Antidiabetic Activity of Some Extracts from *Anisophyllea disticha* Leaves

Muhammad Almurdani, Miftahul Fikriyah, Adel Zamri, Titania T. Nugroho, Jasril Jasril, Yum Eryanti, Hilwan Yuda Teruna

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau
Jl. HR. Soebrantas, Km. 12.5, Pekanbaru, 28293, Indonesia

hyteruna@lecturer.unri.ac.id

**Abstract.** *Anisophyllea disticha* has been being used as a traditional medicine by Talang Mamak tribe in Indragiri Hulu district, Riau province, Indonesia. The aim of this study was to evaluate antidiabetic properties of *A. disticha* leaves. The methanol extract was obtained by maceration, followed by successive partition process and yielded *n*-hexane, dichloromethane, and ethyl acetate fractions. Antidiabetic activity was determined by inhibiting the α-glucosidase activity method. The methanol extract showed high antidibetic activity followed by ethyl acetate fraction with IC$_{50}$ value of 47.765 ± 0.127 and 50.883 ± 0.803 μg/mL, respectively. It could be concluded that leaves of *A. disticha* had antidiabetic properties.

1. **Introduction**

Diabetes mellitus is a complex metabolic disease in the endocrine system characterized by abnormalities in insulin secretion that leads to a decrease glucose tolerance and causes hyperglycemia [1]. This disease is a major public health problem worldwide [2]. One of therapeutic approach to decreasing hyperglycemia is retard the absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes, such as the α-glucosidase enzyme found in the intestine [3]. The α-glucosidase inhibitors can delay the absorption of ingested carbohydrates or glucose, and reduce postprandial glycemia [4].

The tribe of Indonesian peoples have long history of using plants as medicines to treat various diseases. One of them is Talang Mamak tribe, in which using *Anisophyllea disticha* leave to treat fever, headache, diabetic and infectious diseases [5,6]. Some species of *Anisophyllea* were reported has antioxidant, antiinfection and antidiabetic and other activities. Onivogui et al. [7] was reported the ethanol and methanol extracts of the leaves and stem bark from *A. laurina* to show a potency as antibacterial and antifungal. Kargbo et al. [8] was reported that the ethanol crude extract from leaves and stem bark of *A. laurina* have α-glucosidase and α-amylase inhibitory activities.

Secondary metabolites of *Anisophyllea* were isolated and identified such as ellagic acid derivatives, catechins, and procyanidins were found in the *A. dichostyla* leave [9]. Triterpenoid and minor phenolic compounds were foun in the root bark of *A. dichostyla* [10].

*A. disticha* by local name Ribu - ribu is one species of of *Anisophyllea* genus widely found in Indragiri Hulu district, province of Riau, Indonesia and used by Talang Mamak tribe (traditional society of Indragiri Hulu district) to treat various diseases. So far there have been no research reports on the antidiabetic activities of this species. We have an ongoing interest in antidiabetic activities from traditional medicine from Talang Mamak tribe. Here, we report the antidiabetic activity of *A. disticha*
leaves. This species was selected due to their abundance, and lack of information regarding antidiabetic activity.

2. Methodology
The research was conducted by plant collection, extraction and antidiabetic activity assay method.

2.1. Plant collection and Extraction
Fresh leaves of A. disticha was collected from Rakit Kulim of Indragiri Hulu Regency, Riau Province, Indonesia and identify in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. The sample was dried and stored at 4°C until analysis performed. The dry leaves (10 g) were ground into powder and then macerated by using methanol (100 mL) for 48 hour followed by ultrasound for 1 hour and the macerate was concentrated in vacuo at 50°C. The methanol extract was fractionated with n-hexane, dichloromethane and ethyl acetate, respectively.

2.2. Antidiabetic Activity Assay
The antidiabetic assay was determined by using inhibiting activity of α-glucosidase enzyme with p-NPG as a substrate method with a slight modification [11, 12]. Briefly, each of 10 µL of samples or acarbose standard (S₀) at varying concentration (in DMSO) and 10 µL of DMSO (B₀) were added with 40 µL of phosphate buffer (pH 6.8) and 25 µL of p-NPG 20 mM in 96-well microplate as the blank. In same microplate, each of 10 µL of DMSO (B₁) and 10 µL of sample or acarbose standard (S₁) at varying concentration (in DMSO) were added with 40 µL of phosphate buffer (pH 6.8), 25 µL p-NPG 20 mM and 25 µL α-glucosidase 0.2 U/mL. The mixture were incubated for 30 minutes at 37°C. The reaction was stopped by adding 100 µL Na₂CO₃ 0.1 M and then absorbance were measured at a wavelength of 405 nm by microplate reader. The % Inhibition value was calculated by the following formula (1):

\[
% \text{Inhibition} = \left( \frac{(B₁ - B₀) - (S₁ - S₀)}{B₁ - B₀} \right) \times 100
\]

Where \( B₀ \) represents the absorbance of substrate without sample and enzyme, \( B₁ \) represents the absorbance of substrate and enzyme reaction without sample, \( S₀ \) represents the absorbance of substrate and samples without enzyme while \( S₁ \) represents the absorbance of substrate and enzyme reaction contain samples. The IC₅₀ values was calculated using the logarithmic equation (Formula 2) of the calibration curve by plotting of sample concentrations versus % inhibition values. All assays were carried out in triplicate and their results were expressed as mean ± standard deviation.

\[
y = a \ln(x) + b
\]

3. Results and Discussion
Antidiabetic activity assay was determined by inhibition of α-glucosidase enzyme activity. The α-glucosidase inhibition is one therapeutic approach to decreasing postprandial hyperglycemia, this inhibition delay the absorption of ingested carbohydrates, reducing the postprandial glycaemia and insulin peaks [1]. The α-glucosidase inhibitors act against these enzymes in the gut, slowing down the liberation of D-glucose from dietary complex carbohydrates that lowers available glucose for absorption. Hence, they are useful in reducing post-prandial blood glucose in treating prediabetic conditions and delaying the progression of diabetic [13, 14].

The antidiabetic activity of A. disticha leaves showed significantly different \((p < 0.05)\) (Table 1) and logarithmic regression equation can be seen in Figure 1. The methanol extract showed high activity followed by ethyl acetate fraction with IC₅₀ value of 47.765 ± 0.127 and 50.883 ± 0.803 µg/mL, respectively. A. disticha has potential as an antidiabetic agent. Previous study reported A. laurina same genus with A. disticha has α-Glucosidase inhibitory activity[8]. The antidiabetic activity of methanol extract and ethyl acetate fraction of A. disticha leaves were lower than the standard of
antidiabetic acarbose, because it is still in the form of extracts and fractions. It needs further development of isolation of pure compounds to obtain active antidiabetic compounds.

**Figure 1.** Graph of logarithmic regression of antidiabetic activity of *A. disticha* leaves
Table 1. Antidiabetic (Inhibitor α-glucosidase) activity of A. disticha leaves

| Sample                     | IC₅₀ (μg/mL) |
|----------------------------|-------------|
| n-Hexane fraction          | 80.456 ± 0.315 a |
| Dichloromethane fraction   | 70.386 ± 0.239 b |
| Ethyl acetate fraction     | 50.883 ± 0.803 c |
| Methanol extract           | 47.765 ± 0.127 d |
| Acarbose                   | 18.283 ± 0.375 e |

Data were expressed as mean ± standard deviation (n = 3). Same letters in each column mean no significant difference (p < 0.05).

4. Conclusion

The methanol extract and ethyl acetate fraction of A. disticha leaves show good α-glucosidase inhibitory activity. It could be concluded that leaves of A. disticha leaves have antidiabetic properties and ongoing investigation on its secondary metabolites are carried out.

5. Acknowledgements

Thanks to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for supporting this research through postgraduate grant research, Contract Number: 339/UN.19.5.1.3/PP/2018.

References

[1] Pereira D F, Cazarolli L H, Lavado C, Mengatto V, Figueiredo M S R B, Guedes A and Silva F R M B 2011 Effects of flavonoids on α-glucosidase activity: potential targets for glucose homeostasis Nutrition 27(11) 1161-1167

[2] Cetto A A, Jimenez J B and Vazquez R C 2008 Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes Journal Ethnopharmacology 116 27–32

[3] Holman R R, Cull C A and Turner R C 1999 A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years, Diabetes Care 22 960-964

[4] Stuart A R, Gulve E A, Wang M 2004 Chemistry and biochemistry of type 2 diabetes, Chem Rev 104 1255–82

[5] Grosvenor PW, Supriono A and Gray DO 1995 Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial and antifungal activity Journal of Ethnopharmacology 45 97-111

[6] Setyowati F M and Wardah 2009 Keanekearagaman Tumbuhan Obat Masyarakat Talang Mamak di Sekitar Taman Nasional Bukit Tigarupuh, Riau, Biodiversitas 8(3) 228-232

[7] Onivogui G, Diaby M, Chen X, Zhang H, Kargbo M R and Song Y 2015 Antibacterial and antifungal activities of various solvent extracts from the leaves and stem bark of Anisophyllea laurina R. Br ex Sabine used as traditional medicine in Guinea J. Ethnopharmacol 168 287-290

[8] Kargbo M B R, Onivogui G and Song Y 2015 In vitro anti-diabetic activity and phenolic compound profile of ethanol extracts of Anisophyllea laurina R. Br ex Sabine leaves and stem bark, Eur. Acad. Res 2(12)16089-16106

[9] Khallouki F, Haubner R, Hull W E, Erben G, Spiegelhalder B, Bartsch H and Owen R W 2007 Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of Anisophyllea dichostyla R. Br. Food Chem. Toxicol 45(3) 472-485

[10] Khallouki F, Hull W E, and Owen R W 2009 Characterization of a rare triterpenoid and minor phenolic compounds in the root bark of Anisophyllea dichostyla R. Br. Food Chem. Toxicol 47 2007 – 2012

[11] Elya B, Basah K, Mun’im A, Yuliastuti W, Bangun A and Septiana E K 2011 Screening of α-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae,
Euphorbiaceae, and Rubiaceae *Journal of Biomedicine and Biotechnology* 1-6

[12] Sheng Z, Dai H, Pan S, Wang H, Hu Y and Ma W 2014 Isolation and characterization of α-glucosidase inhibitor from *Musa spp.* (Baxijiao) Flowers, *Molecules* 19 10563-10573

[13] Chu Y H, Wu S H and Hsieh J F 2014 Isolation and characterization of α-Glucosidase inhibitory constituents from *Rhodiola crenulata*, *Food Res. Int* 57 8-14

[14] Rengasamy K R R, Aderogba M A, Amoo S, Stirk W A and Staden J V 2013 Potential antiradical and alpha-glucosidase inhibitors from *Ecklonia maxima* (Osbeck) Papenfuss *Food Chem* 14 1412-1415