Study of Potential α-Glucosidase Inhibitor from *Tithonia diversifolia*: *In Vitro*, Pharmacokinetics, Toxicology, and Molecular Docking

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Abstract: *The kipahit plant (*Tithonia diversifolia*) is commonly used in traditional medicine to treat various diseases, particularly diabetes. Investigating the bioactive compounds of* T. diversifolia *as α-glucosidase enzyme inhibitors is very promising to be carried out for antidiabetic drug development. A combination of* in vitro *and* in silico *studies was conducted to determine the inhibitory interaction of these compounds.* In vitro *assay was performed using the spectrophotometry method on methanol extracts and revealed that the stems (IC_{50} = 105.0 ppm) exhibited higher bioactivity than the leaves (IC_{50} > 500 ppm). Metabolite profiling of the methanol extract of the* T. diversifolia *stems revealed 94 compounds, which continued for* in silico *methods (pharmacokinetics and toxicology, followed by molecular docking with flexible-rigid method) for antidiabetic activity and drug-likeness parameters. Among the identified compounds, eight showed promise as drug candidates for inhibiting the α-glucosidase enzyme. The compound 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone was the most effective candidate among the eight candidates, based on its high similarity liked positive control, the lowest binding affinity value (−7.739 kcal/mol), and the inhibition constant (97.0 μM). The research findings suggested that the compounds in* T. diversifolia *had the potential to inhibit the α-glucosidase enzyme and could be developed into antidiabetic drugs.*

Keywords: Tithonia diversifolia *(kipahit); metabolites profiling; α-glucosidase enzyme; pharmacokinetics; molecular docking*

■ INTRODUCTION

Diabetes mellitus (DM), or high blood sugar levels, is one of the biggest health threats in the 21st century, where the projected number of deaths due to diabetes increases [1]. DM treatment can be done in various ways, one of which is by inhibiting the work of the α-glucosidase enzyme, which plays an active role in hydrolyzing complex sugars into simple sugars that cause blood sugar levels to increase [2]. The α-glucosidase is an enzyme that plays a key role in the final steps of carbohydrate digestion. In individuals with type 2 diabetes (noninsulin-dependent), its expression is elevated by 1.5 times, contributing to higher postprandial blood sugar levels [3]. The α-glucosidase inhibitors such as acarbose, miglitol, and voglibose have been used as non-invasive DM treatments but have side effects on the body, namely gastrointestinal disorders, including diarrhea, abdominal pain, and bloating [3-5]. Using plants as an alternative to α-glucosidase inhibitors continues to be pursued, one of which is from the secondary metabolite content of plants that are as efficacious as traditional medicine [4]. One of the plants that has many bioactivity properties is the kipahit.

Kipahit (*Tithonia diversifolia*) is a species in the genus *Tithonia* of the Asteraceae family, known for its invasive growth across multiple continents, including Indonesia [6]. In various countries, the *T. diversifolia* plant has been used as a traditional medicine to treat

diabetes (related to polyuria and polydipsia), malaria, sore throat, fever, hepatitis, stomachache, skin wound infections, anti-inflammatory, and small intestine infections [7-8]. Specially for diabetes treatment, its leaves are commonly administrated orally, either pounded or macerated/infused [9]. The bioactivity of *T. diversifolia*, both from the extract and the compounds, has been widely reported, such as antioxidants [10], antibacterial [11], anti-inflammatory [12], immunomodulatory [13], antimalarial [14], even as an antidiabetic [15]. The diverse bioactivities render the kipahit plant a promising candidate for comprehensive development [9]. According to reports on *T. diversifolia's* antidiabetic bioactivity, its methanol and ethyl acetate extracts have IC_{50} values of 24.59 and 26.30 µg/mL, respectively, and can block the activity of the α-glucosidase enzyme. As a positive control, acarbose has an IC_{50} value of 17.35 µg/mL [7]. Additionally, the plant's tagitinin G, tagitinin H, and tagitinin I compounds have shown antihyperglycemic activity in 3T3-L1 adipocytes through *in vitro* glucose absorption assays, bolstering its potential as diabetic treatments [16].

Looking into the α-glucosidase enzyme inhibitory activity of the compounds in this plant can provide valuable information. However, this process is very time-consuming because it requires efforts to separate and analyze each compound individually to determine its enzyme inhibitory activity. The limited studies correlating compound content with α-glucosidase inhibitory activity pose a challenge in antidiabetic research. Therefore, it is important for a comprehensive understanding of the potential of *T. diversifolia* (kipahit) as an α-glucosidase inhibitor. To explore the interaction between these compounds and enzyme inhibition, a combination of *in silico* and *in vitro* testing methods would be very effective [17].

Non-targeted metabolomics based on LC-MS/MS has been widely used to assess the distribution of metabolites in plants. Compounds identified from metabolomics-based studies can be further studied using *in silico* molecular docking techniques to visualize ligand-protein complex interactions [18]. Furthermore, combining *in vitro* and *in silico* methods to evaluate the α-glucosidase enzyme inhibitory activity of compounds identified through LC-MS/MS can provide additional accurate and verified information and prove further potential in developing alternative antidiabetic drugs from plants. This study aims to identify compounds responsible for the α-glucosidase inhibitory activity of *T. diversifolia* using *in vitro* and *in silico* methods.

■ EXPERIMENTAL SECTION

Materials

T. diversifolia (leaves and stem) were collected from Pagaralam city, South Sumatera, Indonesia. The plant was identified by Dr. Nurainas at the Herbarium Anda, Andalas University, and a voucher specimen has been deposited for reference. The chemicals used were methanol (p.a. grade), sodium carbonate (p.a. grade), phosphate buffer pH 6.8 (p.a. grade), bovine serum albumin (BSA), and dimethylsulfoxide (p.a. grade) were purchased from Merck. The α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich.

Instrumentation

Incubator Biosan PST-60HL was used for the sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader. LC-HRMS/MS analysis was conducted using a Vanquish UHPLC Binary Pump (Thermo Scientific) coupled with a Q Exactive Hybrid Quadrupole Orbitrap High-Resolution Mass Spectrometer (HRMS, Thermo Scientific).

Procedure

Plant material and extraction

The stems and leaves of the plants were dried at room temperature, avoiding direct exposure to sunlight. A total of 500.0 g of *T. diversifolia* (leaves and stems) were finely ground and extracted by maceration using methanol in three repetitions. The filtrates were concentrated using a rotary evaporator. After solvent evaporation under reduced pressure, the methanol extracts yielded 52.07 g from the stems and 89.23 g from the leaves. These extracts were successfully prepared and will be evaluated for their potential to inhibit α-

glucosidase enzyme activity as well as analyzed using LC-MS to identify their chemical constituents.

Inhibition of α-glucosidase using in vitro *test*

The α-glucosidase inhibition assay was performed on extracts from leaves and stems using a spectrophotometric method. In this test, 10 µL of the extracts, at various concentrations in DMSO, were mixed with 10 µL of 10 mM *p*-nitrophenyl glucopyranoside (*p*NPG) substrate and pH 6.8 phosphate buffer. The mixture was incubated at 37 °C for 5 min, followed by the addition of 25 µL of a 0.05 U/mL enzyme solution (prepared in phosphate buffer containing 100 mg of BSA). This was incubated again for 30 min at 37 °C. The reaction was stopped by adding 100 µL of 200 mM Na_2CO_3. Absorbance was measured at 405 nm using a microplate reader [19]. The experiment was performed in triplicate, with blanks (DMSO without sample) and acarbose (positive control) as comparisons. The absorbance of both the sample and blank controls was measured using the same procedure, with the following adjustments: Na_2CO_3 was added after the first incubation, followed by a second incubation. The α-glucosidase enzyme was then added, and absorbance was measured afterward. The percentage of inhibition was calculated according to Eq. (1);

$$\text{Inhibition}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \quad (1)$$

where A_0 is the subtraction of blanko absorbance with the blanko control absorbance and A_1 is the subtraction of sample absorbance with the sample control absorbance. The IC_{50} value was determined using GraphPad Prism v8.0, with a sigmoidal plot of log concentration vs. inhibition.

Metabolite profiling by LC-HRMS/MS

LC-HRMS/MS analysis was conducted using the column employed was an Accucore™ Phenyl-Hexyl (100 mm × 2.1 mm ID × 2.6 µm, Thermo Scientific). The samples were analyzed with a gradient elution program, utilizing a binary mobile phase consisting of A (water with 0.1% formic acid) and B (methanol with 0.1% formic acid). The gradient started at 5% B, linearly increasing to 90% B over 15 min, maintaining 90% B from 15 to 20 min, and then returning to the initial conditions (5% B) until 30 min. The flow rate was set at 0.3 mL/min, with an injection volume of 3 µL and a column temperature of 40 °C. The electrospray ionization source was operated in both positive and negative ionization modes with the following parameters: spray voltage of 3.30 kV, capillary temperature of 320 °C, auxiliary gas heater temperature of 30 °C, and nitrogen gas flows of 32 (sheath), 8 (auxiliary), and 4 AU (sweep). HRMS was performed in full MS/dd-MS2 mode, with a mass range of *m/z* 66.7–1000. Resolving powers were set at 70,000 for full MS and 17,600 for dd-MS2 acquisitions. The LC-MS/MS analysis data were processed using Compound Discoverer 3.2 (Thermo Scientific, Waltham, USA) with in-house and online databases. The steps for identifying metabolites include the selected spectra stage, alignment retention time, detecting unknown compounds, grouping unknown compounds, predicting composition, searching mass lists, filling gaps, normalizing areas, and marking background compounds. The metabolites were detected with a maximum mass error limit of 5 ppm, a minimum intensity of 1,000,000, and an S/N ratio of 3. The MS2 spectrum of the detected metabolites was confirmed using the CFM-ID database to identify the metabolites putatively [20].

In silico *pharmacokinetic, toxicological, and molecular docking*

In silico analysis of the extract of *T. diversifolia* was initially conducted to evaluate pharmacokinetics and toxicology. Subsequently, potential compounds were subjected to molecular docking simulation to assess their inhibitory interactions. The SMILES codes of compounds identified via LC-HRMS/MS were retrieved from the PubChem database. All compounds, represented by their SMILES codes, were screened using the Prediction of Activity Spectra for Substances (PASS) Online database (https://www.way2drug.com/passonline/) to evaluate their potential as antidiabetic agents and α-glucosidase inhibitors. The potential compounds identified through PASS Online screening were further analyzed for their physicochemical properties, drug-likeness, toxicity, and pharmacokinetic profiles using SwissADME (http://www.swissadme.ch/).

Molecular docking simulations were performed with AutoDock Vina (version 1.1.2) to estimate the binding affinities of the identified bioactive compounds to the active site of the α-glucosidase enzyme. The crystallographic structure of α-glucosidase enzyme (PDB code: 3WY1) was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/) in .pdb format. The 3D structures of acarbose (positive control) and the compounds identified through Q-ToF LC-MS analysis were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/) in .sdf format. Molecular docking in this research was carried out using flexible-rigid docking with a site-directed approach. All compounds and acarbose were converted to .pdb format using Chimera software (version 1.15). The molecular docking preparation involved optimizing all essential parameters to ensure the accuracy. Water molecules were removed, and missing hydrogen atoms were added to the protein structure using AutoDockTool (version 1.5.6). Gasteiger charges were assigned, and the final structures were saved in .pdbqt format for docking. For docking simulation, a cubic grid box was generated with dimension of −6.582, −15.388, and 19.464 Å along the X, Y, and Z axes, respectively. The grid box was centered at the active site of the target compound, consisting of 20 points along each axis (X, Y, and Z). The accuracy of the docking protocol was validated by calculating the root mean square deviation (RMSD), yielding the value of 0.4065 Å in the redocking process using a rigid docking model. If the RSMD value was below 2 Å, the docking parameters were considered reliable and accurate. Initial visualization of the superimposed 3D structure of the AG enzyme from 3WY1 and the control interactions was performed using PyMOL software (version 2.4.1). The merged file was saved in .pdb format. Finally, the individual merged .pdb files were analyzed to confirm hydrophilic and hydrophobic interactions and the involved amino acid residues using Biovia Discovery Studio Visualizer [21].

■ RESULTS AND DISCUSSION

Extraction and α-Glucosidase Inhibition Activity from *T. diversifolia*

Extraction of dried stems and leaves of kipahit (*T. diversifolia*) by the maceration using methanol as the solvent following the solvent low-pressure evaporation produced 52.07 and 89.23 g of stems and leaves methanol extract, respectively. The inhibition assay of α-glucosidase enzyme activity was carried out on the stems and leaves of methanol extract utilizing acarbose as a positive control. The results (Fig. 1(a)) indicated that the stem methanol extract exhibited greater inhibitory than the leaves methanol extract, with the IC_{50} value of 105.0 and > 500.0 mg/mL, respectively. The inhibitory ability of leaves methanol extract was only 3% regardless of the maximum concentration of 500 mg/mL (Fig. 1(b)). The inhibitory ability of stem methanol extract was 0.64 times than that of acarbose, which had an IC_{50} value of 67.07 mg/mL. These results are consistent with previous research on *T. diversifolia*'s stems from Chad, a country in Central Africa, which reported that the inhibitory

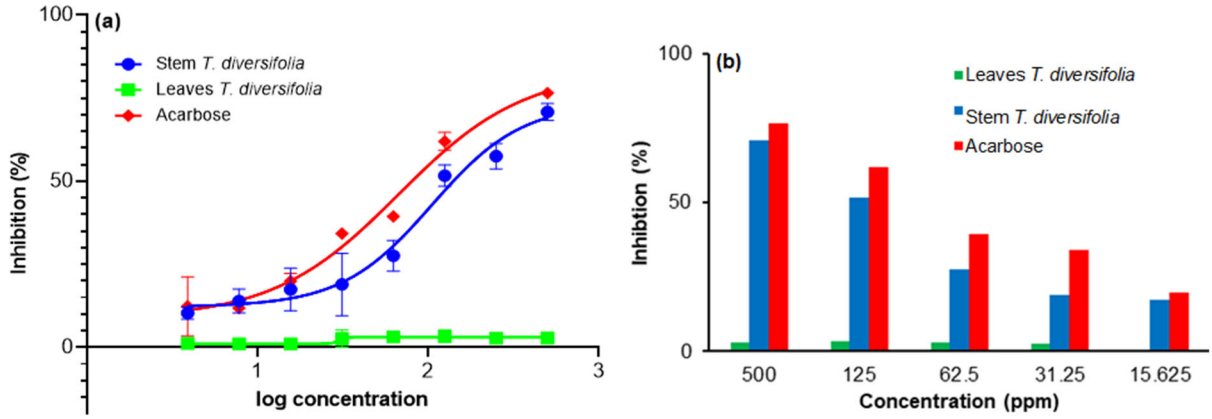

Fig 1. Inhibitory activity of α-glucosidase enzyme in methanol extract of *T. diversifolia* stems and leaves (a) sigmoidal plot using GraphPad Prism and (b) inhibition in different concentrations of extract and acarbose

activity of the methanol extract was 0.71% higher than that of acarbose [7].

Several studies have reported the antidiabetic potential of *T. diversifolia*. Ethyl acetate and methanol extracts of *T. diversifolia*'s stems show α-amylase enzyme inhibitory activities that were 0.76 and 0.84 times greater than acarbose [7]. Furthermore, the water extract of *T. diversifolia* significantly enhanced the release of insulin and lowered blood glucose levels, which were correlated with the hepatic mRNA and protein expression of GLUT2 [22].

Several gallic acid derivative compounds, including protocatechuic and gallic acid, have been reported from *T. diversifolia* [7]. These compounds were shown to inhibit the enzymatic activities of α-glucosidase and α-amylase with IC_{50} values for α-glucosidase were 1.12 and 1.09 μM, and α-amylase were 1.76 and 1.22 μM, respectively [23]. Furthermore, the terpenoid compounds from this plant, such as tagitinin G and I, have shown anti-hyperglycemic activity by enhancing glucose uptake in 3T3-L1 adipocytes without causing toxic effects [16].

Both polar and nonpolar compounds can interact with the α-glucosidase enzyme through distinct mechanisms of inhibition [24]. The polar compounds were reported to interact through hydrogen bonds, electrostatic bonds, or hydrophobic interactions [25]. On the other hand, the nonpolar compounds were predicted based on hydrophobic interactions and cell membrane penetration ability. Since these substances are nonpolar, they dissolve better in lipids, which allows them to pass through intestinal cell membranes and affect the α-glucosidase enzyme's activity [26].

Metabolite Profilling of *T. diversifolia* Stem Extract

Identifying metabolite from the methanol extract of *T. diversifolia*'s stems was conducted using LC-HRMS. Metabolite annotation using Compound Discoverer 3.2 revealed 94 putatively identified metabolites (Table S1). These metabolites included polyketide, phenylpropane, flavonoid, phenolic, terpene, steroid, alkaloid, fatty acid, and alkaloid, while the percentage of each group is shown in Fig. 2(a). Polyketides (21%) were the largest group of compounds found in the stem of *T. diversifolia* followed by phenylpropane (14%), terpenes (14%), alkaloids (13%), and phenolic (10%). Fig. 2(b) presented a Venn diagram comparing compounds identified from the methanol extract of *T. diversifolia* stems with those reported or isolated from several parts of the plant [15]. A total of 8 compounds identified by LC-HRMS have also been previously reported from flowers, roots, and aerial parts of the plant. Stigmasterol and eugenol were detected in the flower, 6-acetyl-2,2-dimethylchromene-8-*O*-D-glucose was found in the aerial parts, and five compounds were identified in the root: (1-(2,2-dimethyl-2*H*-chromen-6-yl)ethanone, (*E*)-*p*-coumaric acid, 6-acetyl-2,2-dimethylchromene, sitostenone, and eupatiochromene.

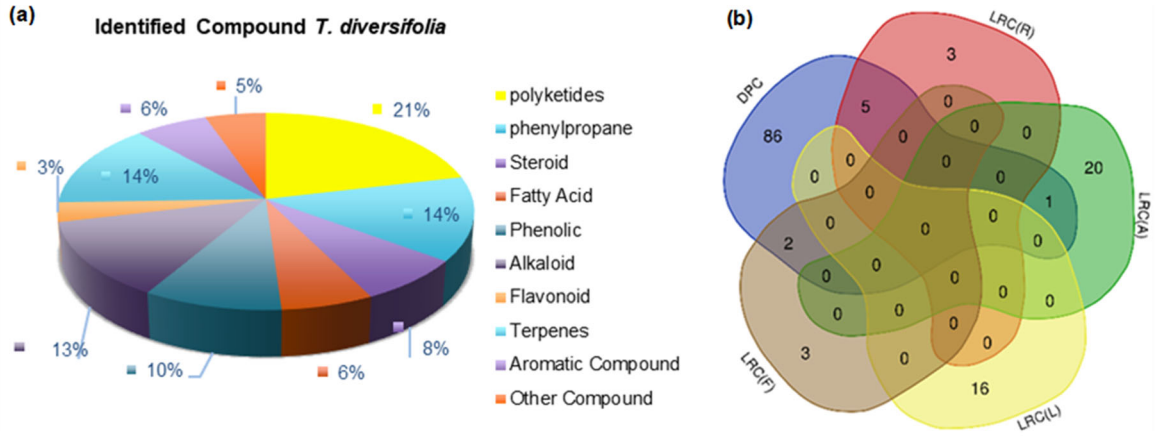

Fig 2. (a) Percentage of compound groups in *T. diversifolia* plants identified using LC-HRMS and (b) Venn diagram of data profiling compound (DPC) of *T. diversifolia* methanol extract against literature review compounds (LRC) of flower [LRC(F)], leaves [LRC(L)], aerial parts [LRC(A)], and roots [LRC(R)]

Several identified compounds were reported to have good activity against type 2 antidiabetic therapy. Cinnamic acid and its derivatives have been identified as potential candidates for antidiabetic drugs. Structural modification of cinnamic acid greatly influences its inhibitory activity against α-glucosidase. Furthermore, substituting a methoxy functional group for 4-hydroxycinnamic acid increased the α-glucosidase inhibitory activity by tenfold [27]. Naringenin, a flavonoid group, inhibited intestinal α-glucosidase while maintaining carbohydrate absorption levels similar to acarbose in rats with type 2 diabetes [28]. The polyketide compound nepodin also stimulated glucose absorption through AMPK activation and subsequent GLUT4 translocation to the cell surface in skeletal muscle [29]. The detected compounds may act synergistically within the extract, contributing to the strong α-glucosidase inhibitory activity observed in vitro for *T. diversifolia* stems.

In Silico Studies: Activity Prediction, Pharmacokinetic, Toxicology, and Molecular Docking

The identified compounds were determined by SMILES through PubChem and continued with PASS Online to predict their activity against antidiabetic and inhibition of the α-glucosidase enzyme. A total of 23 compounds successfully passed the PASS Online screening, with a Probable Activity (PA) value greater than 0.3. Most of these compounds showed higher PA values for antidiabetic activity rather than a-glucosidase inhibition. Thus, it was possible that the antidiabetic activity of the compound was not directly correlated with α-glucosidase enzyme. Table S2 presented the PA values ranging from 0.3 to 0.7, with five compounds classified in moderate criteria (PA 0.5–0.7). The Pa value indicated the structural similarity of the molecule to the most common active compounds that have been tested previously. Compounds with low PA values are less likely to show experimental activity. However, if their activity is confirmed, these compounds may represent a new class for further testing. Therefore, there was no definite correlation between the PA value and the quantitative activity level, especially for typical or novel compounds [30].

Pharmacokinetic and toxicology: Druglikeness parameter

Druglikeness (or drugworthiness) is a concept that describes how likely a chemical compound is to be an effective drug candidate based on its basic physicochemical and pharmacokinetic properties. This evaluation was an essential step in drug development to ensure that the tested compound had a high probability of success in clinical trials and was safe for use in treatment. The drug-likeness parameters of the selected methanol extract compounds of *T. diversifolia* stem that have the potential to be antidiabetic can be seen in Table 1.

Lipinski's rule of five (Ro5) is a set of guidelines used to predict whether a compound would be an effective drug based on its chemical properties [31]. The main parameters of Ro5 include molecular weight < 500 kDa to facilitate penetration of biological membranes, octanol-water partition coefficient (log P) ≤ 5 indicating the lipophilicity of the compound, hydrogen bond donor ≤ 5, and hydrogen bond acceptor ≤ 10 which will affect permeability. A single minor violation of one of those parameters is tolerable [32-33].

Table 1 demonstrated that 21 compounds meet the Lipinski rule, regardless of one violation identified in methyl palmitate, soyasapogenol A, and erucic acid. The other two compounds, azafrin and 6-(α-D-glucosaminyl)-1D-myo-inositol, failed to meet the Lipinski rule criteria and were excluded from further screening, especially ADMET. The next step involved ADMET evaluation using SwissADME software. ADMET analysis plays a vital role in drug development, ensuring that drug candidates are effective and safe, optimizing the dosage to achieve the desired therapeutic effect without causing side effects, and minimizing drug interactions [31]. The compounds selected after going through drug similarity screening whose ADME test can be seen in Table 2.

Absorption was evaluated through gastrointestinal absorption. All test compounds exhibited good absorption in the gastrointestinal tract except erucic acid.

Table 1. Drug-likeness parameters of selected compounds from LC-HRMS of *T. diversifolia* bark extract

Name	Drug-likeness parameters				
	Mol. weight (g/mol)	Log P	Number of H bond donors	Number of H bond acceptors	Lipinski's rule
1-(7-Hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone	234.25	2.12	2	4	0 violation
Sorbic acid	112.13	1.46	1	2	0 violation
3,5-Dimethoxybenzoic acid	182.17	1.79	1	4	0 violation
Carvone	150.22	2.27	1	1	0 violation
Senkyunolide A	192.25	2.69	2	3	0 violation
Methyl palmitate	270.45	4.41	0	2	1 violation
6-Methoxymellein	208.21	2.27	1	4	0 violation
Erucic acid	338.57	5.07	1	2	1 violation
Vanylglycol	184.19	1.61	3	4	0 violation
Nepodin	216.23	1.78	2	3	0 violation
3,4,5-Trimethoxyphenyl acetate	226.23	2.54	0	5	0 violation
(8*E*)-10-Hydroxy-8-decenoic acid	186.25	2.19	2	3	0 violation
Graminiliatrin	434.44	3.23	1	9	0 violation
Geranyl glucoside	316.39	2.97	4	6	0 violation
Phenylglyoxylic acid	150.13	0.91	1	3	0 violation
Nicotinic acid	123.11	0.86	1	3	0 violation
Naringenin	272.25	1.75	3	5	0 violation
Azafrin*	426.59	5.83	3	4	NA
Verrucarol	266.33	2.26	2	4	0 violation
Soyasapogenol A	474.72	3.80	4	4	1 violation
6-(α-D-glucosaminyl)-1D-myo-inositol*	341.31	0.11	9	11	2 violations
N-Stearoylglycine	341.53	4.31	2	3	0 violation
3-Phenylpropanoic acid	149.17	−4.01	0	2	0 violation

Description: * = compound does not meet the Lipinski rule and is not included in the next test; NA not identified in the web system

Table 2. ADME analysis of *T. diversifolia* compound

Name	GI absorp	BBB permeab	P-gp substrate	Cyp substrate inhibitors					Total clearance value	Renal OCT2 substrate
				1A2	2C19	2C9	2D6	3A4		
1-(7-Hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone	high	yes	no	no	no	no	no	no	8.70	ni
Sorbic acid	high	yes	no	no	no	no	no	no	3.46	ni
3,5-Dimethoxybenzoic acid	high	yes	no	no	no	no	no	no	9.06	ni
Carvone	high	yes	no	no	no	no	no	no	9.06	ni
Senkyunolide A	high	yes	no	yes	no	no	no	no	4.53	ni
Methyl palmitate *	high	yes	no	yes	no	no	no	no	6.45	ni
6-Methoxymellein *	high	yes	no	yes	no	no	no	no	7.05	ni
Erucic acid *	low	no	no	yes	no	no	no	no	-0.27	ni
Vanylglycol	high	no	no	no	no	no	no	no	8.73	ni
Nepodin*	high	yes	no	yes	no	no	no	Yes	7.04	ni
3,4,5-Trimethoxyphenyl acetate	high	yes	no	yes	no	no	no	no	4.60	ni
(8*E*)-10-Hydroxy-8-decenoic acid	high	yes	no	yes	no	no	no	no	5.66	ni
Graminiliatrin*	high	no	no	no	no	no	no	yes	13.71	ni

Name	GI absorp	BBB permeab	P-gp substrate	Cyp substrate inhibitors					Total clearance value	Renal OCT2 substrate
				1A2	2C19	2C9	2D6	3A4		
Geranyl glucoside	high	no	no	no	no	no	no	no	2.59	ni
Phenylglyoxylic acid	high	yes	no	yes	no	no	no	no	3.89	ni
Nicotinic acid	high	yes	no	no	no	no	no	no	3.33	ni
Naringenin*	high	yes	yes	yes	no	no	no	yes	8.89	ni
Verrucarol	high	yes	yes	no	no	no	no	no	14.28	ni
Soyasapogenol A	high	no	yes	no	no	no	no	no	10.17	ni
N-Stearoylglycine	high	no	no	no	no	no	no	no	0.05	ni
3-Phenylpropanoic acid	high	yes	yes	no	no	no	no	no	5.52	ni

Description: * = compound were not continued towards toxicity testing; ni = non-inhibitor

Gastrointestinal (GI) absorption is vital in pharmacokinetics because it affects the effectiveness of the drug reaching the target site in the body [34]. The higher GI absorption allows the use of lower doses for therapy. The blood-brain barrier (BBB) permeability refers to the ability of a compound to pass through the BBB and reach the central nervous system (CNS) [35]. P-glycoprotein (P-gp), or multidrug resistance protein 1 (MDR1), is a transport protein in the cell membrane and removes various compounds from cells. Selected compounds from *T. diversifolia* generally can penetrate the BBB or interact with P-gp, allowing compounds to be distributed into brain cells and other cells, indicating their potential as effective drugs.

In ADMET analysis, the metabolism of compounds was assessed through their interaction with cytochrome enzymes found in the liver, namely CYP 1A2, CYP 2C19, CYP 2C9, CYP 2D6, and CYP 3A4. These enzymes metabolize various compounds, including drugs, hormones, and foreign substances (xenobiotics) such as toxins and environmental chemicals [36]. Consequently, the inhibition of CYP enzyme activity will have a negative impact and cause xenobiotic toxicity or the loss of therapeutic benefits from a drug [37]. Table 2 shown that 15 compounds passed the ADMET evaluation, namely 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl) ethanone, sorbic acid, 3,5-dimethoxybenzoic acid, carvone, senkyunolide A, vanylglycol, 3,4,5-trimethoxyphenyl acetate, (8*E*)-10-hydroxy-8-decenoic acid, geranyl glucoside, phenylglyoxylic acid, nicotinic acid, verrucarol, soyasapogenol A, *N*-stearoylglycine, and 3-phenylpropanoic acid. All of these compounds did not inhibit CYP enzyme activity, so they have minimal potential for the risk of side effects or toxicity in the body [38].

Excretion was evaluated based on clearance and inhibition values against OCT2. Total clearance expresses the rate of drug elimination from the body through all possible pathways. A higher total clearance value indicates a faster excretion process for the compound [39]. Organic Cation Transporter 2 (OCT2) is a membrane transporter protein that facilitates organic cations into cells and plays a key role in the kidneys' absorption and elimination of drugs and endogenous substances. Inhibition of OCT2 will affect the elimination of drugs or endogenous substances and has the potential to cause serious side effects or unwanted drug interactions [40]. All selected compounds did not affect the absorption process in the kidneys because they were non-inhibitory to OCT2 [41]. All test compounds also provided good total clearance values except *N*-stearoylglycine and erucic acid (Table 2).

Furthermore, 15 compounds were evaluated for toxicity, including acute oral toxicity in rats (LD_{50}: mol/kg), chronic oral toxicity (LOAEL: mg/kg), hepatotoxicity, ames metagenesis, and dose tolerance (log (mg/kg/day)) (Table 3). Based on hepatotoxicity and ames mutagenesis parameters, 8 compounds were selected for further docking analysis. These compounds were confirmed to be safe regarding both hepatotoxicity and ames mutagenesis., The selected compounds were 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-*2H*-chromen-6-yl)ethanone (**1**), vanylglycol (**4**), 3,4,5-trimethoxyphenyl acetate (**2**), (*8E*)-10-hydroxy-8-decenoate (**5**), geranyl glucoside (**8**),

Table 3. *In silico* toxicity screening of selected *T. diversifolia* compounds

Compound name	LD_{50} (mol/kg)	LOAEL (mg/kg)	Hepatic toxicity	Ames metagenesis	Dose tolerance (log (mg/kg/day))
1-(7-Hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone*	2.17	2.51	Safe	Safe	0.93
Sorbic acid	1.76	2.25	Toxic	Safe	1.83
3,5-Dimethoxybenzoic acid	2.18	2.74	Toxic	Safe	1.64
Carvone	2.18	2.74	Toxic	Safe	1.64
Senkyunolide A	2.16	2.10	Toxic	Safe	0.19
Vanylglycol*	2.05	2.86	Safe	Safe	0.46
3,4,5-Trimethoxyphenyl acetate*	2.28	1.89	Safe	Safe	1.69
(8*E*)-10-Hydroxy-8-decenoic acid*	1.40	2.23	Safe	Safe	0.89
Geranyl glucoside*	1.52	2.81	Safe	Safe	1.30
Phenylglyoxylic acid*	1.67	2.40	Safe	Safe	2.06
Nicotinic acid	1.42	2.40	Toxic	Safe	1.21
Verrucarol	2.64	1.50	Safe	Toxic	-0.41
Soyasapogenol A*	2.75	2.08	Safe	Safe	0.42
N-Stearoylglycine*	1.80	2.49	Safe	Safe	1.56
3-Phenylpropanoic acid	2.40	3.90	Toxic	Safe	1.15

Description: * = compounds selected for molecular docking

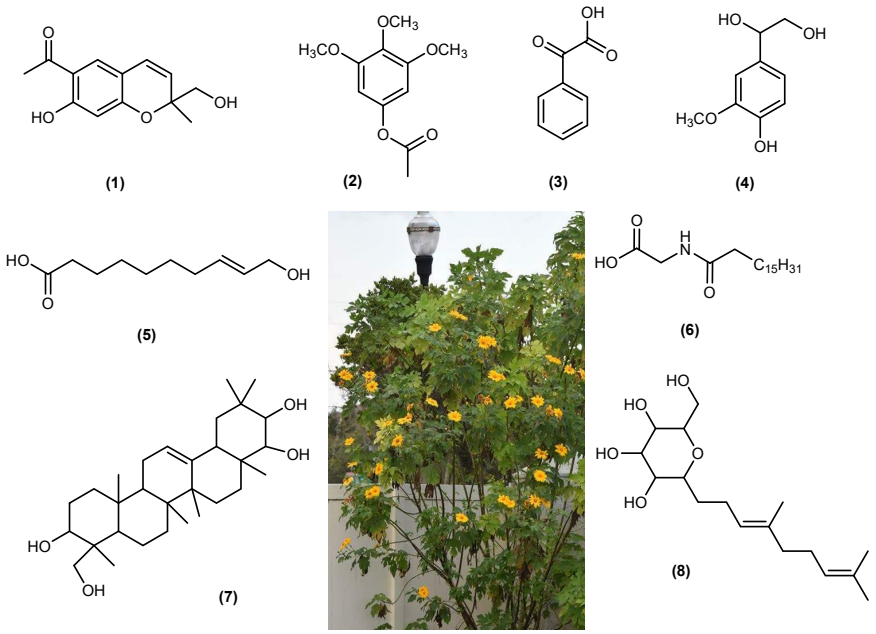

Fig 3. Potential α-glucosidase inhibitory compounds from *T. diversifolia* plant

phenylglyoxylic acid (**3**), soyasapogenol A (**7**), and *N*-stearoylglycine (**6**) (Fig. 3). Ames mutagenesis test was needed to assess the mutagenic potential of a compound. Hepatoxicity is a very important parameter in minimizing liver damage that can lead to organ failure, even death [42]. Acute and chronic toxicity parameters were important for determining safe doses [43].

Molecular docking results analysis

Molecular docking was used to predict the position and orientation of selected compounds (ligands) when binding to the α-glucosidase enzyme receptor and to assess the strength of the interaction as a binding energy value. The increasingly negative binding energy value reflects the conformational stability of the α-glucosidase

ligand-enzyme complex. The rigid-flexible docking approach is a combination of molecular docking techniques where the receptor is prevented from moving during the simulation. In contrast, the flexibility is applied to the receptor residues. This method enables a detailed analysis of binding interactions, providing insights into the ligand-receptor interaction [44-45]. Tables 4 and S3 showed the interaction of amino acid residues and the binding affinity values of selected compounds of a methanol extract of kipahit stems.

Table 4. Molecular docking analysis on selected compounds from *T. diversifolia* stem

Compound	Type of ligand interaction with a receptor					
	Hydrogen bond	Type	Hydrophobic bond	Type	Others bond	Type
Acarbose	ARG200	conventional	PHE166	phi-sigma	ARG400	unfavorable
(positive control)	VAL335	conventional	ILE146	alkyl		
	TYR389	conventional	PHE166	phi-alkyl		
	ARG400	conventional	PHE206	phi-alkyl		
	ASP202	conventional				
	LEU227	conventional				
	GLU271	conventional				
	PRO230	carbon				
1-(7-Hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethenone (**1**)	ARG400*	conventional	PHE147	phi-alkyl	-	-
	GLY228	conventional	PHE166*	phi-alkyl		
	HIS332	conventional	ILE146*	phi-alkyl		
	ASP202*	conventional				
3,4,5-Trimethoxyphenylacetate (**2**)	ARG400*	conventional	PHE206*	phi-alkyl	-	-
	GLU271*	carbon	TYR389	phi-alkyl		
	GLY228	carbon	ILE146*	phi-alkyl		
			PHE166*	phi-phi T shape		
Phenylglyoxylic acid (**3**)	ARG400*	conventional	PHE166*	phi-sigma	ASP333	phi-anion
	HIS332	conventional	PRO230	alkyl		
	ASP333	conventional	ILE146*	alkyl		
Vanylglycol (**4**)	ARG200*	conventional	ILE146*	alkyl	-	-
	ASP333	conventional	PHE166*	alkyl		
	GLU271*	conventional				
	GLY228	carbon				
(8*E*)-10-Hydroxy-8-decenoicacid (**5**)	ARG200*	conventional	PHE206	phi-alkyl	-	-
	GLU271*	conventional	ILE146*	phi-alkyl		
	HIS332	conventional	PHE206*	phi-alkyl		
	THR226	conventional				
N-Stearoylglycine (**6**)	LEU227*	conventional	PHE297	alkyl	ASN301	unfavorable
			PHE147	alkyl		
			PHE166*	alkyl		
			ILE146*	alkyl		
			PHE206*	phi-alkyl		
			TYR235	phi-alkyl		
Soyasapogenol A (**7**)			VAL335	alkyl	LEU300	unfavorable
			VAL334	alkyl	GLY228	unfavorable
			PHE297	alkyl	ASP333	unfavorable
			PRO230	alkyl	PHE166	unfavorable
			PHE397	phi-alkyl		
			PHE147	phi-alkyl		
			ILE146*	phi-alkyl		
			TYR389	phi-alkyl		
			ARG400	phi-alkyl		

Compound	Type of ligand interaction with a receptor					
	Hydrogen bond	Type	Hydrophobic bond	Type	Others bond	Type
Geranylglucoside (**8**)	ASN301	conventional	PHE297	alkyl	-	-
	LEU227*	conventional	TYR389	alkyl		
	ASP333	carbon	PHE206*	alkyl		
			ILE146*	alkyl		

Table 5. Inhibition constant and binding affinity of molecular docking toward α-glucosidase

Compound	binding affinity	Inhibition constants (μM)
Acarbose (positive control)	−5.123	98.0
1-(7-Hydroxy-2-(hydroxymethyl)-2-methyl-*2H*-Chromen-6-yl)ethenone (**1**)	−7.739	97.0
3,4,5-Trimethoxyphenylacetate (**2**)	−5.671	97.8
Phenylglyoxylicacid (**3**)	−6.368	97.6
Vanylglycol (**4**)	−6.173	97.6
(8*E*)-10-Hydroxy-8-decenoicacid (**5**)	−5.709	97.8
N-Stearoylglycine (**6**)	−6.452	97.5
Soyasapogenol A (**7**)	3.091	101.2
Geranylglucoside (**8**)	−7.665	97.1

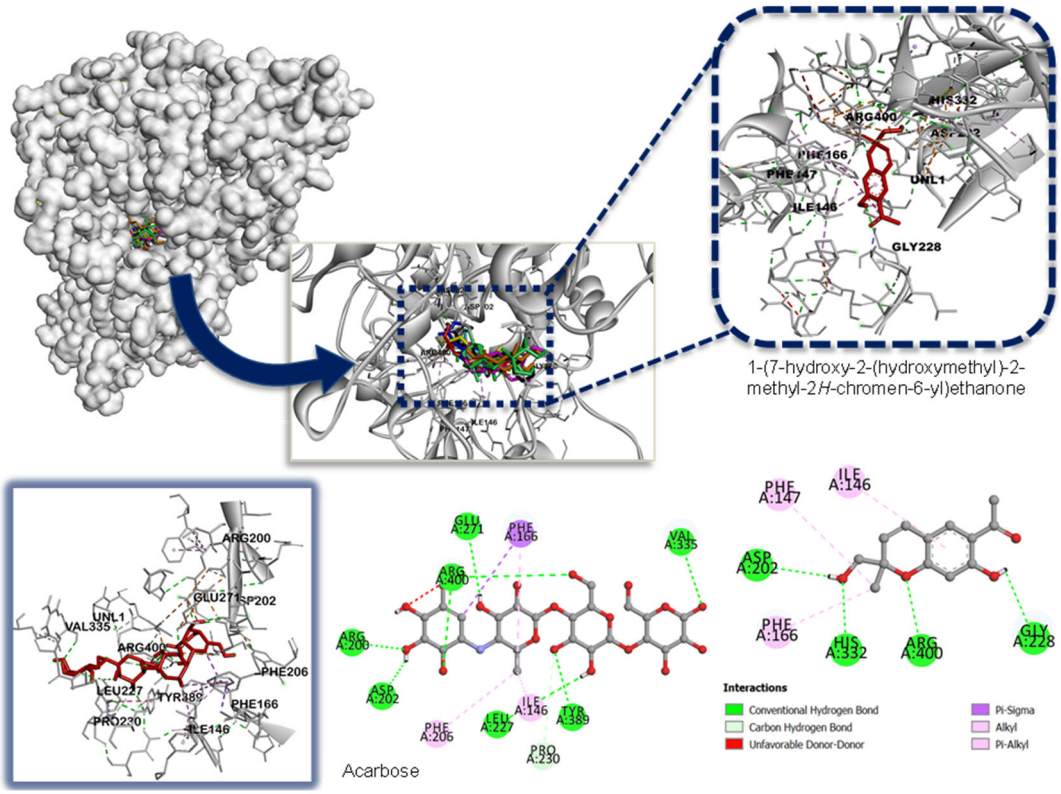

Fig 4. Molecular docking visualization of all selected compounds superimposed on the a-glucosidase enzyme receptor with compound 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone as the best interaction and acarbose as a positive control

Table 4 showed that compounds **1**, **2**, **4**, **5**, and **6** were similar to acarbose in terms of the amino acid residues that interact with it through hydrogen bonds and hydrophobic forces. These interacting amino acid

residues provide insight into the stability of the ligand-receptor complex and help predict whether the ligand can be a viable drug candidate [46]. The binding affinity values represented in Table 5 showed that most compounds except for soyasapogenol A (**7**) have more negative binding affinity values (−5.671 to −7.739 kcal/mol) compared to acarbose as a reference (−5.123 kcal/mol). This suggested that the binding stability of these compounds was better than that of acarbose. The compound with the most negative binding affinity was 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone (**1**), which interacts with seven amino acid residues, i.e., ARG400, GLY228, HIS332, ASP202, PHE147, PHE166, and ILE146. Meanwhile, the soyasapogenol A (**7**) compound has a positive binding energy, indicating a weak interaction with the enzyme α-glucosidase. This compound's interactions are primarily hydrophobic and other interactions, with some exhibiting minimal resemblance to acarbose.

The inhibition constant analysis reveals the ligand's capacity to inhibit the receptor. The inhibition constant value was directly related to the binding affinity, so compound **1** was the most effective at inhibiting α-glucosidase (Table 5). The molecular docking visualizations for 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-2*H*-chromen-6-yl)ethanone (**1**) and acarbose with α-glucosidase were shown in Fig. 4, while the results for other compounds can be found in Table S3.

■ CONCLUSION

The stem of *T. diversifolia* had the potential to be antidiabetic because of its ability as an inhibitor of the α-glucosidase enzyme with an IC_{50} value of 105.0 ppm. Metabolite profiling using LC-HRMS identified 94 compounds, with the most abundant the polyketide group. *In silico* analysis of these compounds revealed 8 potential candidates for inhibiting α-glucosidase. Among these, the polyketide compound containing a chromen ring, 1-(7-hydroxy-2-(hydroxymethyl)-2-methyl-*2H*-chromen-6-yl)ethanone, emerged as the most promising candidate based on its interaction similarity with positive controls (similar interaction residues: ARG400, ASP202, PHE166, ILE146), binding affinity values (7.739 kcal/mol), and inhibition constant parameters (97.0 μM).

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■ CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Daniel Alfarado: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. Shaum Shiyan: Writing – review & editing, Resources, Project administration, Methodology. Ferlinahayati: Writing – review & editing, Validation, Conseptualization, Resources, Methodology, Funding acquisition. All authors have read and agreed to the final version of this manuscript.

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