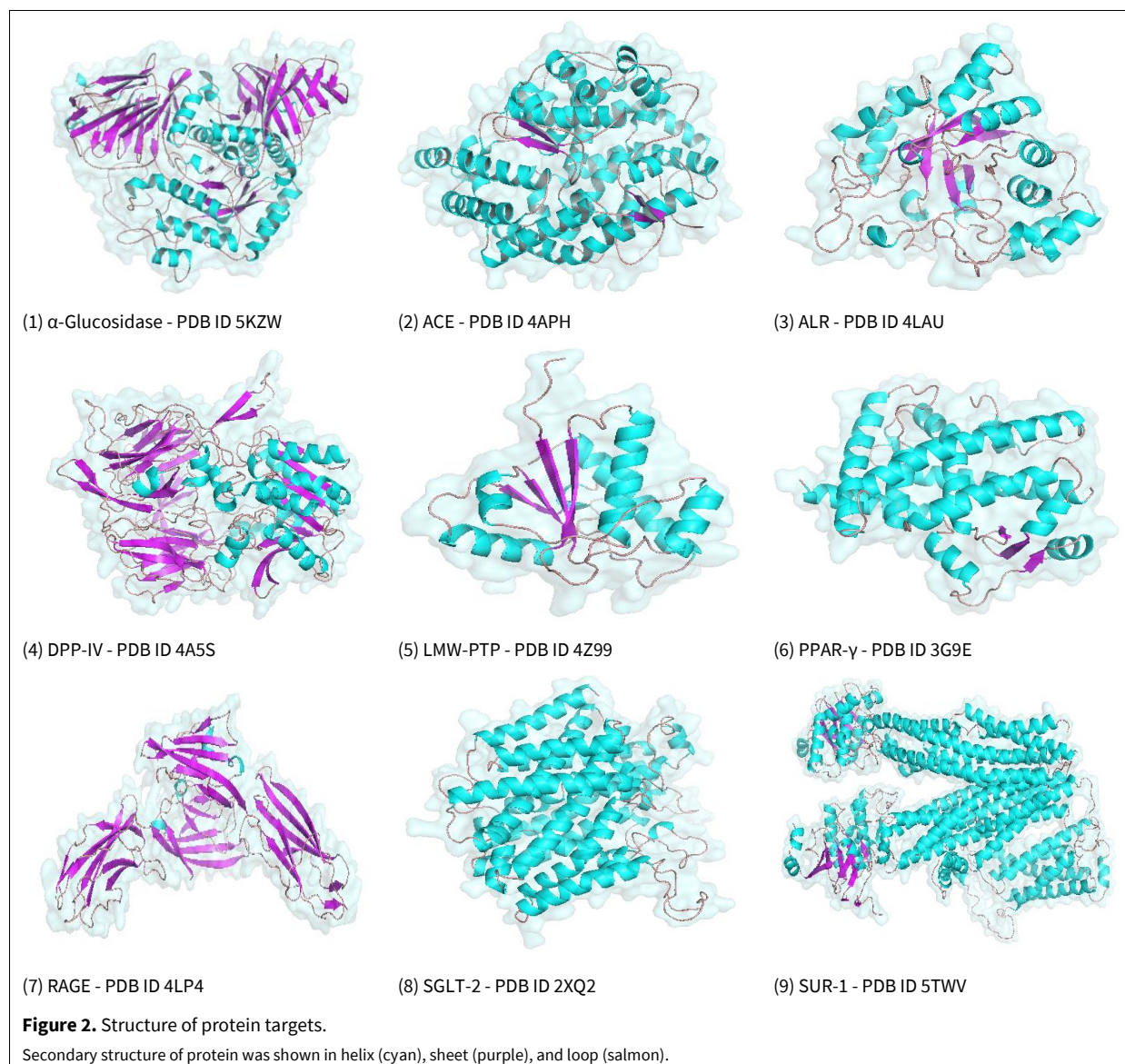


(Prabhu et al., 2017), aminoguanidine as RAGE inhibitor (Arfin et al., 2018), dapagliflozin as SGLT-2 inhibitor (Yue et al., 2021), gliquidone as SUR1 inhibitor (sulfonylurea), the standard treatment for diabetic nephropathy (Jadoon et al., 2019).

All these ligand molecules were retrieved from the PubChem (<https://pubchem.ncbi.nlm.nih.gov>) with structure data format (.sdf). The ligand molecules were converted into the 3D structure using OpenBabel version 2.4.1 software. This tool is used to generate 3D structure (protein data bank (.pdb) format) from 2D structure (.sdf format). The 2D structure and PubChem ID of phytoconstituents of *Tithonia diversifolia* were shown in Fig. 1. To qualify the selected phytoconstituents in *Tithonia diversifolia* as drug candidates, the properties of Lipinski's rule of five were calculated using SwissADME website (<http://www.swissadme.ch/>) (Attique et al., 2019). Only those structures were selected for docking that strictly followed Lipinski's rule.

Protein preparation

3D structures of diabetes-related proteins were retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (<http://www.rcsb.org>) as following: α -glucosidase (PDB ID 5KZW), ACE (PDB ID 4APH), ALR (PDB ID 4LAU), DPP-4 (PDB ID 4A5S), LMW-PTP (PDB ID 4Z99), PPAR- γ (PDB ID 3G9E), RAGE (PDB ID 4LP4), SGLT-2 (PDB ID 2XQ2), and SUR1 (PDB ID 5TWV). Since SGLT2 did not have experimental crystal structures, these structures were modelled using SWISS-MODEL (<https://swissmodel.expasy.org>) before they were docked to the respective ligands. The initial search for templates homology modelling of human SGLT-2 was searching revealed *Vibrio parahaemolyticus* SGLT-2 (PDB ID: 2XQ2) as the best template for modelling with a sequence identity of 99.45%. The ligands and water molecule occupying protein were removed using PyMOL Stereo 3D Zalman software version 4.30. and further checked for Ramachandran plot analysis (favored region) using the MolProbity website (<https://molprobity.biochem.duke.edu>). The structures of the protein target were shown in Fig. 2.



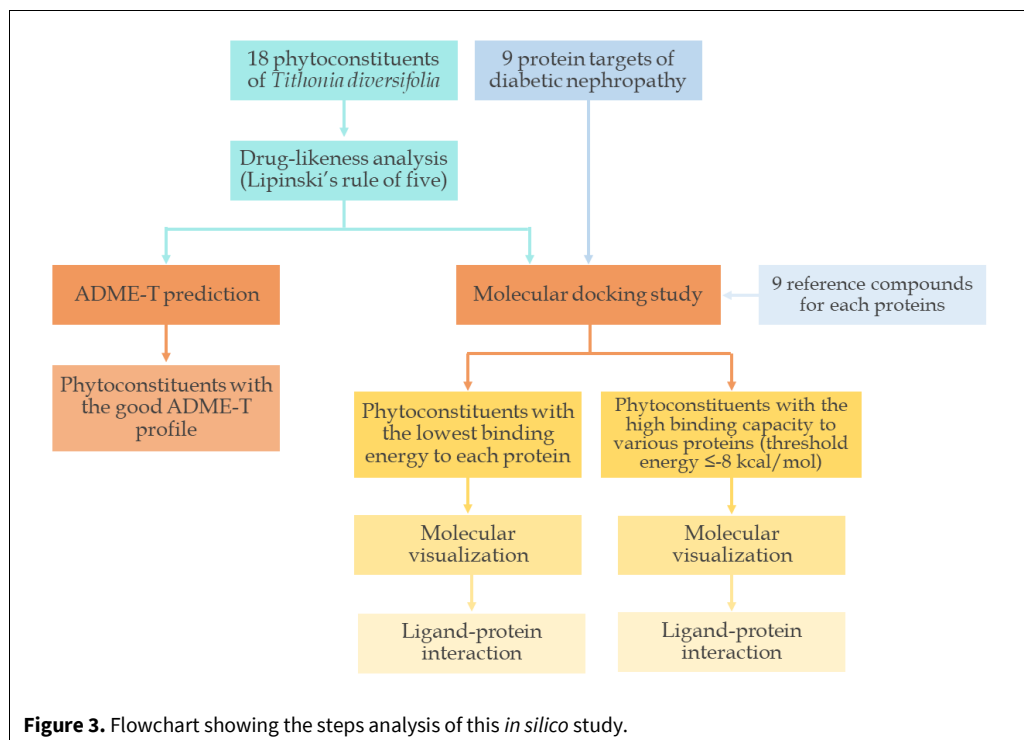
Molecular docking studies

PyRx-Virtual Screening Tool (Autodock Vina) version 8.0 was used to perform molecular docking for predicting free binding energy (Adianingsih and Kharisma, 2019). 18 phytoconstituents of *Tithonia diversifolia* that passed the drug-likeness criteria then were docked with nine protein targets, including α -glucosidase (acarbose as reference compound), ACE (lisinopril as reference compound), ALR (epalrestat as reference compound), DPP-4 (sitagliptin as reference compound), LMW-PTP (ethyl-3,4-dephostatin as reference compound), PPAR- γ (tesaglitazar as reference compound), RAGE (aminoguanidine as reference compound), SGLT-2 (dapagliflozin as reference compound), and SUR1 (gliquidone as reference compound). The docked molecules were visualized using PyMOL Stereo 3D Zalman software version 4.30. Bonding interaction and the residues of proteins in-

involved in the interaction with the ligands were analyzed using LigPlot+ version 1.4.5.

ADME-T prediction

The absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties of the 18 phytoconstituents of *Tithonia diversifolia* were screened using three different web servers to predict the important pharmacokinetic properties. The web servers used were Admetsar.v2 (<https://lmmd.ecust.edu.cn/admetsar2/>), ADMETlab 2.0 (<https://admetmesh.scbdd.com/>), and pkCSM (<https://biosig.unimelb.edu.au/pkcsml>) (Awaluddin et al., 2017; Shah et al., 2020). ADME-T properties include absorption: human intestinal absorption (HIA), human oral bioavailability (HOB), CaCo-2 permeability, water solubility (logS), and subcellular localization; distribution: P-glycoprotein (P-gp) substrate and inhibitor, and blood-brain barrier (BBB) permeability; metabolism: CYP450 (2C9, 2D6, 3A4)



substrate and CYP450 (1A2, 2C9, 2D6, 2C19, 3A4) inhibitor; excretion: renal organic cation transporter-2 (rOCT-2) substrate and inhibitor; toxicity: human ether-a-go-go-related gene (HERG) inhibition, AMES toxicity, carcinogens, and hepatotoxicity.

Data analysis

The step of data analysis is summarized in Fig. 3. The purposes of data analyses were (1) to propose the molecular anti-diabetic mechanism of *Tithonia diversifolia* by identifying the phytoconstituents with lowest energy binding to each protein, (2) to identify the phytoconstituents in *Tithonia diversifolia* with the high binding capacity (≤ -8 kcal/mol) to various diabetes-related proteins, and (3) to predict the phytoconstituents in *Tithonia diversifolia* with good ADME-T properties.

RESULTS

Drug likeness analysis of phytoconstituents in *Tithonia diversifolia*

Lipinski's rule of five is used to analyze whether a molecule has the potential to be developed as a drug (drug-likeness). This rule predicts that the molecule has a high probability to be developed as a drug if there are no more than two rule violations on the following criteria: there are no more than five H-bond donors (HBD) and 10 H-bond acceptors (HBA), the molecular weight (MWT) is smaller than 500, and the calculated Log P (CLogP) is smaller than 5 (Lipinski et al., 2012;

Naglah et al., 2020). The result prediction of drug analysis is shown in Table 1. The molecular weight, the number of hydrogen bond acceptors and donors, and the lipophilicity property for all the phytoconstituents in *Tithonia diversifolia* in accordance with Lipinski's rule of five. Based on the results, we selected all these ligands for the molecular docking study.

Molecular docking study and molecular interaction analysis

Ramachandran plot was used to validate the residues accuracy and reliability in both Psi and Phi angles. For a good model structure, the percentage of the protein residues that are in the most favored regions obtained should be over 90% (Laskowski et al., 1993). The number of residues in favored region of selected proteins was 792/815 (97.18%) for α -glucosidase, 542/549 (98.72%) for ACE, 290/293 (99.0%) for ALR, 673/692 (97.25%) for DPP-4, 152/156 (97.44%) for LMW-PTP, 246/254 (96.85%) for PPAR- γ , 412/414 (99.52%) for RAGE, 461/510 (90.39%) for SGLT-2, and 1302/1362 (95.59%) for SUR1. All the protein was valid thus could be used in the molecular docking study.

Molecular docking was carried out to evaluate the anti-diabetic effect of *Tithonia diversifolia* phytoconstituents on various targets of diabetic nephropathy. The docked binding energy of the selected ligands against each protein is summarized in Table 2 and Fig. 4. The binding free energies of the compound were between -5.1 and -9.5 Kcal/mol. In this study,

Table 1. Lipinski's rule of five for the phytoconstituents in *Tithonia diversifolia*.

No.	Compound	Lipinski's rule of five				No. of rule violations	Drug-Likeness
		MW	HBD	HBA	cLog P _{o/w}		
		≤500 Dalton	≤5	≤10	≤5	≤ 2 violations	
1	α-Humulene	204.35	0	0	4.26	0	Yes
2	β-Caryophyllene	204.35	0	0	4.24	0	Yes
3	β-Gurjunene	204.35	0	0	4.35	0	Yes
4	β-Pinene	136.23	0	0	3.42	0	Yes
5	5-Caffeoylquinic acid	354.31	6	9	-0.42	0	Yes
6	Camphene	136.23	0	0	3.43	0	Yes
7	Catechin	290.27	5	6	0.83	0	Yes
8	Diversifolin	350.41	1	6	2.3	0	Yes
9	Eucalyptol	154.25	0	1	2.67	0	Yes
10	Gallic acid	170.12	4	5	0.21	0	Yes
11	Germacrene D	204.35	0	0	4.3	0	Yes
12	Hispidulin	300.26	3	6	2.12	0	Yes
13	Myrcene	136.23	0	0	3.43	0	Yes
14	Tagitinin A	368.42	2	7	1.77	0	Yes
15	Tagitinin C	348.39	1	6	1.95	0	Yes
16	Tagitinin F	348.39	1	6	2.11	0	Yes
17	Tirotundin	352.42	1	6	2.42	0	Yes
18	Tithonine	298.29	1	5	2.72	0	Yes

we revealed the compounds with the highest binding affinity for α-glucosidase was diversifolin, tagitinin F, and tirotundin; for ACE was tagitinin C; for ALR and DPP-4 were hispidulin; for LMW-PTP was β-gurjunene and caryophyllene; for PPAR-γ and SGLT2 were tithonine; for RAGE was β-caryophyllene and α-humulene; and for SUR1 was tagitinin C. The docked complexes with the highest binding affinity are visualized in Figs. 5-6, and their interaction is shown in Table 3.

Molecular interactions of the amino acid residues of α-glucosidase, ACE, ALR, and DPP-4 with the highest binding affinity compounds against each protein are presented in Fig. 5. Diversifolin, tagitinin F, and tirotundin (-8.1 Kcal/mol) achieved the lowest free energy binding than acarbose (-7.6 Kcal/mol), an α-glucosidase inhibitor. Those three compounds showed a similar binding site to Glu866, Glu869, and Leu868 amino acid residue of α-glucosidase (Fig. 5a-c). Tagitinin C (-8.9 Kcal/mol) exhibited a lower free binding energy to ACE than lisinopril (-7.9 Kcal/mol), an ACE inhibitor. Both tagitinin C and lisinopril showed the same binding site at Val380 and Phe457 (Fig. 5d). Hispidulin (-9.5 Kcal/mol) showed a lower free energy binding than epalrestat (-7.3 Kcal/mol), an ALR inhibitor. Both hispidulin and epalrestat in-

teracted with the same amino acid residue Tyr48 at a distance of 2.75 Å and 2.95 Å (Fig. 5e). Sitagliptin (-8.9 Kcal/mol), a DPP-4 inhibitor, had lower binding energy than hispidulin (-8.7 Kcal/mol) and other phytoconstituents in *Tithonia diversifolia*. The DPP-4-hispidulin complex formed five hydrogen bonds but presented a different binding site from the DPP-4-sitagliptin complex (Fig. 5f).

Molecular bonding of the amino acid residues of LMW-PTP, PPAR-γ, RAGE, SGLT-2, and SUR1 with the highest binding affinity compounds against each protein are presented in Fig. 6. Most constituents of *Tithonia diversifolia* showed a lower free binding energy to LMW-PTP than ethyl-3,4-dephostatin. The free binding energy to LMW-PTP was lowest for β-gurjunene and caryophyllene -7.0 Kcal/mol and largest for ethyl-3,4-dephostatin with -5.9 Kcal/mol. Both ligands presented the same hydrophobic interaction with control to LMW-PTP at Asp129, Gly14, and Tyr131 (Fig. 6a-b). Tithonine (-8.6 Kcal/mol) had greater free binding energy compared to the control PPAR-γ agonist, tesaglitazar (-8.7 Kcal/mol). Tesaglitazar also interacted with the same amino acid residue at Ser342 with hydrogen bond and hydrophobic interaction at Leu330, Cys285, Leu340, Val339 (Fig. 6c). The free binding energy between RAGE and

Table 2. Summary of molecular docking result.

Ligands	Binding energy (kcal/mol)								
	α -glucosidase	ACE	ALR	DPP-4	LMW-PTP	PPAR- γ	RAGE	SGLT-2	SUR1
α -Humulene	-6.2	-7.4	-8.2	-7.2	-6.6	-6.3	-7.4	-6.5	-7.5
β -Caryophyllene	-6.4	-7.4	-7.7	-7.1	-7.0	-6.8	-7.4	-6.9	-7.6
β -Gurjunene	-6.3	-7.6	-7.8	-8.0	-7.0	-7.2	-7.0	-6.8	-7.6
β -Pinene	-5.4	-5.9	-6.5	-6.3	-5.5	-5.6	-5.4	-5.3	-6.6
5-Caffeoylquinic acid	-7.4	-8.3	-7.3	-8.3	-6.2	-7.0	-6.5	-7.8	-7.5
Camphene	-5.1	-5.7	-6.2	-5.8	-5.3	-5.6	-5.0	-5.3	-6.7
Catechin	-7.4	-8.3	-8.4	-8.4	-6.5	-8.2	-7.2	-7.6	-7.9
Diversifolin	-8.1	-8.7	-8.1	-7.9	-6.0	-6.9	-7.2	-7.1	-8.3
Eucalyptol	-5.3	-6.0	-6.1	-6.0	-5.4	-5.4	-5.4	-5.3	-6.6
Gallic acid	-6.1	-6.1	-7.2	-6.0	-5.4	-5.5	-5.6	-5.7	-5.9
Germacrene D	-6.6	-7.2	-7.6	-7.3	-6.5	-6.8	-7.1	-6.4	-7.7
Hispidulin	-7.2	-8.0	-9.5	-8.7	-6.6	-7.9	-6.9	-8.0	-8.1
Myrcene	-5.3	-5.2	-6.6	-5.5	-4.3	-5.6	-5.3	-5.1	-6.0
Tagitinin A	-8.0	-8.6	-8.3	-7.4	-5.7	-6.6	-6.7	-7.3	-8.3
Tagitinin C	-7.8	-8.9	-7.9	-7.5	-5.8	-7.2	-6.6	-7.8	-8.7
Tagitinin F	-8.1	-8.1	-9.4	-8.0	-6.0	-7.1	-6.5	-7.5	-8.4
Tirotundin	-8.1	-8.4	-8.4	-7.7	-5.8	-7.2	-7.0	-7.2	-8.1
Tithonine	-7.3	-8.4	-9.2	-8.4	-6.5	-8.6	-7.2	-8.1	-8.4
Acarbose ^a (ref)	-7.6								
Lisinopril ^b (ref)		-7.9							
Epalrestat ^c (ref)			-7.3						
Sitagliptin ^d (ref)				-8.9					
Ethyl-3,4-dephostatin ^e (ref)					-5.9				
Tesaglitazar ^f (ref)						-8.7			
Aminoguanidine ^g (ref)							-4.1		
Dapagliflozin ^h (ref)								-8.1	
Gliquidone ⁱ (ref)									-10.7

Abbreviations: ref - reference compound, ^a - α -glucosidase inhibitor, ^b - ACE inhibitor, ^c - ALR inhibitor, ^d - DPP-4 inhibitor, ^e - LMW-PTP inhibitor, ^f - PPAR- γ agonist, ^g - RAGE inhibitor, ^h - SGLT-2 inhibitor, ⁱ - sulfonylurea

aminoguanidine, a RAGE inhibitor, was -4.1 Kcal/mol, which greater than the free binding energy of *Tithonia diversifolia* constituents and RAGE. The lowest free binding energy from these constituents was -7.4 Kcal/mol for β -caryophyllene and α -humulene. Both β -caryophyllene and α -humulene did not form any hydrogen bond with RAGE (Fig. 6d-e). The free binding energy between SGLT2 and dapagliflozin was -8.1 Kcal/mol. Tithonine showed the same free binding energy with dapagliflozin. Both tithonine and dapagliflozin were interacted with amino acid residue Ser392 by a hydrogen bond (Fig. 6f). Docking analysis showed that the lowest free bonding energy was obtained by tagitinin C with -8.7

Kcal/mol, which was lower than gliquidone, the SUR1 inhibitor, with -10.7 Kcal/mol (Fig. 6g).

The bar chart in Fig. 4 showed a binding capacity of phytoconstituents in *Tithonia diversifolia* on nine proteins related to diabetic nephropathy. In terms of value, the compound is classified as a compound with a high binding capacity if the free binding energy is <-8 kcal/mol (Vo et al., 2016). In this study, we used a cut-off of around -8.0 kcal/mol. The good binding energy belonged to the bar of ACE, which owned nine compounds—followed by ALR, which had eight compounds with free binding energy $\leq$ -8 kcal/mol – seven compounds for SUR1, six compounds for DPP-4, four compounds for α -glucosidase, two compounds

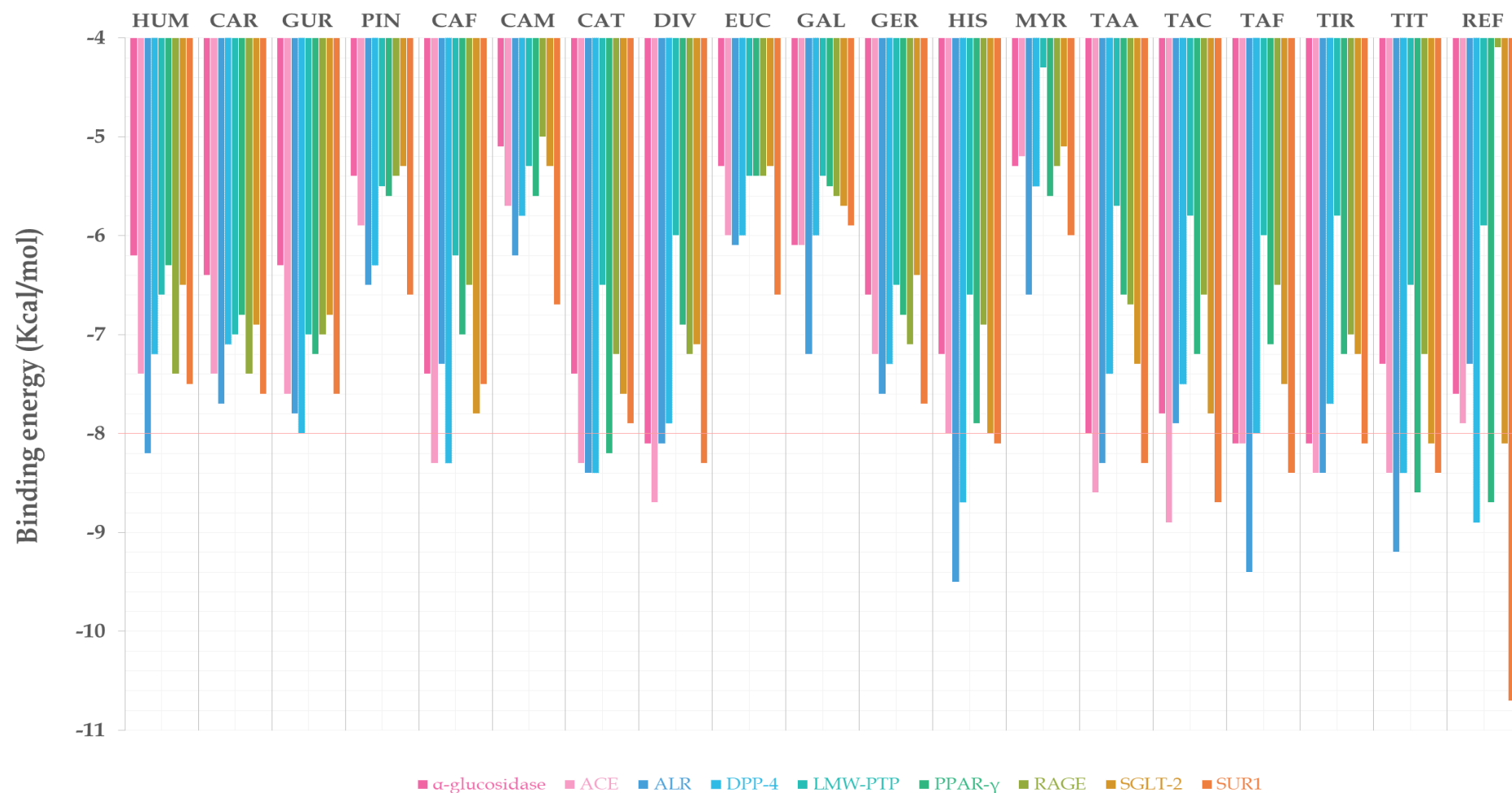


Figure 4. Binding energy of phytoconstituents in *Tithonia diversifolia* and reference compound with diabetes-related protein targets.

Abbreviations: HUM - α -humulene, CAR - β -caryophyllene, GUR - β -gurjunene, PIN - β -pinene, CAF - 5-caffeoylquinic acid, CAM - camphene, CAT - catechin, DIV - diversifolin, EUC - eucalyptol, GAL - gallic acid, GER for germacrene D, HIS for hispidulin, MYR for myrcene, TAA for tagitin A, TAC for tagitin C, TAF for tagitin F, TIR for tirotundin, TIT for tithonine, and REF for reference compound.

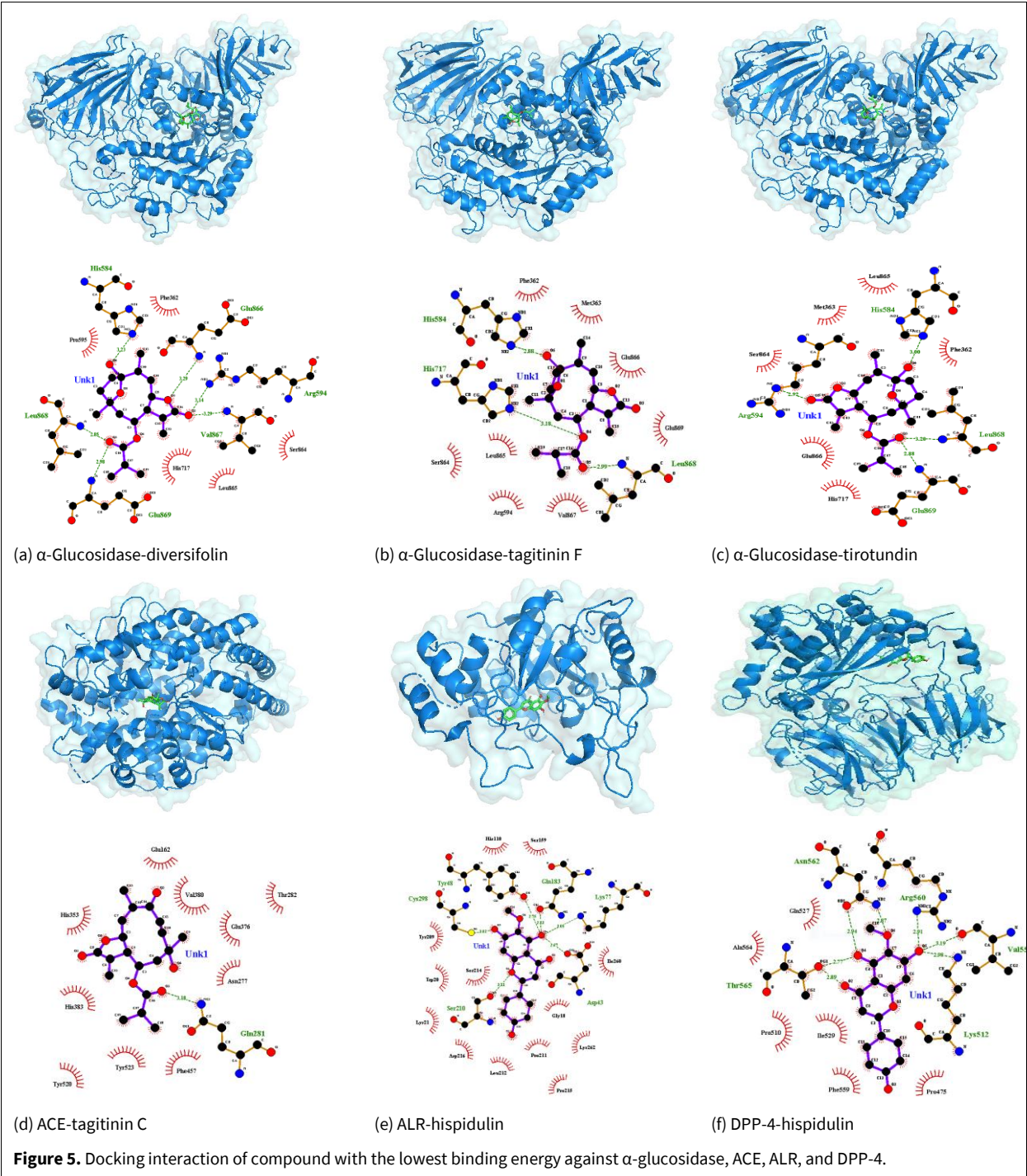


Figure 5. Docking interaction of compound with the lowest binding energy against α-glucosidase, ACE, ALR, and DPP-4.

Table 3. Ligand interaction of docked molecules with the lowest binding energy.

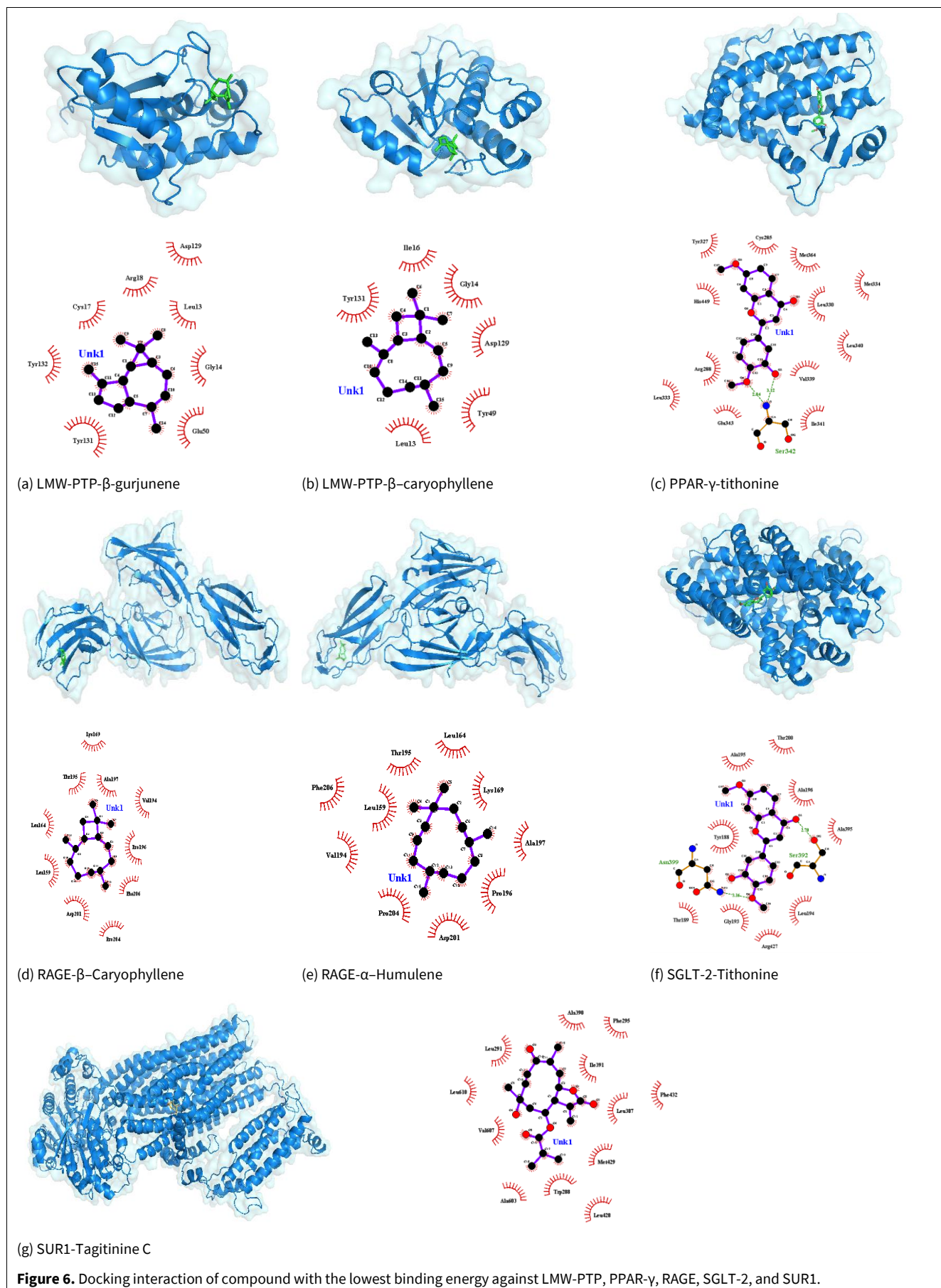
No.	Target	Compound	Hydrogen bonding				Hydrophobic interaction
			Total	Amino acid	Ligand atom	Distance (Å)	
1	α-Glucosidase	Diversifolin	6	Glu866(N)	O	3.29	Ser864, His717, Leu865, Phe362, Pro595
				Arg594(N)	O	3.14	
				Val867(N)	O	3.29	
				Glu869(N)	O	2.98	

Table 3. Ligand interaction of docked molecules with the lowest binding energy (continued...)

No.	Target	Compound	Hydrogen bonding				Hydrophobic interaction
			Total	Amino acid	Ligand atom	Distance (Å)	
2	ACE	Tagitinin F	3	Leu868(N)	O	2.85	Val867, Ser864, Leu865, Arg594, Phe362, Met363, Glu866, Glu869
				His584(N)	O	3.23	
				Leu868(N)	O	2.99	
				His717(N)	O	3.18	
				His584(N)	O	2.88	
		Tirofundin	4	Arg594(C)	O	2.92	Phe362, His717, Glu866, Ser864, Met363, Leu865
				Glu869(N)	O	2.88	
				Leu868(N)	O	3.20	
				His584(N)	O	3.00	
				Leu868(N)	O	3.20	
		Acarbose (reference)	6	Ile98(N)	O	3.23	Tyr110, Ala93, Ala97, Lys96, Asp95, Gly546, Pro545, Tyr543
				Ile98(O)	N	3.17	
				Ile98(O)	O	2.81	
				Asp319(O)	O	2.92, 2.81	
				Val544(O)	O	2.90	
				Pro94(O)	O	3.03	
				Cys127(N)	O	3.05	
				Trp126(N)	O	3.10	
				Gln281(N)	O	3.18	
				Gln281(N)	O	3.18	
		Lisinopril (reference)	6	His513(N)	O	3.28	Thr282, Glu376, Phe457, Val380, Glu162, Asn277, Tyr523, His383, Tyr520, His353
				His353(N)	O	3.16	
				Tyr523(O)	O	2.88	
				Glu162(O)	O	3.15	
				His383(N)	O	3.24	
3	ALR	Hispidulin	6	Asp415(O)	N	3.01	Ile260, Ser159, His110, Tyr209, Trp20, Ser214, Gly18, Lys262, Pro211, Pro215
				Asp43(O)	O	2.67	
				Lys77(N)	O	3.05	
				Gln183(O)	O	2.83	
				Tyr48(O)	O	2.75	
				Cys298(S)	O	3.02	
				Ser210(O)	O	3.11	
		Epalrestat (reference)	1	Tyr48(O)	O	2.95	Gln49, Trp219, Trp79, Val47, Phe122, Trp20, Ala299, Cys298, Trp111, Leu300
				Tyr48(O)	O	2.95	
				Tyr48(O)	O	2.95	
4	DPP-4	Hispidulin	5	Thr565(O)	O	2.77, 2.89	Ile529, Gln527, Ala564, Pro510, Phe559, Pro475
				Asn562(N)	O	2.87	
				Asn562(O)	O	2.94	
				Arg560(N)	O	2.91	
				Val558(O)	O	3.19	
				Lys512(N)	O	2.98	

Table 3. Ligand interaction of docked molecules with the lowest binding energy (continued...)

No.	Target	Compound	Hydrogen bonding				Hydrophobic interaction
			Total	Amino acid	Ligand atom	Distance (Å)	
5	LMW-PTP	Sitagliptin (reference)	2	Ser630(O)	N	3.20	His740, Tyr547, Gly741, Trp629, Arg125
				Tyr662(O)	N	2.96	
		β-Gurjunene	-	-	-	-	Asp129, Arg18, Leu13, Gly14, Glu50, Tyr131, Tyr132, Cys17
		β-Caryophyllene	-	-	-	-	Ile16, Gly14, Asp129, Tyr49, Leu13, Tyr131
6	PPAR-γ	Ethyl-3,4-dephostatin (reference)	2	Arg18(N)	O	3.13, 2.95, 3.03	Tyr49, Ser47, Leu13, Asp129, Cys12, Tyr131, Gly14, Cys17, Ile16
				Glu50(O)	O	2.91	
		Tithonine	1	Ser342(N)	O	3.12, 2.84	Met334, Met364, Leu330, Cys285, Leu340, Val339, Ile341, Tyr327, His449, Arg288, Leu333, Glu343
		Tesaglitazar (reference)	6	His449(N)	O	2.91, 3.01	Phe282, Leu453, Leu469, Cys285, Ile326, Leu340, Val339, Leu330, Ile341, Glu343, Leu333
				Ser342(N)	O	3.04	
				Arg288(N)	O	2.81	
				Ser289(O)	O	2.83	
				His323(N)	O	2.81	
				Tyr473(O)	O	2.90	
7	RAGE	β-Caryophyllene	-	-	-	-	Pro204, Asp201, Leu159, Leu164, Thr195, Ala197, Val194, Pro196, Phe206, Lys169
		α-Humulene	-	-	-	-	Pro204, Val194, Lys169, Ala197, Pro196, Asp201, Leu159, Leu-164, Thr195, Phe206
		Aminoguanidine (reference)	1	His-217 (N)	N	3.13	Phe210, Ile120, Tyr118, Gln119, Pro212, Tyr150, Ser149, Pro151
8	SGLT-2	Tithonine	2	Ser392(O)	O	2.70	Ala395, Thr200, Ala196, Ala195, Leu194, Tyr188, Arg427, Gly193, Thr189
				Asn399(N)	O	3.16	
		Dapagliflozine (reference)	3	Ser396(O)	O	2.92	Lys154, Ile397, His80, Ile456, Phe453, Ser74, Asp158, Gly79, Gln204, Ala389, Gly77
				Ser392(O)	O	3.02, 2.97	
9	SUR-1			Ser393(O)	O	2.98, 3.31	
		Tagitinin C	-	-	-	-	Phe295, Phe432, Ile391, Ala390, Leu387, Met429, Leu428, Trp288, Ala603, Val607, Leu291, Leu610
		Gliquidone (reference)	-	-	-	-	Gln219, Leu218, Trp1247, Ala1244, Met233, Phe1182, Ala1185, Phe236, Ala235, Ala1243, Leu1189, Phe1240, Asp1188, Thr239



for PPAR- γ and SGLT-2. The bottom in the chart is LMW-PTP and RAGE, which all compounds had low results (> -8 kcal/mol).

This part of the results also indicated that 5-caffeoylquinic acid, catechin, diversifolin, hispiduline, tagitinin A, tagitinin C, tagitinin F, tirotundin, and tithonine had a high binding capacity (≤ -8 kcal/mol) to selected protein targets. Sitagliptin, tesaglitazar, dapagliflozin and gliquidone are the only reference compounds, which had a high binding capacity to each protein targets. Tithonine had a high binding capacity to six proteins (ACE, ALR, DPP-4, PPAR- γ , SGLT-2, SUR1) and had a higher binding affinity than reference compounds towards those proteins, except to PPAR- γ and SUR1. Hispidulin and tagitinin F had a high binding capacity to five proteins (hispidulin to ACE, ALR, DPP-4, SGLT-2, SUR1; tagitinin F to α -glucosidase, ACE, ALR, DPP-4, SUR1). Both hispidulin and tagitinin F had a higher binding affinity than reference compounds towards ACE and ALR. Catechin, diversifolin, tagitinin A, and tirotundin had a high binding affinity to four proteins (catechin to ACE, ALR, DPP-4, PPAR- γ ; diversifolin to α -glucosidase, ACE, ALR, SUR1; tagitinin A and tirotundin to α -glucosidase, ACE, ALR, SUR1). Catechin had a higher binding affinity than reference compounds to those protein, except to α -glucosidase. Both tagitinin A and tirotundin had a higher binding affinity than reference compounds against those protein, except to SUR1. 5-Caffeoylquinic acid and tagitinin C had a high binding affinity to two proteins (5-caffeoylquinic acid to ACE, DPP-4; tagitinin C to ACE, SUR1). Both tagitinin A and tirotundin had a higher binding affinity than reference compounds against ACE. These results indicated that phytoconstituents in *Tithonia diversifolia* is potential against diabetes and its complication such as diabetic neuropathy.

ADME-T results

The results of computed *in silico* ADME-T of phytoconstituents in *Tithonia diversifolia* are shown in Tables 4-8. We used three different ADME-T prediction web servers to compare and predict the most accurate results. The results of the human intestinal absorption (HIA) test indicated that all the compounds have a high permeation across the membrane, whereas only nine of eighteen compounds have good human oral bioavailability (HOB) (Table 4). On the *in vitro* Caco-2 cell permeability test, all the compounds exhibited good permeation, except 5-caffeoylquinic acid, catechin, and gallic acid. All the compounds are expected to be soluble in water, except α -humulene,

β -caryophyllene, β -gurjunene, and germacrene D. All compounds have various subcellular localization.

Distribution profile prediction is presented in Table 5. All compounds revealed not to inhibit P-glycoprotein (P-gp), whereas only 5-caffeoylquinic acid and hispiduline were P-gp substrate and not BBB permeant. Catechin and gallic acid also did not show potential to cross BBB. All compounds were non-inhibitor for CYP2C19 and non-substrate for CYP3A4 (Table 6). Both hispiduline and tithonine were inhibitors for CYP1A2, CYP2C9, CYP2C19, and CYP3A4. They were also substrate for CYP3A4. Other CYP3A4 substrates were β -gurjunene, diversifolin, tagitinin C, tagitinin F, and tirotundin. However, only 5-caffeoylquinic acid was a substrate for CYP2C9.

All the compounds were neither rOCT2 substrate nor rOCT2 inhibitor (Table 7). None of the compounds were hepatotoxic and non-inhibitor for HERG (Table 8). Catechin, diversifolin and tithonine are all mutagenic, based on the AMES toxicity test. Many compounds were not carcinogenic, except for α -humulene, eucalyptol, germacrene D, and tagitinin F, which show the opposite results of carcinogen properties from two different web servers since pkCSM webserver could not predict the carcinogen properties.

In this analysis, we revealed that β -gurjunene, tagitinin A, tagitinin C, tagitinin F, and tirotundin showed a better pharmacokinetic and toxicity profile than other compounds. Based on molecular docking study and ADME-T prediction, we concluded that four compounds (tagitinin A, tagitinin C, tagitinin F, tirotundin) were showed both high binding affinity to various diabetes-related proteins and good ADME-T profile.

DISCUSSION

Drug likeness analysis

Drug development and discovery cannot be separated from drug-likeness analysis, which focuses on evaluating the solubility, absorption, and permeability of drugs given orally, although metabolic processes can change the physicochemical properties of drugs (Wu et al., 2016). Lipinski's rule of five is the common rule used in the drug-likeness analysis. The rule has four parameters with different criteria. Molecular weight ≤ 500 g/mol describes the compound having good permeability to the blood and intestinal barrier, then increases the permeation time through the lipid bilayer. The profile of hydrogen bond donor ≤ 5 and hydrogen bond acceptor ≤ 10 indicates that the compound is able to be absorbed well.

Table 4. Absorption prediction output.

Compound	HIA				HOB		CaCo-2 permeability			Water solubility (logS)				
	(+) ^a	(+) ^b	> 30% ^c	Conclusion	(+) ^a	(+) ^a	> -5.15 log unit ^b	> 0.90 log Papp in 10 ⁻⁶ cm/s ^c	Conclusion	(≥-5) ^a	> -5 mol/L ^b	-4~-0.5 log mol/L ^c	Conclusion	Subcellular localization ^a
α-Humulene	+	+	94.68%	Yes	Yes	+	-4.42	1.42	Yes	-5.04	-5.87	-5.19	No	Lysosome
β-Caryophyllene	+	+	94.84%	Yes	Yes	+	-4.51	1.42	Yes	-4.68	-6.16	-5.55	No	Lysosome
β-Gurjunene	+	+	96.88%	Yes	Yes	+	-4.60	1.40	Yes	-5.12	-6.14	-5.72	No	Lysosome
β-Pinene	+	+	95.52%	Yes	Yes	+	-4.46	1.38	Yes	-4.32	-4.38	-4.19	Yes	Lysosome
5-Caffeoylquinic acid	+	-	36.37%	Yes	No	-	-6.12	-0.84	No	-2.45	-1.19	-2.44	Yes	Mitochondria
Camphene	+	+	94.14%	Yes	Yes	+	-4.46	1.38	Yes	-3.43	-4.71	-4.34	Yes	Lysosome
Catechin	+	+	68.82%	Yes	No	-	-5.97	0.28	No	-3.10	-2.99	-3.11	Yes	Mitochondria
Diversifolin	+	+	97.12%	Yes	No	+	-4.73	1.27	Yes	-3.74	-2.40	-3.61	Yes	Mitochondria
Eucalyptol	+	+	96.50%	Yes	Yes	+	-4.41	1.48	Yes	-2.00	-1.91	-2.63	Yes	Lysosome
Gallic acid	+	+	43.37%	Yes	Yes	-	-5.72	-0.08	No	-1.09	-1.22	-2.56	Yes	Mitochondria
Germacrene D	+	+	95.59%	Yes	Yes	+	-4.47	1.43	Yes	-5.02	-5.69	-5.68	No	Lysosome
Hispiduline	+	+	84.65%	Yes	No	+	-4.88	-0.04	Yes	-3.22	-3.63	-3.40	Yes	Mitochondria
Myrcene	+	+	94.69%	Yes	No	+	-4.40	1.40	Yes	-3.44	-4.17	-4.49	Yes	Nucleus
Tagitinin A	+	+	95.33%	Yes	No	+	-4.90	0.39	Yes	-3.26	-1.51	-3.48	Yes	Mitochondria
Tagitinin C	+	+	97.79%	Yes	No	+	-4.70	1.33	Yes	-2.55	-2.17	3.20	Yes	Mitochondria
Tagitinin F	+	+	97.60%	Yes	No	+	-4.77	1.28	Yes	-2.68	-2.60	-3.53	Yes	Mitochondria
Tirotundin	+	+	96.97%	Yes	No	+	-4.73	1.27	Yes	-3.74	-2.02	-3.64	Yes	Mitochondria
Tithonine	+	+	94.94%	Yes	Yes	+	-4.68	1.21	Yes	-3.56	-4.30	-3.71	Yes	Mitochondria

Output resulted from three web servers: Admetsar.v2 (a), ADMETlab 2.0 (b), and pkCSM (c). Abbreviations: HIA - human intestinal absorption, HOB - human oral bioavailability, CaCo-2: characterization of the human colon carcinoma cell line.

Table 5. Distribution prediction output

Compound	P-gp substrate				P-gp inhibitor				BBB permeability			
	(-) ^a	(-) ^b	(-) ^c	Conclusion	(+) ^a	(+) ^b	(+) ^c	Conclusion	(-) ^a	(-) ^b	< -1 logBB ^c	Conclusion
α-Humulene	-	-	+	No	-	-	-	No	+	-	0.663	Yes
β-Caryophyllene	-	-	-	No	-	-	-	No	+	+	0.733	Yes
β-Gurjunene	-	-	-	No	-	-	-	No	+	+	0.819	Yes
β-Pinene	-	-	-	No	-	-	-	No	+	+	0.818	Yes
5-Caffeoylquinic acid	-	+	+	Yes	-	-	-	No	-	+	-1.407	No
Camphene	-	-	-	No	-	-	-	No	+	+	0.787	Yes
Catechin	-	-	+	No	-	-	-	No	-	-	-1.054	No
Diversifolin	-	-	-	No	-	-	-	No	+	+	-0.383	Yes
Eucalyptol	-	-	+	No	-	-	-	No	+	+	0.368	Yes
Gallic acid	-	-	-	No	-	-	-	No	-	-	-1.102	No
Germacrene D	-	-	-	No	-	-	-	No	+	-	0.723	Yes
Hispiduline	-	+	+	Yes	-	-	-	No	-	-	-1.12	No
Myrcene	-	-	-	No	-	-	-	No	+	+	0.781	Yes
Tagitinin A	-	-	-	No	-	-	-	No	+	+	-0.32	Yes
Tagitinin C	-	-	-	No	-	+	-	No	+	+	-0.431	Yes
Tagitinin F	-	-	-	No	-	-	-	No	+	+	-0.376	Yes
Tirotundin	-	-	-	No	-	-	-	No	+	+	-0.386	Yes
Tithonine	-	-	-	No	+	-	-	No	-	-	-0.469	No

Output resulted from three web servers: Admetsar.v2 (a), ADMETlab 2.0 (b), and pkCSM (c). Abbreviations: P-gp - P-glycoprotein, BBB - blood brain barrier

Table 6. Metabolism prediction output

Compound	CYP450 2C9 substrate			CYP450 2D6 substrate			CYP450 3A4 substrate			CYP450 1A2 inhibitor			CYP450 2C9 inhibitor			CYP450 2D6 inhibitor			CYP450 2C19 inhibitor			CYP450 3A4 inhibitor					
	a	b	Con	a	b	c	Con	a	b	c	Con	a	b	c	Con	a	b	c	Con	a	b	c	Con	a	b	c	Con
α-Humulene	-	+	Yes/No	-	+	-	No	-	-	-	No	-	+	-	No	-	+	-	No	-	-	-	No	-	+	-	No
β-Caryophyllene	-	-	No	-	+	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
β-Gurjunene	-	+	Yes/No	-	+	-	No	+	-	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
β-Pinene	-	+	Yes/No	-	+	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
5-Caffeoylquinic acid	+	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Camphene	-	+	Yes/No	-	+	-	No	-	-	-	No	-	+	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Catechin	-	+	Yes/No	+	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Diversifolin	-	-	No	-	-	-	No	-	+	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Eucalyptol	-	+	Yes/No	-	+	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Gallic acid	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Germacrene D	-	+	Yes/No	-	-	-	No	-	+	-	No	-	-	-	No	-	+	-	No	-	-	-	No	-	-	-	No
Hispiduline	-	+	Yes/No	-	-	-	No	+	-	+	Yes	+	+	+	Yes	+	+	+	Yes	-	+	-	No	+	-	+	Yes
Myrcene	-	+	Yes/No	-	+	-	No	-	-	-	No	-	+	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Tagitinin A	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Tagitinin C	-	-	No	-	-	-	No	-	+	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Tagitinin F	-	-	No	-	-	-	No	-	+	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Tirotundin	-	-	No	-	-	-	No	-	+	+	Yes	-	-	-	No	-	-	-	No	-	-	-	No	-	-	-	No
Tithonine	-	+	Yes/No	-	+	-	No	+	-	+	Yes	+	+	+	Yes	+	+	+	Yes	-	+	-	No	+	+	+	Yes

Output resulted from three web servers: Admetsar.v2 (a), ADMETlab 2.0 (b), and pkCSM (c). Abbreviations: Con - Conclusion, CYP450 - cytochrome 450.

Table 7. Excretion prediction output

Compounds	rOCT2 substrate	rOCT2 inhibitor
	c	a
α -Humulene	No	No
β -Caryophyllene	No	No
β -Gurjunene	No	No
β -Pinene	No	No
5-Caffeoylquinic acid	No	No
Camphene	No	No
Catechin	No	No
Diversifolin	No	No
Eucalyptol	No	No
Gallic acid	No	No
Germacrene D	No	No
Hispiduline	No	No
Myrcene	No	No
Tagitinin A	No	No
Tagitinin C	No	No
Tagitinin F	No	No
Tirotundin	No	No
Tithonine	No	No

Output resulted from two web servers: Admetsar.v2 (a) and pkCSM (c). Abbreviations: rOCT2 - renal organic cation transporter-2.

The value exceeding the criteria indicates that the compound is soluble in a polar solvent such as water through hydrogen bonds (Pollastri, 2010). The logP parameter is related to the lipophilicity or hydrophobicity of a compound. Rachmania et al. (2015) stated that a logP value that exceeds five describes a compound that is highly lipophilic, thus triggering high toxicity because the drug molecule is retained for a long time in the lipid bilayer membrane and is widely distributed in the body. In contrast, the negative value of logP describes a compound that is hydrophilic so that it can cause difficulty for a molecule to be absorbed. The nature of a compound molecule can be said to have a good absorption and permeation profile if the compound is at a logP value of ≤ 5 and is not negative, because the compound can penetrate the hydrophilic outer layer of the membrane and enter the hydrophobic lipid bilayer layer (Rachmania et al. al., 2015). All compounds of *Tithonia diversifolia* meet the drug-likeness criteria in the Lipinski rule, so it can be concluded that the 18 compounds have good absorption to be taken orally.

Molecular docking studies

Tithonia diversifolia (Hemsl.) A. Gray has been traditionally used in the traditional medicine as an anti-

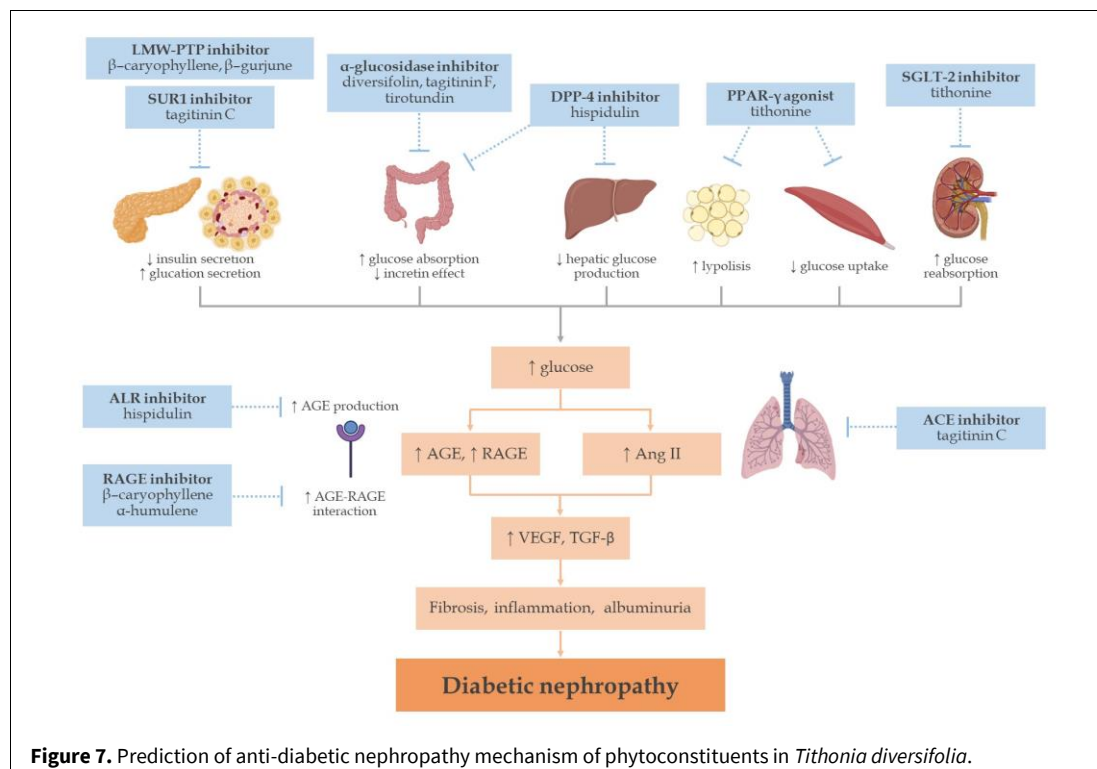
inflammatory and against other illnesses. Several reports that exist on the identification of *Tithonia* constituent point that the tagitinins isolated from *Tithonia diversifolia* are the most studied sesquiterpene lactones (STL) due to their wide spectrum of pharmacological activity. Controlling hyperglycemia is the key factor in the management of diabetes and its complication, such as diabetic nephropathy. Several target proteins, such as α -glucosidase, ACE, ALR, DPP-4, LMW-PTP, PPAR- γ , RAGE, SGLT2, and SUR1, play an important role in the occurrence of hyperglycemia.

Binding affinity is examined in this molecular docking study to determine the strength of binding between ligand and protein. It is necessary to identify the binding affinity because it describes the accumulation of binding strength between the ligand and the protein receptor. Meng (2011) states that the smaller the binding affinity value, the higher the binding affinity between the receptor and the ligand. In this study, we also performed the identification of the conformational binding interactions that occur between docked molecules, namely hydrogen bonds and hydrophobic interactions.

Table 8. Toxicity prediction output.

Compounds	HERG inhibition				AMES toxicity				Carcinogens (binary)			Hepatotoxicity			
	(-) ^a	(-) ^b	(-) ^c	Conclusion	(-) ^a	(-) ^b	(-) ^c	Conclusion	(-) ^a	(-) ^b	Conclusion	(-) ^a	(-) ^b	(-) ^c	Conclusion
α-Humulene	-	-	-	No	-	-	-	No	-	+	Yes/No	-	-	-	No
β-Caryophyllene	+	-	-	No	-	-	-	No	-	-	No	-	-	-	No
β-Gurjunene	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
β-Pinene	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
5-Caffeoylquinic acid	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
Camphene	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
Catechin	-	-	-	No	+	+	-	Yes	-	-	No	-	-	-	No
Diversifolin	-	-	+	No	-	+	+	Yes	-	-	No	+	-	-	No
Eucalyptol	-	-	-	No	-	-	-	No	-	+	Yes/No	-	-	-	No
Gallic acid	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
Germacrene D	-	-	-	No	-	-	-	No	-	+	Yes/No	-	-	-	No
Hispiduline	-	-	-	No	-	-	-	No	-	-	No	+	-	-	No
Myrcene	-	-	-	No	-	-	-	No	+	+	Yes	-	+	-	No
Tagitinin A	-	-	+	No	-	-	+	No	-	-	No	-	-	+	No
Tagitinin C	-	-	+	No	-	-	+	No	-	-	No	+	-	-	No
Tagitinin F	+	-	+	Yes	-	-	+	No	-	+	Yes/No	+	-	-	No
Tirotundin	-	-	-	No	-	-	-	No	-	-	No	-	-	-	No
Tithonine	-	-	-	No	+	+	-	Yes	-	-	No	+	-	-	No

Output resulted from three web servers: Admetsar.v2 (a), ADMETLab 2.0 (b), and pkCSM (c). Abbreviations: HERG: human ether-a-go-go-related gene



Based on binding affinity, we predicted the mechanism of action of phytoconstituents in *Tithonia diversifolia* as anti-diabetic nephropathy based on the highest binding affinity, which is shown in Fig. 7.

Hyperglycemia induces RAAS activation, including ACE activation, leading to an increase in blood pressure by angiotensin-II (Ang-II) production. Ang-II increases the vascular endothelial growth factor (VEGF), transforming growth factor beta-1 (TGF-β), and lipid accumulation which is involved in kidney damage in patients with diabetic nephropathy. ACE inhibitor (ACEI) showed the renoprotective effect thus could prevent diabetic nephropathy (Soetikno et al., 2014; Hsu et al., 2017; Ramanjaneyulu et al., 2013). Based on the molecular study approach, tagitinin C possesses the most inhibitory activity against ACE, better than lisinopril. This compound also inhibits SUR1, but the potency was lower than gliclazide. SUR1 is an ATP-binding cassette (ABC) transporter that serves as the regulatory subunit of an ion channel. SUR1/Kir6.2 activity regulates insulin secretion in pancreatic β-cell (Aittoniemi et al., 2009). Inhibition of ATP-sensitive potassium (K_{ATP}) channels by sulfonylureas causes depolarization of the β-cell membrane. It triggers the voltage-gated Ca^{2+} channels opening, eliciting Ca^{2+} influx, and increasing the intracellular Ca^{2+} , thus stimulating the exocytosis of insulin-containing secretory granules (Proks et al., 2002).

ALR is responsible for the reduction of glucose to sorbitol. In hyperglycemia, an imbalance of cofactor involved in the polyol pathway results in deleterious effects such as advanced glycation end product (AGEs) formation. AGEs are considered as a major cause of diabetic nephropathy (Adianingsih et al., 2016). Epalrestat is an example of ALR inhibitor. Inhibition of ALR showed a potential therapeutic strategy to prevent diabetic nephropathy (El Gamal et al., 2017; Kajal and Singh, 2018; Antony and Vijayan, 2015). The most active compound against ALR is hispidulin. In this present study, hispidulin also has the potency as DPP-4 inhibitor, although lower than sitagliptin. DPP-4 is a ubiquitous enzyme that inactivates endogenous incretin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These hormones secreted by the enteroendocrine cells of the gut regulate glucose homeostasis by modulating islet hormone secretion (Pratley and Salsali, 2007). A cohort study showed that the administration of DPP-4 inhibitor reduces urine albumin excretion and mitigates the reduction of estimated glomerular filtration rate (eGFR) in type 2 diabetes mellitus (T2DM) patients (Kim et al., 2016). A previous study by Purnomo et al. (2014) of DPP-4 inhibitory activity of *Tithonia diversifolia* in vitro has shown that an ethanolic extract has an inhibitory concentration 50 (IC_{50}) value of 15,385.27 μ g/mL. A recent study by Ejeloni et al. (2022) concluded that *Tithonia diversifolia* saponin-rich extract (TDS) is an agonist to Takeda G-protein bile acid receptor 5 (TGR5), which increases GLP-1 ex-

pression and insulin release in diabetic model mice. Wang et al. (2020) has demonstrated the role of hispidulin on stimulating GLP-1 secretion and ameliorating hyperglycemia in diabetic mice.

Insulin receptor (IR) is a tyrosine kinase that phosphorylates multiple target proteins known as insulin receptor substrate (IRS) and initiates various intracellular signaling pathways. Insulin signaling is counteracted by tyrosine phosphatases that dephosphorylate and inactivate the IR (Kasibhatia et al., 2007). Protein tyrosine phosphatase 1B (PTP1B) has been validated for its involvement in obesity and diabetes. LMW-PTP is a small (18 kDa) cytosolic class II PTP with two isoforms, LMW-PTP-A and LMW-PTP-B, resulted from alternative splicing. Low LMW-PTP enzymatic activity protects against hyperlipidemia in obese patients and protects against hyperglycemia in diabetes. Selective LMW-PTP chemical inhibitors would be highly valuable for assessing activity-dependent LMW-PTP functions and its potential as a drug target (Barr, 2010; Standford et al., 2017). Ethyl-3,4-dephostatin is a control drug that used as LMW-PTP inhibitor in a study by Ren et al. (2017). Based on in silico analysis, we hypothesized that β -caryophyllene and β -gurjunene are potential LMW-PTP inhibitors. Research conducted by Saifudin et al. (2012) showed that β -caryophyllene 8R,9R-oxide isolated from *Blumea balsamifera* exhibit the significant activity of PTP1B inhibitor with an IC_{50} value of 25.8 μ M.

Not only inhibit LMW-PTP, β -caryophyllene also has the potential as RAGE inhibitor, as same as α -humulene. AGE-RAGE signaling plays a role in the pathophysiology of diabetic nephropathy and other diabetes complications. Chronic hyperglycemia induces AGE formation. AGE deposition in kidney and its interaction with RAGE in podocyte, mesangial and endothelial cells induce ROS production and initiate the various signaling cascade, leading to transcription factor activation such as nuclear factor κ B (NF- κ B) and forkhead box O4 (FOXO4) (Daroux et al., 2010; Sanajou et al., 2018). AGE-RAGE interaction in podocytes reduces the nephrin expression in the slit diaphragm. Nephrin plays a pivotal role in the glomerular filtration barrier integrity. Nephrin reduction causes the podocyte loss from the glomerular barrier membrane, podocyte apoptosis, and simplification of podocyte foot processes, leading to proteinuria development (Daroux et al., 2010; Müller-Krebs et al., 2012). RAGE activation could be attenuated by inhibiting AGE formation or RAGE blockade (Fatchiyah et al., 2015; Adianingsih et al., 2016). In this study, two ligands in *Tithonia diversifolia*, β -caryophyllene and α -humulene, have the potential as RAGE inhibitors, better aminoguanidine as control. Cho et al. (2015)

showed that β -caryophyllene suppresses the RAGE expression in hepatic injury.

This study demonstrates that tithonine has the potential as PPAR- γ analog and SGLT2 inhibitor. PPAR- γ is expressed in podocyte, mesangial and endothelial cells in kidney. Many studies demonstrated that thiazolidinedione (TZD), a PPAR- γ agonist has a beneficial role in the attenuation of diabetic nephropathy development (Jia et al., 2014). A PPAR- γ agonist is a promising target for treating diabetic nephropathy. PPAR- γ analog increases the insulin sensitivity, has the anti-proliferative and anti-inflammation in kidney (Singh and Mohanty, 2018). The potency of tithonine as PPAR- γ analog is lower than tesaglitazar, an PPAR- γ analog used in this study. Research from Lin (2012) showed that tagitinin A and tirotundin, the sesquiterpene lactone isolated from *Tithonia diversifolia* has an anti-diabetes effect with mechanism as PPAR analogue.

SGLT2 is one of the glucose transporters that is upregulated in diabetic patients. It is located in the early proximal convoluted tubule of the kidney and is accountable for approximately 90% of glucose reabsorption resulting in hyperglycemia and reduced glucosuria (Rosenwasser et al., 2013). Dapagliflozin is a drug used to inhibit SGLT-2. In this study, tithonine showed good potency as SGLT-2 inhibitor compared to dapagliflozin. Diversifolin, tagitinin F dan tirotundin serve as a potential α -glucosidase inhibitor. They have a higher binding affinity than acarbose. Inhibition of α -glucosidase in the intestine could decrease the digestion process result in the decrease of post-prandial blood glucose (Kumar et al., 2011).

ADME-T analysis

The most critical challenge for drugs to be distributed in the body is their ability to be absorbed in the gastrointestinal tract. HIA is a parameter in predicting the absorption of a drug molecule in the human intestine after oral administration, which is evaluated by the level of excretion in urine, bile, and feces. The molecule has good absorption if it is absorbed in at least 90% into the human bloodstream (Borad et al., 2020). In addition, the Caco-2 permeability parameter represents an important challenge faced by oral drugs due to movement across the barrier through the intestinal epithelium originating from human colonic adenocarcinomas that have multiple transport pathways (Pham The et al., 2011). Drugs that have undergone the absorption process are then distributed throughout the body.

The role of the plasma membrane ATP-binding cassette transporter known as P-glycoprotein (P-gp) affects the drug transport process. The activation of P-

gp results in the efflux of the drug outside the cells, so the therapeutic effect is not achieved (Ojo et al., 2021). Another important factor in the distribution process is the ability of the drug to penetrate the blood-brain barrier (BBB) (Borad et al., 2020). Although the compound *Tithonia diversifolia* does not target brain receptors, it will be less advantageous to avoid complications of diseases directly related to the central nervous system. According to Prasad et al. (2014), hyperglycemia conditions can trigger changes in BBB transport function (e.g., glucose, insulin, choline, amino acids), integrity (tight junctions' disruption), and oxidative stress in central nervous system microcapillaries. It was also mentioned that Alzheimer's disease is related to impaired insulin signaling in the brain. Thus, the presence of compounds that can penetrate the BBB can show physiological activity and neurogenesis in the brain that can overcome neurodegenerative diseases. An example of an anti-diabetic drug that can be applied is liraglutide as a GLP-1 analog that acts as a neuroprotective (Prasad et al., 2014; Hunter and Holscher, 2012).

The distributed drug is then metabolized. CYP450 plays a role in the metabolism of drugs and xenobiotics in the liver and intestines. When a compound is found as a substrate for at least one type of CYP450 enzyme, then the compound is anticipated to be easily metabolized by the respective CYP450 enzyme (Babatomiwa et al., 2020). It was also stated that drug interactions occur due to the inhibition of one of the isoforms of the enzyme, which ultimately results in hepatotoxicity (May and Schindler, 2016). The excretion process involves a transporter protein, namely renal organic cation transporter-2 (rOCT-2). These proteins act as organic cation transporters, which work to transport drugs that have a positive charge of polar cations from the blood into tubular epithelial cells to be excreted in the kidneys (Koepsel, 2004). The human liver is the primary site of metabolism that affects toxic agents and various drugs. Hepatotoxicity parameters indicate various types of damage to the liver that can cause organ failure or eventually lead to death. Thus, consideration of hepatotoxicity is essential in the development and drug discovery stages if taken orally (Ojo et al., 2021).

This study demonstrated that various compounds in the *Tithonia diversifolia* leaf could work synergistically in the treatment of diabetic nephropathy because it has various mechanisms of action on several diabetes-related protein targets and has the potential to compete with existing conventional drugs. In addition, compounds that meet the criteria for drug-likeness and a good ADME-T profile can support the development of anti-diabetic drugs from *Tithonia diversifolia*.

CONCLUSION

The phytoconstituents in *Tithonia diversifolia* (Hemsl.) A. Gray demonstrates potential anti-diabetic properties by binding to the protein targets for diabetic nephropathy. Further research is needed to support the development of *T. diversifolia* as therapy of diabetes and its complications.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Adianingsih OR	Khasanah U	Anandhy KD	Yurina V
Concepts or ideas	x			
Design	x	x		
Definition of intellectual content	x	x		
Literature search	x	x	x	x
Experimental studies	x	x	x	
Data acquisition	x		x	
Data analysis	x	x	x	
Statistical analysis	x	x	x	x
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

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