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Original Article

A preliminary study on α-glucosidase inhibitory and antidiabetic activity of Indonesian *Toona sinensis* bark extract in alloxan-induced diabetic rats

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Abstract

This study aimed to determine the antidiabetic activity of *Toona sinensis* bark extract. Spectrophotometry was used to measure the inhibitory activity of α-glucosidase. Its antidiabetic activity was determined *in vivo*. Phytochemical analysis showed that the ethanol and water extracts contained flavonoids, saponins, and phenolic hydroquinones. In addition, alkaloids and tannins were found in the ethanol extract. Inhibition of α-glucosidase activity showed that the half maximal inhibitory concentration values for the ethanol extract and water extract were 0.60 µg/mL and 3.60 µg/mL, respectively. Blood analysis revealed that a dose of 150 mg/kg body weight (BW) of the ethanol extract reduced the blood glucose level by 70.8%. Meanwhile, glibenclamide (0.25 mg/kg BW) and 300 mg/kg BW of the ethanol extract decreased the levels by 69% and 52%, respectively. We concluded that the ethanol extract of *T. sinensis* has more potential as a herbal remedy at a dose of 150 mg/kg BW than at 300 mg/kg BW.

Keywords: alloxan, antidiabetic, α-glucosidase, diabetic rats, *Toona sinensis*

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by an inherited or acquired deficiency in insulin production by the pancreas or by the ineffectiveness of the insulin that is produced (Nagappa, Thakurdesai, Rao, & Singh, 2003). The two main types of diabetes are type 1 and type 2. Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-immune destruction of pancreatic cells. It is more likely to occur in family members of affected patients (Bottini, Vang, Cucca, & Mustelin, 2006). Type 2 diabetes, which accounts for more than 90% of the cases, is caused by resistance to the action of insulin combined with impaired insulin secretion (Warren, 2004).

DM can be treated by anti-diabetic drugs. Some of these drugs are derived from plants and spices that have antioxidant and antidiabetic activities (Minaiyan, Ghannadi, Mohavedian, & Hakim-Elahi, 2014; Rates, 2001; Zolfaghari, Shokoohinia, Sadeghi, Mahmoudzadeh, & Minaiyan, 2012). In Indonesia, the alternative medications that are used locally are usually derived from herbaceous plants. One of these plants that may have potential for drug development is *Toona sinensis* (Meliaceae) which is widely distributed in Southeast Asia (Edmonds & Staniforth, 1998). All parts of the plant, including the seeds, bark, root bark, petioles, and leaves, are claimed to have medicinal efficacy (Cho *et al.*, 2003a, 2003b). *T. sinensis* leaves have been used to treat enteritis, dysentery, metabolic diseases, general infections, and itching (Perry, 1980). The bark is used as an astringent and depurative agent, the powdered root is used as a corrective, and the fruits are used to treat eye infections (Perry, 1980).

Previous reports have demonstrated that *T. sinensis* leaf extracts have multiple applications that include anti-proliferation of human lung adenocarcinoma cells (A549) (Chang, Hung, Huang, & Hsu, 2002), hypoglycemic effects (Chang *et al.*, 2002; Fan *et al.*, 2007), treatment of diabetes-associated high blood pressure (Yang, Hwang, & Hong, 2003), augmenting uptake of glucose in 3T3-L1 adipocytes

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(Hseu *et al.*, 2008; Hsu, Yang, Hwang, & Hong, 2003), and antioxidant activities using different antioxidant models (Hseu *et al.*, 2008). The highest dose tested (5.0 g/kg body weight [BW]) did not show an acute lethal effect in mice (Liao *et al.*, 2007).

All previous studies were reported on plants cultivated in Taiwan or mainland China. The potential of *T. sinensis* from Indonesia, especially the bark as an antidiabetic agent, has not yet been studied. This is important because environmental conditions and geographic variations are known to affect the chemical composition of plants (Figueiredo, Barroso, Pedro, & Scheffer, 2008). Here, we report the phytochemical components, α-glucosidase inhibitory activity, and antidiabetic activity of Indonesian *T. sinensis* bark extract using alloxan-induced diabetic Sprague-Dawley rats as a bioassay.

2. Materials and Methods

2.1 Plant material

In March 2011, bark was stripped from a *T. sinensis* trunk in the town of Sumedang (6°51′35″S, 107°55′15″E; altitude 650 m), West Java, Indonesia. The plant was identified and deposited at the Department of Forest Engineering, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia with the voucher specimen number SF.03.2011. The chipped bark (5000 g) was dried at 50 °C until the moisture content was <10%. It was then ground in a Wiley mill (Thomas Scientific, New Jersey, USA). The resultant meal was sieved through 40- and 80-mesh screens.

2.2 Extraction of *T. sinensis* bark

The bark meal (1.5 kg air dried) was macerated three times with 70% (v/v) ethanol (Sigma-Aldrich, Darmstadt, Germany) at room temperature for 48 h (Ningappa, Dinesha, & Srinivas, 2008). An aqueous extract was produced by heating a mixture of bark powder and water (1:10) at 100 °C for 4 h. The extracts were filtered and concentrated using a rotary evaporator (Eyela N-1100, Tokyo, Japan) at 40 °C. The crude extracts were used in our biological assays. Water and 70% (v/v) ethanol were used as solvents for safety purposes for human medical applications.

2.3 Qualitative phytochemical analyzes

The extracts were screened for the presence of secondary metabolites such as alkaloids, saponins, flavonoids, phenolic hydroquinones, triterpenoids, and tannins. All solvents used were analytical grade (Merck, Darmstadt, Germany). Phytochemical contents were detected qualitatively using Harborne's procedures (1987).

2.3.1 Alkaloids

The extract (100 mg) was combined with 3 mL of chloroform and three drops (~150 µL) of ammonia. The chloroform fraction was separated and acidified with 10 drops (500 µL) of H_2SO_4 (2.0 M). Three H_2SO_4 fraction samples were each combined with Dragendoff, Meyer, or Wagner reagents. The presence of alkaloids was indicated by the formation of a white precipitate upon addition of the Meyer reagent, an orange precipitate with Dragendorff reagent, and a brown precipitate with Wagner reagent.

2.3.2 Saponins

Aliquots of 100 mg extract were added to 2 mL of H_2O and heated for 5 min. The mixtures were cooled and then stirred for >10 minutes. The appearance of foam in more than 30 min indicated the presence of saponins.

2.3.3 Flavonoids

Aliquots of 100 mg extract were soaked with 2 mL of 30% (v/v) methanol, heated, and then filtered. Filtrates were combined with 1 drop (50 µL) of concentrated H_2SO_4. A red color indicated the presence of flavonoids.

2.3.4 Phenolic hydroquinone

Aliquots of 100 mg extract were soaked with 2 mL of 30% (v/v) methanol, heated, and then filtered. The filtrates were combined with 1 drop (~50 µL) of NaOH 10% (w/v). Formation of a red color indicated the presence of phenolic hydroquinones.

2.3.5 Triterpenoids

Aliquots of 100 mg extract were combined with 2 mL of 30% ethanol, heated and filtered. The filtrates were evaporated and then diethyl ether was added. Liebermann-Burchard reagent (3 drops [~150 µL] of acetic acid anhydride and 1 drop [~50 µL] of concentrated H_2SO_4) was added to the ether layer. A reddish-violet pigment indicated the presence of triterpenoids.

2.3.6 Tannins

Aliquots of 100 mg extract were combined with 2 mL of H_2O and heated for 5 min. The mixtures were filtered and the filtrates combined with $FeCl_3$ 1% (b/v). The presence of tannins was indicated by the formation of a dark-blue or greenish-black color.

2.4 α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed as described previously (Sancheti, Sancheti, Bafna, & Seo, 2011) using an ELISA test kit (Bio-Rad, Singapore). Acarbose was used as the standard or positive control in a series of concentrations (2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 µg/mL). A standard solution, a blank, and sample concentrations of 12.5, 6.2, 3.1, and 1.5 µg/mL were placed into 50 µL capacity microplate wells (96-well microplate type, Bio-Rad, Singapore). To each of the wells, 50 µL of 100 mM phosphate buffer (pH 7.0) was added. All chemical reagents were purchased from Merck (Darmstadt, Germany). A total of 25 µL of α-glucosidase at a concentration of 1.0 mg/mL in 100 mM phosphate buffer (pH 7.0) was placed into the microplate wells. The enzyme substrate that contained 50 µL of 100 mM phosphate buffer (pH 7.0) and 25 µL of 500 µM 4-nitrophenyl α-D-glucopyranoside in 100 mM phosphate buffer (pH 7.0)

was added to start the assay. All treatments were incubated at 37 °C for 30 min. The enzyme reaction was stopped by adding 100 µL of 200 mM Na_2CO_3. All tests were replicated three times. The reaction product was measured with a microplate reader (Bio-Rad, Singapore) at 400 nm. The percentage inhibition was then calculated to determine the half maximal inhibitory concentration value (IC_{50}) value from the following equation: % inhibition = [1−(absorbance of sample/absorbance of positive control)]×100.

2.5 Tested animals

Male Sprague-Dawley rats (250–350 g) obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia were fed with a standard laboratory diet and distilled water *ad libitum* for an acclimatization period of 2 months until the age of 3.5–4 months. All animal experiments were approved by the ethics committee of the animal laboratory, Department of Biochemistry, Bogor Agricultural University, and performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Body weight of the rats was measured on days 4, 7, 10, and 14 before treatment with alloxan.

2.6 Experimental design

The animals were randomly divided into five groups with four rats in each group. Untreated normal rats were in Group A. These rats were intraperitoneally administered NaCl 0.9% (w/v) (Merck, Darmstadt, Germany) and orally administered distilled water 1.0 mL daily for 14 days. Diabetic control rats in Group B were orally administered distilled water 1.0 mL daily for 14 days. The diabetic rats in Group C were orally administered glibenclamide (0.25 mg/kg) daily for 14 days. The diabetic rats in Group D were orally administered ethanol extract (150 mg/kg) daily for 14 days. Finally, the diabetic rats in Group E were orally administered ethanol extract (300 mg/kg) daily for 14 days.

The rats in Groups B through E were injected intraperitoneally with alloxan (Sigma-Aldrich, Darmstadt, Germany) 150 mg/kg on the first day. Treatment with the extracts and glibenclamide was started 48 h after the alloxan injection. Blood samples were obtained from the tail vein of the rats after fasting for 16 h. The blood glucose levels were measured using an Accu-Check® glucometer (Miles Inc, New York, USA). Fasting blood glucose and body weight were measured on days 0, 4, 7, 10, and 14 after induction (Cing, 2010).

2.7 Histopathology

Necropsies were conducted at the Laboratory of Histopathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia. The results were analyzed at the Veterinary Research Institute (Balitvet), Bogor, Indonesia. All animals were sacrificed by cervical dislocation on day 14. The pancreases were excised, isolated, and subjected to histopathological studies and microscopy (Bansal *et al.*, 2012). Pancreatic tissues were immediately removed and washed with ice-cooled saline and then fixed in 10% (v/v) neutral formalin (Merck, Darmstadt, Germany). Sections were stained in hematoxylin (Sigma-Aldrich, Darmstadt, Germany) and eosin (Sigma-Aldrich, Darmstadt, Germany), mounted, and observed under a microscope (CX-21 Halogen Olympus, Tokyo, Japan).

2.8 Statistical analysis

This study used a completely randomized design with five treatment groups and four replications. As a measure of inhibitory activity, the concentrations required for IC_{50} of α-glucosidase's activity were determined. Values reported of five experiments are the mean±standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA, PASW Statistics 18.0.0, Hong Kong). Duncan's test was used for multiple comparisons. The values were considered to be significantly different when $P<0.05$.

3. Results

3.1 Phytochemical constituents

Phytochemical assays of the ethanol extract of *T. sinensis* bark revealed the presence of flavonoids, tannins, phenolic hydroquinones, saponins, and alkaloids, whereas the hot water extract tested positive for the presence of flavonoids, phenolics hydroquinones, and saponins (Table 1). The average yields of the ethanol and hot water extracts of *T. sinensis* were 4.8 and 2.6% w/w, respectively.

Table 1. Phytochemical constitutes of *T. sinensis* bark extracts.

Test	Extracts	
	70% ethanol	Hot water
Alkaloids	+	−
Flavonoids	+	+
Phenolic hydroquinone	+	+
Saponins	+	+
Triterpenoids	−	−
Tannins	+	−

(+) Positive; (−) negative.

3.2 α-Glucosidase inhibition

The effect of *T. sinensis* bark extracts against α-glucosidase was evaluated and the results, expressed as IC_{50} values, indicated that the ethanol extract possessed a high potency with an IC_{50} value of 600 ng/mL. This value was higher than the hot water extract (3.60 µg/mL). Nevertheless, neither extract was better than acarbose (positive control), which gave a value of 80 ng/mL (Table 2).

Table 2. Inhibitory effect of *T. sinensis* bark extracts and acarbose against α-glucosidase.

Samples	IC_{50} (µg/mL)
70% ethanol extract	0.60^a
hot water extract	3.60^b
acarbose	0.08^c

The different letters on the IC_{50} values indicate statistical significance ($P>0.05$).

3.3 Body weight and food intake

Generally, the body weights of the rats in all treatment groups increased in the adaptation period (day 14 to day 0) (Figure 1). After 14 days of induction (day 0 to day 14), the body weight decreased in all groups except in the normal group. However, the food intake (g/rat/day) of diabetic rats increased after treatment (data not shown).

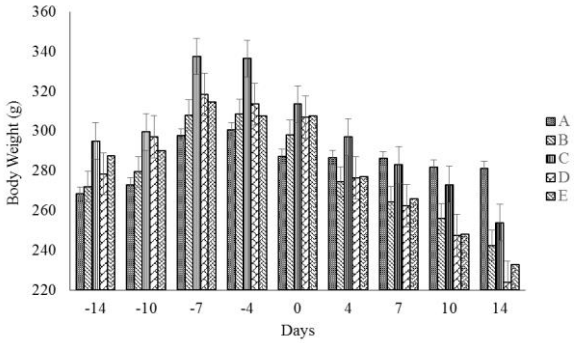

Figure 1. Body weight of rats before and after treatment with ethanolic extracts of *T. sinensis* bark. Data are presented as mean±SEM. A, normal; B, hyperglycemia; C, glibenclamide (0.25 mg/kg BW); D, extract 150 mg/kg BW; E, extract 300 mg/kg BW. * P<0.05 (ANOVA).

3.4 Blood glucose level

Blood glucose measurements were started on day 4. At three days after treatment induction (day 7), the blood glucose levels in groups B, C, D, and E had decreased by 16.6%, 30.4%, 32%, and 11%, respectively (Figure 2). Blood glucose levels measured again on days 10 and 14 after induction in groups B, C, D, and E had decreased by 56.2%, 69%, 71%, and 52%, respectively.

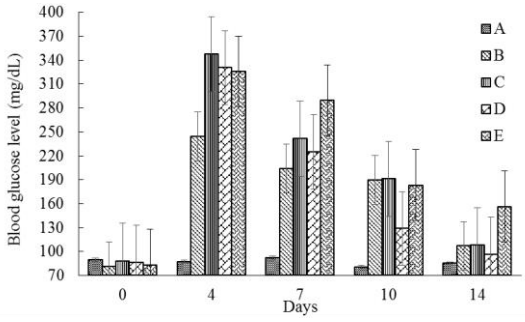

Figure 2. Blood glucose levels after 0−14 days of treatment with ethanolic extracts of *T. sinensis* bark. Data are presented as mean±SEM. A, normal; B, hyperglycemia; C, glibenclamide (0.25 mg/kg BW); D, extract 150 mg/kg BW; E, extract 300 mg/kg BW. * P<0.05 (ANOVA).

3.5 Histological studies

The histochemistries of the pancreases of each group are shown in Figure 3. In Group A, the histological section showed whole cells and normal tissues. In the hyperglycemia Group B, hemorrhaging was observed in the

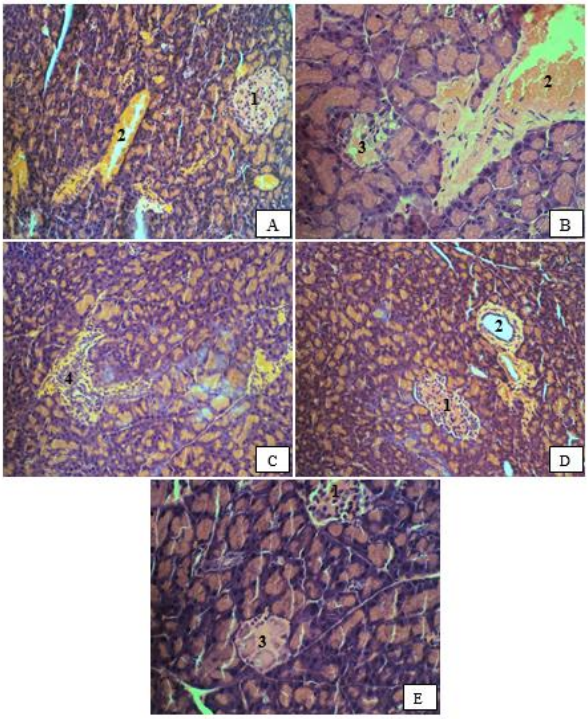

Figure 3. Histopathological sections of the rat pancreases after different treatments. Data are shown at a magnification of 40×. (A) Normal: (1) islets of Langerhans, (2) blood vessel. (B) Hyperglycemia: (2) blood vessel (congestion), (3) acinar cell (necrosis). (C) Glibenclamide: (4) infiltration of inflammatory cell. (D) Extract 150 mg/kg BW: (1) islets of Langerhans, (2) blood vessel. (E) Extract 300 mg/kg BW: (1) islets of Langerhans (hemorrhage), (2) acinar cell (necrosis).

islets of Langerhans while the acinar cell nuclei showed lysis. In Group C (glibenclamide group), the acinar cells were still normal. Also in Group C, the Langerhans cells appeared normal in size but their nuclei were partially damaged. The sections of Group D showed a more normal appearance, as indicated by the presence of normal blood vessels and acinar cells. However, in Group E at 300 mg/kg BW the acinar cells were necrotic and hemorrhaging occurred in the islets of Langerhans.

4. Discussion

The ethanol and hot water extracts of *T. sinensis* contained flavonoids, triterpenoids, alkaloids, tannins, and phenols which are all known to be bioactive antidiabetic agents (Battu *et al.*, 2007; Nagappa *et al.*, 2003). Wang, Yang, and Zhang (2007) reported that the phenolic compounds present in *T. sinensis* were gallic acid and its derivatives, gallotannins, and flavonoids (especially quercetin and rutin). Alkaloids, flavonoids, terpenes, and anthraquinones have all been found to have a role in inhibiting α-glucosidase activity (Chen, Luo, Cui, Zhen, & Liu, 2000; Kunyanga *et al.*, 2011; Luo, Wu, Ma, & Wu, 2001). The phytochemical analysis also showed a slight difference in secondary metabolites of extracts from Indonesia and other countries. The difference lies primarily in the variety of secondary metabolites produced

by the plant. This is probably due to variation in the environmental and geographic condition among the countries (Figueiredo *et al.*, 2008).

The α-glucosidase inhibitory activity of *T. sinensis* extracts might be caused by the phytochemical constituents in the extracts. Yin, Zhang, Feng, Zhang, and Kang (2014) reported that flavonoids, terpenes, quinones, and phenols all have antidiabetic activity. Recent studies have determined that flavonoid compounds such as xanthones, flavanones, flavans, anthocyanins, and chalcones have α-glucosidase inhibitory activity (Ichiki *et al.*, 2007; Jong-Anurakkun, Bhandari, Hong, & Kawabata, 2008; Kato *et al.*, 2008; Lee, Lin, & Chen, 2008; Seo *et al.*, 2007; Zhang & Yan, 2009). Another report on antidiabetic activity indicated that phenolic compounds such as stilbenoids had α-glucosidase inhibitory potency (Lam, Chen, Kang, Chen, & Lee, 2008). According to Zhao, Zhou, Chen, and Wang (2009), the most effective compound from *T. sinensis* that can act as an antidiabetic agent is gallic acid, followed by procyanidin and catechin.

The ethanol extract had a smaller IC_{50} than the hot water extract, implying that some secondary metabolites in this extract interacted with the α-glucosidase. Since the enzyme consists mainly of protein, it is believed that tannins in the ethanol extract decrease the enzymatic activity as a result of enzyme complexation. In addition, some studies also suggested that tannins can act as potential inhibitors (Adamczyk, Simon, Kitunen, Adamczyk, & Smolander, 2017). This result guided us to examine the ethanol extract in an *in vivo* experiment.

The animal experiment examined food intake, blood glucose level, and histopathology. The body weight increase during the adaptation period suggests that rats in all treatments were normal and healthy during the ongoing adaptation. In this study, treatments were conducted over a period of 14 days because the effect of alloxan is best observed during the first two weeks after induction. Although the body weights of the rats decreased after induction, their food intake remained the same. Further, the blood glucose levels increased after alloxan-induction treatment which indicated that the rats were diabetic. Zajac, Shrestha, Patel, and Poretsky (2010) reported that the general characteristics of type 1 diabetes were an increased food intake, decreased body weight, and damage to the pancreas which suggested that the diabetic condition in rats could improve the food efficiency ratio (weight gain/food intake) by reducing food intake and decreasing the body weight (Sheng *et al.*, 2017).

The reduced blood glucose levels in the glibenclamide group showed the effect of glibenclamide treatment. Glibenclamide specifically acts on pancreatic β cells by increasing insulin secretion. It binds to the transmembrane complex that consists of sulfonylurea receptors in the liver and adenosine triphosphate-sensitive potassium ion channels. This process closes the potassium channels, causing membrane depolarization, opening of the calcium channels, and an increase in the concentration of intracellular free calcium. Increased calcium levels trigger the activation of proteins regulating insulin secretion in the pancreas. Sufficient amounts of the insulin can lower blood sugar levels by inhibiting endogenous glucose production and increasing glucose uptake in insulin-sensitive tissues (Krentz & Bailey, 2005; Obici & Martins, 2010).

Blood glucose assays showed that the decreased blood glucose levels caused by treatment with ethanol extract at a dose of 150 mg/kg BW was greater than at a dose of 300 mg/kg BW. Administrating the bark extract at a dose of 300 mg/kg BW was less optimal ($P<0.05$). The presence of a pro-oxidant effect arising from administering large amounts of antioxidant is suspected to cause decreased blood glucose levels (Maddux *et al.*, 2000). However, in the 150 mg/kg BW group and glibenclamide 0.25 mg/kg BW group the amount of decrease was the same. Accordingly, 150 mg/kg BW was concluded to be less effective than glibenclamide. This also suggested that only small compounds in the crude extract contribute to the antidiabetic activity. Here, quercetin from *T. sinensis* leaves exhibits significant antihyperglycemic and liver cell-protective effects in a high-carbohydrate/high-fat diet in an alloxan-induced mouse model of diabetes.

According to Eliakim-Ikechukwu and Obri (2009), alloxan selectively destroys β cells in the islets of Langerhans and induces type 1 DM. In the glibenclamide group, the acinar and Langerhans cells appeared normal. However, the Langerhans nucleus was partially damaged. The presence of some β cells in this group indicated that glibenclamide has anti-hyperglycemic activity in alloxan-induced diabetic rats by stimulating insulin secretion (Rao, Sudarshan, Rajasekhar, Nagaraju, & Rao, 2003).

Histopathological sectioning also showed that the administration of bark extract at a dose of 150 mg/kg BW provided the best treatment effect on the pancreas of rats compared to the glibenclamide and the 300 mg/kg groups. Acinar cell necrosis and hemorrhaging of the islets of Langerhans at 300 mg/kg BW might be due to damage caused by the pro-oxidant activity. Yang *et al.* (2006) reported that *T. sinensis* extracts could generate reactive oxygen species, especially hydrogen peroxide which is a potent pro-oxidative agent.

5. Conclusions

In conclusion, this study revealed that both ethanol and hot water extracts of *T. sinensis* contain phytochemical substances that were reported to be antidiabetic agents. Our findings also demonstrate that *T. sinensis* bark has antidiabetic activity. Further investigations are required to identify the bioactive compounds responsible for this activity. In addition, a toxicological analysis is needed for further development.

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