Antidiabetic Activity and Identification of Active Compounds of *Toona sinensis* Leaf and Bark Extracts and Molecular Inhibition Tests

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Abstract. Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia, and its global prevalence continues to increase. One potential alternative treatment approach is the use of medicinal plants such as *Toona sinensis*. This study aimed to evaluate the α-glucosidase inhibitory activity of ethanol extracts of *T. sinensis* leaves and stem bark using in vitro and *in silico* analyses. The test showed that the stem bark extract had an IC_{50} value of 200.83 µg/mL, which was lower than that of the leaves, and contained 30 secondary metabolite compounds based on LC-MS/MS results. In contrast, the leaf extract contained only 22 compounds. These compounds were predicted to be bioactive by using SMILES and pChEMBL. The prediction results showed that several compounds, particularly those from the flavonoid and phenolic groups, have potential as α-glucosidase inhibitors. This finding indicates that the stem bark of *T. sinensis* has more potential as an antidiabetic phytotherapy candidate than its leaves.

1 Introduction

Diabetes mellitus is no longer just a health issue but a growing global crisis. This condition is characterized by high blood glucose levels owing to complex disturbances in the metabolism of carbohydrates, lipids, and proteins. Data from the International Diabetes Federation (IDF) are truly concerning; in 2021, more than 460 million people worldwide were living with diabetes [1], and this number is projected to exceed 700 million by 2045. Indonesia has also experienced a significant impact from this condition, with the prevalence of diabetes mellitus reaching 10.3 million in 2021, and is expected to continue rising to 16.7 million cases by 2045 [2]. To address this major challenge, various strategies have been developed to manage diabetes. A holistic approach that includes dietary regulation, regular physical activity, medication use, routine blood sugar monitoring, and ongoing health education is key [3]. In addition, the role of glucose-lowering drugs, such as metformin and glibenclamide, is crucial. Metformin, an oral antidiabetic of the biguanide class [4], has long been the primary choice in the management of diabetes mellitus. Although modern medications have proven effective, there has been a significant shift in interest towards the search for alternatives, as the side effects caused by synthetic chemical drugs often pose difficulties for patients, ranging from digestive issues to the risk of hypoglycemia, which can

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affect quality of life. The strong hereditary belief in the efficacy of natural ingredients in many cultures of Indonesia is a driving factor for the use of herbal medicine. Globally, countries such as China have demonstrated substantial progress in the use of herbal medicines both traditionally and in modern contexts; for example, one plant consumed by Chinese people to lower glucose levels is *Toona sinensis*.

Toona sinensis, also known as surian leaves, has been identified as a plant with the potential for use as an antidiabetic herbal medicine owing to its diverse phytochemical content, such as terpenoids, flavonoids, and phenylpropanoids, which have been linked to various pharmacological activities, including antidiabetic, antitumor, anticancer, antioxidant, and anti-inflammatory effects [5]. Surian leaves (*Toona sinensis*) are known to contain the major flavonoid quercetin. Quercetin has been shown to inhibit α-glucosidase activity in vitro. Quercetin has also shown potential in overcoming hyperglycemia in animal experiments, [6] demonstrating that *T. sinensis* can reduce blood glucose levels in rats by 72.83% and 70.61%. In this study, the bark of surian reduced glucose levels more significantly than the leaves, and both were able to maintain the body weight of the rats. In addition, a study [7] found phytochemical compounds, such as flavonoids, triterpenoids, saponins, and tannins, in both the bark and leaves of surian. An inhibition test of the extract against α-glucosidase based on IC_{50} was also performed, with the highest IC_{50} value found in the ethanol extract of the leaves and bark (approximately 177.98). This was attributed to the presence of hydrolyzed tannins in the leaves, whereas condensed tannins were present in the bark extract.

Numerous in vitro and in vivo studies have been conducted to explore the potential of active compounds found in the leaves and bark of *T. sinensis* for the treatment of diabetes mellitus. However, studies involving in vitro and in silico testing to investigate the potential of active compounds from surian plants originating from Indonesia as antidiabetic agents have not yet been conducted. Therefore, understanding of the potential of Indonesian *T. sinensis* leaf and bark extracts for drug development remains limited. Therefore, the authors consider it important to conduct molecular docking studies of inhibitors such as acarbose for antidiabetic purposes. This step is crucial in the effort to identify new drugs that are more effective and safer for the treatment of diabetes mellitus.

2 Materials and method

1.1 Materials

In this study, the leaves and bark of the Surian (*Toona sinensis* Merr.) aged 25 years obtained from Sumedang Regency, West Java, Indonesia.

1.2 Sample Preparation and Extraction

The samples were dried without direct exposure to sunlight, ground into a powder with a 60 mesh size, and the dry powder was stored in a closed container. Extraction was performed with 70% EtOH using the maceration method for 24 h, repeated three times, and then evaporated using a rotary evaporator. The resulting extract was stored at temperatures below 20 °C.

1.3 α-Glucosidase Enzyme Inhibitory Activity [8]

The tested sample was dissolved in DMSO and diluted with phosphate buffer at pH 7 to produce an extract solution with a concentration of 1% (b/v). After that, 100 mM phosphate buffer and 0.5 mM p-NPG substrate solution were added, followed by incubation for 30 minutes. Next, 200 mM Na2CO3 was added and the absorbance of the solution was measured at a wavelength of 410 nm. Acarbose 1% (b/v) was used as the positive control. The percentage inhibitory activity was calculated using the following equation:

$$\%\text{Inhibition} = (B-S/B) \times 100$$

Description:
B = Absorbance of blank minus absorbance of blank control (B1-B0)
S = Absorbance of sample minus absorbance of sample control (S1-S0)
The IC_{50} value was calculated using the linear regression equation y = ax + b, with the variable y set to 50 and the value of x representing the IC_{50}.

1.3 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) analysis [9]

Samples showing activity in α-glucosidase inhibition testing and total phenolic analysis, using 5µL of sample solution at a concentration of 1,000 µg/mL with a flow rate of 0.2 mL/min, were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS) for 23 min at 20°C using a mobile phase consisting of a mixture of acetonitrile and water and a stationary phase with a C18 column.

1.4 *In silico* Analysis

The 3D structure of the receptor was downloaded from the Protein Data Bank website (rcsb.org) with the code 2QMJ and prepared by removing the water molecules. The structure of the test ligand was obtained from the LC-MS/MS results and saved in sdf format; the natural ligand was separated from the receptor using Yasara Structure [10]. Gridbox validation using the PDB file was performed with sizes ranging from 3 to 10 Å at 1 Å intervals. The natural ligand was removed from the grid box and reinserted into a separate file. The redocking process was set in the dock_runlocal.mcr file and was carried out 50 times. After validation, the selected gridbox should have the most positive binding energy value, indicating a strong potential interaction between the ligand and receptor. The virtual screening process was continued by combining the best receptors from the gridbox validation results with the prepared ligands. Each ligand was then separated and saved as a *. sce format. Docking was performed 50 times by opening the dock_runscreening.mcr file in the YASARA Structure folder. The docking results are saved in a working folder in *. txt format for binding energy values and *. yob format for receptor-ligand interaction visualization. 3D visualization was performed using PyMol, with the receptor displayed as a surface, the ligand as a stick, and colored protein functional domains.

1.5 Prediction of Toxicity Properties

The predicted toxicity properties are acute oral (LD50), toxicity class, hERG (*human Ether-a-go-go-Related Gene*) II inhibitor, chronic oral, and carcinogenicity, using pKCSM to determine ligands that pass toxicity screening, pass the test, and exhibit toxic properties.

1.6 Data Analysis

The research design used was a completely randomized design (CRD) analyzed using Microsoft Excel, ANOVA test, and further tested using Duncan's Multiple Range Test (DMRT) with SPSS.

3 Results and discussion

3.1 α-Glukosidase enzyme inhibition activity

Inhibition of the α-glucosidase enzyme begins with enzyme screening to obtain the best enzyme to use, followed by sample screening to determine the test concentration value. In this study, a concentration range of 25–500 (μg/mL) was determined, with seven positive control points (using samples) and one blank point (without samples and replaced with DMSO). If the r value reaches 0.97–0.99, the IC_{50} calculation will be carried out on both the samples and acarbose [10].

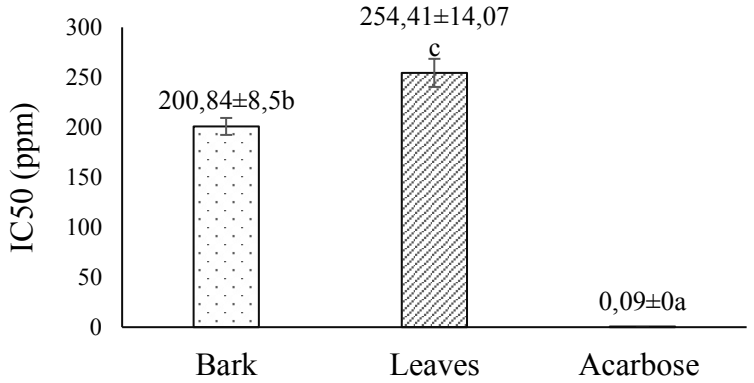

Fig. 1. Comparison of IC_{50} values of *Toona sinensis* in bark, leaves, and acarbose

The IC_{50} value in the bark was higher than that in the leaves, at approximately 200.84 ppm for the bark, while in the leaves it reaches 254.41 ppm. The difference in IC_{50} between these two extracts was due to the higher content of secondary metabolites found in the bark than in the leaves. This difference is the opposite of the findings in [7] because the trees tested in this study were older than those in the previous research. Therefore, the phytochemical content of flavonoids in the bark was higher, resulting in a greater ability to inhibit α-glucosidase. In this study, acarbose was found to have a very strong potential for enzyme inhibition, as reflected by its much lower IC_{50} value.

3.2 Secondary Metabolite Profile of Leaves and Stem Bark of *Toona sinensis* Based on LC-MS/MS Analysis

The results of LC-MS/MS analysis showed the presence of secondary metabolite groups in both the bark and leaves. The presence of the same secondary metabolites indicates that both have a similar chemical basis for providing pharmacological effects. However, differences in relative abundance and retention time, which indicate the presence of isomers or different matrix effects in the analysis, explain why previous studies [7] found differences in effectiveness between bark and leaves. The bark contains higher concentrations of certain

active compounds, resulting in a more optimal reduction in blood glucose levels [6]. LC-MS/MS analysis showed that quercetin was only detected in the leaves, with an abundance of 0.566%, whereas it was not found in the bark. This finding supports the hypothesis that quercetin is present in leaves [5].

Table 1. LC-MS/MS Results of *T. sinensis* Leaves and Bark

Name	RT (min)	% Abundance	Compound Group	RT (min	% Abundance
Dihydroxymandelic acid	5,971	64,30	Phenolic	6,239	0,68
Gallic acid	1,968	10,8	Phenolic	6,251	33,8
Quercitrin	9,412	6,1	Flavonoid	1,062	0,53
Cianidanol	6,22	0,98	Flavonoid	4,583	1,89
Kinoprene	18,724	0,352	Fatty Acids	28,943	0,57
Curcumene	20,485	0,488	Prenol Lipids Terpenoid	1,095	2,06

Apart from phenolics and flavonoids, this section also identifies several compounds from the terpenoid and benzenoid groups, which are known to have higher enzymatic and pharmacological activities. The IC_{50} value of the stem bark extract was lower than that of the leaf extract, indicating a stronger potential for α-glucosidase enzyme inhibition. This is most likely influenced by the presence of active compounds in greater variety and quantity, as well as the synergy between these compounds [11].

3.3 Molecular Docking Simulation of the α-Glucosidase Enzyme

Molecular docking was performed on 20 test compounds from leaves, 29 compounds from bark, and one reference compound against 2QMJ. Before the analysis, grid box size validation was carried out to determine the optimal grid box size and to serve as a method validation parameter for molecular docking [12]; the best grid box size obtained was 2 Å, with RMSD and binding ΔG values of 1.516 Å and -7.797 kcal/mol, respectively.

Table 2. Molecular Docking Results of *T. sinensis* Leaves and Bark

Name	Compound Group	ΔGvalues [kkal/mol]	
		Leave	Bark
Dihydroxymandelic acid	Phenolic	-6,178	-6.178
Gallic acid	Phenolic	-5,935	-5,935
Quercitrin	Flavonoid	-7,828	-7,828
Cianidanol	Flavonoid	-7,397	-7,397
Kinoprene	Fatty Acids	-5,871	-5,871
Curcumene	Prenol Lipids Terpenoid	-6,011	-6,011

Description: = ligands pass molecular docking selection

The bark was analyzed in 50 runs with 29 test ligands downloaded from PubChem using SMILES. The difference in the number of ligands passing the molecular docking selection in the bark compared to the leaves was due to the higher number of secondary metabolite compounds found in the bark of *Toona sinensis*. Molecular docking against antidiabetic

inhibitor targets, such as the enzyme targeted by acarbose, showed that quercitrin passed this test. The sugar moiety attached to the aglycone in quercitrin has been shown to enhance its solubility and absorption in the small intestine through interactions with sodium-dependent glucose transporter receptors.

Quercitrin also has the potential to alleviate liver injury induced by N-acetyl-p-aminophenol (APAP). This compound acts by inhibiting the Janus kinase (JNK) and p38 signaling pathways while simultaneously activating defense genes and suppressing pro-inflammatory genes in HepG2 cells and animal models [13]. Given the involvement of the liver in glucose metabolism and the increased susceptibility of people with diabetes to liver damage, the hepatoprotective activity of *T. sinensis* leaf extract shows great potential as a liver-protective raw material, especially in diabetic patients. In addition to quercitrin, several other tested ligands that were identified as having passed the molecular docking test were mostly derived from the bark of *T. sinensis*. This study supports the notion that the bark possesses superior compounds with potential antidiabetic properties.

Table 3. Test ligands that passed the molecular docking test found in the leaves and bark of *T. sinensis*

Name	Compound Group	ΔGvalues [kkal/mol]	Sample
Gambiriin A1	Flavonoid	-9,565	Bark
(+)- Procyanidin B2	Flavonoid	-9,025	Bark
Tiliroside	Flavonoid	-8,571	Bark
3-5-6-7-tetrahydroxy-2	Prenol Lipids Terpenoid	-8,189	Leave
Procyanidin C1	Flavonoid	-8,100	Bark
3-hydroxy-2	Phenylpropanoids	-7,904	Bark

3.4 Ligand Toxicity as a Test Compound *In Silico*

Prediction of the toxicity of active compounds is a selection parameter for compounds with potential as α-glucosidase inhibitors. Toxicity prediction is carried out based on several parameters such as acute toxicity (LD50), toxicity class, carcinogenicity, hERG II inhibitor, and chronic oral toxicity [14].

Table 4. Predicted toxicity of test ligands found in the leaves and bark of T. sinensis

Name	Carcinogenic	hERG II inhibitor	Chronic Oral (LOAEL)	Toxicity	
				LD_{50} (mol/kg)	Class
Dihydroxymandelic acid	-	No	1.517	1,881	4
Gallic acid*	+	No	306	2218	4
Quercitrin*	+	No	3022	2,586	5
Cianidanol	-	No	2.5	2.428	6
Kinoprene	-	No	3043	1453	6
Curcumene	-	No	1297	1873	4
Gambiriin A1*	-	Yes	385	2719	5
(+)- Procyanidin B2*	-	Yes	4349	2482	5

Tiliroside*	-	Yes	4096	2578	5
3-5-6-7-tetrahydroxy-2	-	No	3529	1128	6
Procyanidin C1*	-	Yes	7463	2482	5
3-hydroxy-2	-	No	2462	1982	6

Description: = ligands pass molecular docking selection
= the ligand did not pass the toxicity screening
= ligands that have toxic properties

The ligands that passed the toxicity selection and molecular docking tests are 3-5-6-7-tetrahydroxy and 3-hydroxy compounds found in the leaves and bark of *T. sinensis*.

4 Conclusion

The bark extract of *Toona sinensis* showed a higher antidiabetic potential than the leaf extract, with a lower IC_{50} value and a greater number of secondary metabolite compounds. In silico bioactivity predictions supported the possibility that these compounds act as α-glucosidase inhibitors, making the bark of *T. sinensis* a potential candidate for the development of antidiabetic phytotherapy.

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