INHIBITION OF α-GLUCOSIDASE ACTIVITY AND THE TOXICITY OF *Tristaniopsis merguensis* Griff. LEAF EXTRACT

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Received: December 2, 2019 Accepted: June 11, 2020 Published: June 30, 2020

Abstract : This study aims to determine the antidiabetic activity and toxicity of the acetone extract of *Tristaniopsis merguensis* Griff leaf. The antidiabetic test was the α-glucosidase inhibition method, while the toxicity test used the Brine Shrimp Lethality Test (BSLT) method. The acetone extract possessed antidiabetic activity with an IC_{50} value of 8.83 ± 0.31 (μg/mL). This value is not much different from the positive control of quercetin which has an IC_{50} value of 6.04 ± 0.14 (μg/mL). The characteristics of the FT-IR spectrum of acetone extract showed that *Tristaniopsis merguensis* leaf has the groups Ar-OH (phenolic), -OH (hydroxyl), C=O (ketone) and C=C (aromatic). Based on the toxicity test, the *Tristaniopsis merguensis* leaf acetone extract has an LC_{50} value of 959.25 ppm which means that the acetone extract is toxic. Therefore, the acetone extract of *Tristaniopsis merguensis* might be the potential agent of antidiabetic.

Keywords : Inhibition of α-glucosidase, acetone extract, *Tristaniopsis merguensis* Griff., Brine Shrimp Lethality Test, BSLT

Abstrak : Penelitian ini bertujuan untuk mengetahui aktivitas antidiabetes dan toksisitas dari ekstrak aseton daun *Tristaniopsis merguensis* Griff. Uji aktivitas antidiabetes ditentukan berdasarkan metode inhibisi enzim α-glucosidase, sedangkan toksisitas ditentukan berdasarkan metode *Brine Shrimp Lethality Test* (BSLT). Ekstrak aseton memiliki aktivitas antidiabetes dengan nilai IC_{50} 8,83 ± 0,31 (μg/mL). Nilai ini tidak jauh berbeda dengan kontrol positif quersetin yang memiliki nilai IC_{50} 6,04 ± 0,14 (μg/mL). Karakteristik spektrum FT-IR ekstrak aseton menunjukkan bahwa daun *Tristaniopsis merguensis* memiliki gugus fungsi Ar-OH (fenolik), -OH (hidroksil), C=O (keton) dan C=C (aromatik). Berdasarkan uji toksisitas, ekstrak aseton daun *Tristaniopsis merguensis* memiliki nilai LC_{50} sebesar 959,25 ppm yang berarti bahwa ekstrak aseton bersifat toksik. Oleh karena itu, ekstrak aseton dari *Tristaniopsis merguensis* berpotensi untuk dijadikan agen antidiabetes.

Kata kunci : Inhibisi α-glucosidase, ekstrak aseton, *Tristaniopsis merguensis* Griff., Brine Shrimp Lethality Test, BSLT

Introduction

The *Tristaniopsis merguensis* Griff in Bangka Belitung is known as the Pelawan tree. This tree has been used by many people of Bangka to get Pelawan honey and Pelawan mushrooms. Based on reports from the people of Bangka, mushrooms and honey Pelawan has several properties as a cough and diabetes medicine. Besides, the main composition of the Pelawan tree is the phenolic composition. Phenolic compounds, in general, have good antioxidant activity. Based on the research of Verotta *et al*., (2001) secondary metabolites of the genus *Tristaniopsis* containing tannins 1.04%, flavonoids 0.03% saponins 0.95%. The majority of the secondary metabolite of the genus *Tristaniopsis* is phenolic compounds such as tannins and flavonoids. Phenolic compounds generally have certain biological activities.

In Indonesia, diabetes mellitus type 2 is a chronic disease that is increasing every year. This disease is caused by many factors, such as food, diet, physical activity, and age (Kahn, 1996). One of treatment diabetes mellitus is inhibiting the performance of the α-glucosidase enzyme. This enzyme contributes to breaking down carbohydrates into glucose. If the inhibition of the enzyme α-glucosidase, blood sugar levels can be reduced. Therefore, α-glucosidase (AGI) enzyme inhibitors are needed.

Acarbose is one of the synthetic α-glucosidase inhibitors, but it has been reported to cause many side effects such as gastrointestinal and hepatic problems (Feng, 2011). Long-term use can increase levels of glutamate oxalate transaminase and or glutamate pyruvate transaminase in 15% of patients using acarbose (Sun *et al*., 2017). Therefore, the development of α-glucosidase inhibitors from natural ingredients is needed to reduce the side effects of synthetic drugs such as acarbose.

In recent years, many studies of α-glucosidase inhibitors derived from natural compounds. It has been reported that antioxidant compounds with high IC_{50} such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, and phenolics have α-glucosidase inhibitor properties (Kumar, 2011). One of the active compound as antioxidants and α-glucosidase inhibitors is the phenolic group such as ellagitannin. *Terminalia chebula* Retz. identified as containing ellagitannins such as chebulanin, chebulagic acid, and chebulinic acid. These compounds are proven to be potent α-glucosidase inhibitors (Goa, 2008). On the other hand, bark extracts from *Tristaniopsis calobuxus* (*Myrtaceae*) also contain many phenolic compounds, such as ellagic acid, (+)-gallocatechin, (-)-gallocatechin, and (-)-epigallocatechin (Bellosta, 2003). The acetone extract of *T. merguensis* leaf itself has been reported to have a total phenolic content of 215.22 mg gallic acid equivalent (GAE) /g dry extract and IC_{50} value of 22.1454 µg / mL (Roanisca *et al*., 2019). The high content of phenolic and antioxidant *T. merguensis* leaves is also expected to have an impact on its antidiabetic activity

with the hope that in the future it can be used as herbal medicine. One indicator of the efficacy of herbal drugs is knowing their toxicity. Therefore, *T. merguensis* which is a superior plant in Bangka Belitung is expected to know the antidiabetic activity and toxicity.

Research Methods

Materials and Tools

The materials used in this study included the leaves of *T. merguensis*, methanol p.a Merck, technical acetone, enzyme α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), quercetin dihydrate, dimethyl sulfoxide (DMSO) p.a. Merck, p-nitrophenyl-α-D-glucopyranoside, seawater, KBr, Na_2CO_3 Merck, Na_2HPO_4 Merck, NaH_2PO_4 Merck, *Artemia salina*, and filter paper. While the tools used in this study included erlenmayer, rotary vacuum evaporator glass IKA RV-10, measuring, Büchner funnel, dropper, test tube, spatula, measuring flask, analytical balance, stirring rod, test tube rack, hot plate, UV-Vis spectrophotometer 1800 Shimadzu, and FT-IR spectrophotometer thermolyne.

Extract Preparation

T. merguensis leaf samples were from Kimak Village, Bangka Regency. The sample was dried for two weeks in the open air and avoided to be exposed to direct sunlight. Then the leaf samples ware mashed into dry powder. The dried powder of the leaves was taken 1 kg and macerated using 10 L acetone solvent for 3 x 24 hours. Every 1 x 24 hours, a change of solvent was made on the same powder. The filtrate obtained was evaporated with a vacuum rotary evaporator until thick acetone extract was obtained (Mahardika & Roanisca, 2018). The characteristic of *T. merguensis* extract was based on FT-IR spectrum measurements.

α-Glucosidase Inhibition Activity

Antidiabetic activity testing was carried out based on enzymatic reactions in vitro from Dewi *et al.*, 2013. This test begins with the preparation of reagents such as phosphate buffer pH 7.0 from Na_2HPO_4 and NaH_2PO_4 solution, making 4 mg p-nitrophenyl-α-D-glucopyranoside solution, preparation of α-glucosidase enzyme, and making 0.2 mg Na_2CO_3 solution. Extract samples were dissolved in 1 mL DMSO at various concentration. The concentrations were 7.5; 5; 2.5; and 1 µg/mL. Inhibition of α-glucosidase activity was carried out by adding a 5 µL test solution with p-nitrophenyl-α-D-glucopyranoside as much as 250 µL and phosphate buffer pH 7 0.1 M as much as 495 µL. This solution was homogenized and preincubated for 5 minutes at 37^{o}C. The reaction started with the addition of 250 µL of α-glucosidase solution (0.062 units), which was dissolved in a pH 7.0 phosphate buffer. Incubation was continued for 15 minutes. The reaction was stopped by adding 1 mL of Na_2CO_3 0.2 M. Enzyme activity was measured at λ

400 nm from the absorbance of p-nitrophenol. Measurements were made three times. The positive control used quercetin as a comparison (Dewi *et al.*, 2013).

The percentage of inhibition was determined based on the following equation 1:

$$\%\ inhibisi = \frac{(C-S)}{C}\ x\ 100 \quad \text{.....(1)}$$

Where, C= blank absorbance (DMSO)
S= Sample absorbance (the difference between absorption with and without enzymes)

Inhibition of the sample against concentration was plotted to get a straight line equation. The strength of inhibition was expressed in IC_{50} values. This value was obtained from the value of x with y = 50 from the results of the linear equation.

Toxicity Test

This toxicity test is based on the Brine Shrimp Lethality Test (BSLT) method. Preparation of the test solution was carried out, making 1000 ppm by dissolving 50 mg extract in seawater 50 mL. Its solution was diluted with various concentrations of 5, 10, 20, 40, 50, 100, 125 ppm. Then a vial tube was prepared, then put a test solution from each concentration variation of 10 ml and 10 larvae of *A. salina* are added. 2 days old until. Each concentration was calculated as the percentage of larvae that died within 24 hours and compared with a control solution using seawater. Toxicity is determined by looking at the LC_{50} value that can kill *A. Sanila* larvae up to 50% through the calculation of probit analysis (probability unit) (Albuntana *et al.*, 2011).

FTIR Analysis

FT-IR analysis is based on the KBr pellet method. The dried extract was mixed in KBr then made pellets using manual pressing (Shimadzu). Furthermore, it was analyzed using an FT-IR thermolyne spectrophotometer (Purwakusumah *et al*., 2014).

Results and Discussion

Inhibition of α-Glucosidase Activity

Antidiabetic activity is carried out in vitro by the α-glucosidase enzyme inhibition method. A-glucosidase inhibition is one approach to reduce postprandial hyperglycemia, thereby delaying glucose absorption and controlling hyperglycemia in diabetic patients (Dewi *et al.*, 2013). The ideal of the antidiabetic compound must have hypoglycemic properties. Therefore in-vitro testing of inhibition of this enzyme can be used as a reference in antidiabetic screening. The results of this test are shown in **Table 1**. Measurement of inhibition was carried out three times at each concentration.

Table 1. The results of α-glucosidase enzyme inhibition

Sample	Inhibition (%) of concentration (µg/mL)					IC_{50} (µg/mL)
	1	2,5	5	7,5	10	
Quercetin	7.42±0.85	27.01±3.23	45.22±1.33	61.06±0.31	77.59±1.10	6.04±0.14
Acetone Extract	12.05±0.33	7.67±0.03	22.90±0.38	38.29±2.78	-	8.83±0.31

Antidiabetic testing of acetone extract of *T. merguensis* leaves has antidiabetic activity with an IC50 value of 8.83 ± 0.31 µg / mL. This value is not much different from the positive control quercetin, which has an IC_{50} value of 6.04 ± 0.14 µg / mL. Quercetin is a phenolic compound with a class of flavonoids that has been reported to actively inhibit the enzyme α-glucosidase, which plays a role in controlling blood sugar (Khuankaew, 2014). The smaller the IC_{50} value, the better the activity of the compound.

Table 2. The Activity of α-glucosidase enzyme inhibition from *Myrtaceae* Extract

No.	Sample	Solvent	IC_{50} (µg/mL)
1.	*Tristaniopsis merguensis*	Acetone	8.83±0.31
2	*Syzygium sumatranum (Myrtaceae)* (Saraswati, 2010)	Methanol	13.99
3	*Syziygium hyntum (Myrtaceae)* (Saraswati, 2010)	Methanol	23.38
4	*Syziygium paucifunctatum (Myrtaceae)* (Saraswati, 2010)	Methanol	12.69
5.	*Eugenia jambolana (Myrtaceae)* (Ahmed *et al*., 2009)	Distilled water	68.3± 1.4
6.	*Psidium guajava (Myrtaceae)* (Nkobole *et al*., 2011)	Acetone	62.7 ± 0.19
7.	*Syziygium cumini (Myrtaceae)* (Saraswati, 2010)	Methanol	8.17

T. mergensis is included in the *Myrtaceae* family. Based on the α-glucosidase inhibition test on plants in one family such as *S. sumatranum*, *S. hyntum*, *S. paucifunctatum*, *S. Cumini*, *P. Guajava*, and *E. jambolana* (Table 2), the α-glucosidase inhibition activity of *T. merguensis* leaf extract in this study was better than the antidiabetic activity of methanol in *S. sumatranum*, *S. hyntum*, *S. paucifunctatum*, and *P. Guajava* acetone extracts, and water extracts from E. jambolana. But the antidiabetic activity in this study was no better than the methanol extract in *S. Cumini*. Ethanol extract *S. cumini* itself has been reported to contain many active compounds that are preventing degenerative diseases such as antidiabetic (Sari *et al*., 2018). When compared with plants in one family *Myrtaceae*, the acetone extract of *T. mergensis* leaves has the potential as an antidiabetic.

Extract Characterization Based on FT-IR Analysis

The acetone extract of *T. merguensis* Grifft was obtained by the functional group analysis using FT-IR spectrophotometer. FT-IR spectrum of acetone extract of *T. merguensis* leaves is shown in **Figure 1** and **Table 3**.

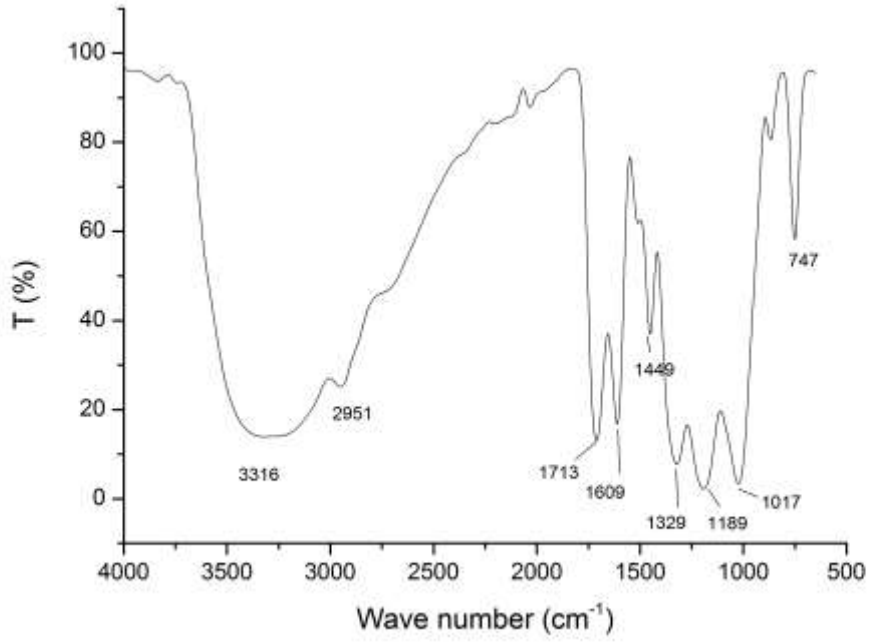

Figure 1. FT-IR spectrum of acetone extract of *T. merguensis* leaves

The wavenumber at 3316 which broad at 3400-3100 cm^{-1} is a vibration -OH stretching group-broadband due to the interaction of hydrogen bonds. The -OH group may be derived from phenols (Ar-OH), aromatics from phenols are shown to have wavenumber at 751 cm^{-1}. In addition, the absorption of C-H aromatic stretching uptake, which is generally located at 3160-3050 cm^{-1} may be overlap with the -OH group. The presence of aromatic compounds is also supported by the appearance of wavenumber at 1609 cm^{-1} which is vibrations of C=C stretching. Based on this analysis, the acetone extract of *T. merguensis* is thought to contain aromatic compounds such as phenolic compounds or polyphenols (Roanisca *et al.*, 2019).

Table 3. Vibration mode of functional groups from FT-IR Spectrum

Wavenumber (cm^{-1})	Vibration Mode
3316	-OH stretching
2951	CH_3 stretching, asymmetric CH_2 stretching
1713	C=O stretching
1609	C=C aromatic stretching
1449	CH_2 bending
1329	C-H bending
1018	C-O alcohol stretching
747	C-H aromatic bend

Based on this spectrum, *T. merguensis* extract contains CH_3 (methyl) and CH_2 (methylene) groups which are shown wavenumber at 2951 cm^{-1} which is the vibration of CH_3 stretching and asymmetric CH_2 stretching (Maobe *et al.*, 2013). This is also supported by the wavenumber 1449 to 1329 cm^{-1} which is the vibration of CH_2 bending and C-H bending. Therefore, the extract of *T. merguensis* contains methyl and methylene groups.

In addition, the extract of *T. merguensis* also contains a group C=O (carbonyl) is characterized by the appearance of wavenumber 1713 cm^{-1}. It is thought to be the wavenumber of the ketone carbonyl group. It is because there are no wavenumber 1280-1150 cm^{-1} which is a characteristic of the C-O-C ester group, or the absence of a wavenumber of about 2800 cm^{-1} which is a characteristic of C-H aldehydes.

Based on FTIR spectrum data analysis (**Figure 1**), *T. merguensis* extract contains compounds that have Ar-OH (phenolic), -OH (hydroxyl), C = O (ketone) and C=C (aromatic) groups. The results of Mahardika & Roanisca (2019) study, phytochemical *T. merguensis* leaf extract contains alkaloids, tannins and flavonoids. When compared to the phytochemical test, it is assumed that the signal comes from the compound.

Toxicity Test

In this study, the toxicity test was carried out using the Brine Shrimp Lethality Test (BSLT) method. This test was treated on the *T. merguensis* acetone extract. BSLT test results were performed by calculating the percentage of *Artemia salina* larvae mortality against acetone extract of *T. merguensis* leaves. The results are shown in **Table 4**. The greater the extract concentration, the higher the percentage of *A. salina* deaths.

Table 4. Percentage of mortality of *A. salina* larvae against acetone extract of *T. merguensis* leaves

Concentration (ppm)	Mortality (%)	Log of Concentration	Probit Value
5	10	0.699	3.72
10	10	1.000	3.72
20	15	1.301	3.96
40	20	1.602	4.16
50	20	1.699	4.16
100	30	2.000	4.48
125	30	2.097	4.48
0	0	0	0

Determination of toxicity is based on probit analysis by determining the linear line equation between log concentration (x) and probit value (y). The linear equation obtained is used to determine the concentration of death of 50% *A. salina*

(LC_{50}). The results of the *T. merguensis* leaf extract test had an LC_{50} value of 959.252 ppm. LC_{50} values between 30-1000 ppm are categorized as toxic; it is mean that *T. merguensis* leaf extract is toxic (Ningdyah, Alimuddin, & Jayuska, 2015). The value of R^2 (coefficient of determination) obtained is 0.9552 which means that 95.52% of deaths can be caused by changes in the concentration of *T. merguensis* extract (Dhone *et al.*, 2018). When compared with species in one family (*Myrtaceae*), the toxicity of *T. merguensis* extract is lower than the ethanol extract of *Syzygium samarangense* and ethyl acetate of extract *Eugenia uniflora* with LC_{50} values of 170.01 ppm and 79.43 ppm (Swantara *et al.*, 2016).

A. salina absorbs compounds in the leaves of *T. merguensis* through the digestive tract. This absorption process through the cell membrane. Then the toxicity compound from the extract enters the cell, causing a failure in the metabolism of *A. salina*. The increased concentration of the extract can cause many toxic compounds to spread widely in the body of *A. salina* so that it can cause death. This metabolic failure can cause death and can be observed within 24 hours to cause the death of 50% *A. salina* (Ningdyah *et al*., 2015).

Conclusions

Based on the results of the study, the antidiabetic testing of the acetone extract of *T. merguensis* has antidiabetic activity with an IC_{50} value of 8.83 ± 0.31 (µg / mL). This value is not much different from the positive control, quercetin which has an IC_{50} value of 6.04 ± 0.14 (µg / mL). The characteristics of functional groups from the acetone extract of *T. merguensis* using FTIR that have the Ar-OH (phenolic), -OH (hydroxyl), C= O (ketone) and C = C (aromatic) groups. Based on the toxicity test, the acetone extract of *T. merguensis* leaves has an LC_{50} value of 959.252 ppm which means that the acetone extract is toxic. Therefore, the acetone extract of *T. mergensis* leaves has the potential as an antidiabetic.

Acknowledgements

This research partially funded by Ministry of Research, Technology and Higher Education through Penelitian Dosen Pemula (PDP) in accordance with the agreement/contract number 052/SP2H/LT/DRPM/2019 dan 187.O/UN.50.3.1/PP/2019 and we gratefully acknowledge the Institute of Research and Community Service from Universitas Bangka Belitung.

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