

## Natural Antidiabetic of Tunjuk Langit (*Helminthostachys zeylanica*) Rhizome Extracts

Fania E. Ridhasya<sup>1</sup>, Muhammad Almurda<sup>1</sup>, Rudi Hendra<sup>1,2</sup>, Hilwan Y. Teruna<sup>1,2</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences,  
University of Riau Pekanbaru, Riau, Indonesia

<sup>2</sup>Scientific Consortium of Drug Discovery and Development,  
University of Riau, Pekanbaru, Riau, Indonesia

### Abstract

The use of medicinal plants in treating diabetes mellitus is increasing in Indonesia. Plenty of plants from different regions may have antidiabetic effect, including *Helminthostachys zeylanica*. This plant is commonly used as a traditional medicine to treat inflammation, cough, dysentery, and malaria in Talang Mamak tribe, Indragiri Hulu, Riau, however in China it is used to treat diabetic. Thus, we examined whether the extract of *H. zeylanica* originated from Riau have potential antidiabetic activity. We assessed the  $\alpha$ -glucosidase inhibitory activity of the extract of *H. zeylanica* rhizome. The results showed the antidiabetic values of n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and ethanol (EtOH) extracts were  $380.88 \pm 0.09$ ;  $190.76 \pm 0.22$ ;  $61.18 \pm 0.59$ ;  $47.86 \pm 0.06$ ; and  $60.78 \pm 0.02$ , respectively. Acarbose were used as standard with antioxidant values of  $19.73 \pm 0.07$ . It can be concluded that the methanol extract is potential to be proposed as antidiabetic.

Keywords:  $\alpha$ -glucosidase, antidiabetic, *H. zeylanica*

### Introduction

Diabetes is a chronic metabolic disease which is caused by abnormalities in insulin action. Chronic hyperglycemia in diabetes can cause degenerative in several organ such as kidney, retina etc. There are various medications to treat diabetes mellitus such as gene therapy, insulin, and antidiabetic drugs. However, antidiabetic drugs possess adverse reactions. Therefore, an alternative medicine with less adverse reaction is needed.<sup>1</sup> Different tribes in Indonesia have been using plants to treat various diseases. Talang Mamak tribe in

Riau use *H. zeylanica* to treat inflammation, dysentery, cataracts, early-stage tuberculosis, syphilis, diabetic, and malaria.<sup>2</sup>

*H. zeylanica* contains saponins, flavonoids, stilbenes and phenolics and shows various biological activities such as antioxidant,<sup>3,4,5</sup> anti-inflammation,<sup>6,7,8</sup> anti-osteoporotic,<sup>9</sup> and antihyperuricemia.<sup>10</sup> Furthermore, phenolics and flavonoids inhibit  $\alpha$ -glucosidase enzyme which is responsible in glucose levels.<sup>11</sup> Therefore, we studied whether the extracts of *H. zeylanica* originated from Riau have

potential antidiabetic activity. The results of this study may be beneficial for the use of *H. zeylanica* as an antidiabetic drug.

## Material and Methods

### Extract Preparation

*H. zeylanica* were collected in Kelayang District, Indragiri Hulu Regency, Riau Province. Rhizome dried powder (100 g) were cold extracted using *n*-hexane, dichlorometane, ethyl acetate (EtOAc), methanol (MeOH), and ethanol (EtOH), respectively, and filtered. 10 mL of filtrate was prepared for antidiabetic assay.

### *In vitro* $\alpha$ -glucosidase inhibition assay

Enzyme solution was prepared by dissolving 1 mg of  $\alpha$ -glucosidase in 100 mL of phosphate buffer (pH 7) which contained 200 mg of bovine serum albumin. Prior to use, 1 mL of enzyme solution was diluted 25 times with phosphate buffer (pH 7). The reaction mixture was prepared in the microplate wells which consisted of 25  $\mu$ L of 20 mM p-nitrophenyl-D-glucopyranose as substrate and 50  $\mu$ L of 100 mM phosphate buffer (pH 7). Briefly, each extract was dissolved in DMSO and aliquots of samples (10  $\mu$ L) was added to the reaction mixture to final concentrations of: 31.25  $\mu$ g/mL, 62.5  $\mu$ g/mL, 125  $\mu$ g/mL, 250  $\mu$ g/mL, 500  $\mu$ g/mL, 1000  $\mu$ g/mL. Solution of 1% acarbose (Glucobay®) was prepared with phosphate buffer pH 7. Then it was mixed

with 2N HCl of equal volume (1:1) and was centrifuged. Aliquots of supernatant (10  $\mu$ L) was taken and added into the reaction mixture at final concentration of 0.0625  $\mu$ g/mL; 0.125  $\mu$ g/mL; 0.25  $\mu$ g/mL; 0.5  $\mu$ g/mL; and 1  $\mu$ g/mL. Blanks, controls and each concentration of samples were done in triplicate. The mixture was incubated at 37°C for 5 minutes, and then 25  $\mu$ L of enzyme solution was added into the reaction mixture and incubated further for 15 minutes. Enzyme reaction was stopped by adding 100  $\mu$ L of 0.1M Na<sub>2</sub>CO<sub>3</sub>. Blanks, controls, and samples absorbance of the p-nitrophenol product was measured by microplate reader spectrophotometer at 410 nm wavelength.

## Results and Discussion

The result is presented in Table 1.

The  $\alpha$ -glucosidase inhibition activity was conducted based on the basic principle of enzymatic reaction, the hydrolysis of p-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) substrate by the  $\alpha$ -glucosidase enzyme to p-nitrophenol (yellow color) and glucose.<sup>11</sup> We found that MetOH extract has the highest antidiabetic activity, weaker than acarbose. Acarbose is an antidiabetic drug that works by inhibiting the activity of the  $\alpha$ -glucosidase enzyme in compete directly with polysaccharides to cover the active side of the enzyme.<sup>12</sup> Thus, we propose that

**Table 1. Antidiabetic Activity of Extracts from *H.zeylanica***

Sample	IC50 ( $\mu$ g/mL)
n-hexane extract	380.88 $\pm$ 0.09
DCM extract	190.76 $\pm$ 0.22
EtOAc extract	61.18 $\pm$ 0.59
MetOH extract	47.86 $\pm$ 0.06
EtOH extract	60.78 $\pm$ 0.02
Acarbose	19.73 $\pm$ 0.07

MetOH extract of *H. zeylanica* might have similar activity with that of acarbose.

*H. zeylanica* contains various flavonoid compounds<sup>3</sup> that have ability to inhibit  $\alpha$ -glucosidase enzymes.<sup>13</sup> Inhibition of  $\alpha$ -glucosidase activity by various phenolic compounds has been widely explained in the literature.  $\alpha$ -glucosidase is effectively inhibited by flavonols,<sup>11</sup> luteolin, myricetin, and quercetin.<sup>14</sup>

### Conclusion

The methanol extract of *H. zeylanica* rhizome might be potential in inhibiting  $\alpha$ -glucosidase (IC<sub>50</sub> 47.86  $\pm$  0.06 ppm). Therefore, this plant can be proposed as antidiabetic medicine.

### Acknowledgments

Authors thank to all colleagues who fully supported this research.

### Funding

This study was funded by Postgraduate Research Grant from the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia 2019 with contract number: 808/UN.19.5.1.3/ PT.01.03/2019.

### Conflict of Interest

None declared.

### References

1. Aquarista NC. Perbedaan karakteristik penderita diabetes melitus tipe 2 dengan dan tanpa penyakit jantung koroner. *Jurnal Berkala Epidemiologi*. 2017;4(1):37–47.
2. Hartini S. *Helminthostachys zeylanica* (L.) Hook : Potensinya Sebagai Obat Masa Depan. *Warta Kebun Raya*. 2011;11(1):34–37.
3. Huang YL, Yeh PY, Shen CC, Chen CC. Antioxidant flavonoids from the rhizomes of *Helminthostachys zeylanica*. *Phytochemistry*. 2003;64(7):1277–1283.
4. Chen CC, Huang YL, Yeh PY, Ou JC. Cyclized Geranyl Stilbenes from the Rhizomes of *Helminthostachys zeylanica*. *Planta Medica*. 2003;69(10):964–967.
5. Fitrya, Anwar L, Eliza, Muharni. Ugonin J Flavonoid From Tunjuk Langit (*Helminthostachys zeylanica* Linn.) Root Extract. *Indonesian Journal of Chemistry*. 2010;10(2):233–238.
6. Huang YC, Hwang T, Chang C, Yang Y, Shen C, Liao W, Liaw C. Anti-inflammatory Flavonoids from the Rhizomes of *Helminthostachys zeylanica*. *Journal of Natural Products*. 2009;72(7):1273–1278.
7. Chen C, Yang C, Tsai Y, Liaw C, Chang W, Hwang T. Ugonin U stimulates NLRP3 inflammasome activation and enhances inflammasome-mediated pathogen clearance. *Redox Biology*. 2017;11: 263–274.
8. Su L, Li Y, Li H, Dai W, Liu D, Cao L, Li R. Anti-inflammatory Prenylated Flavonoids from *Helminthostachys zeylanica*. *Chemistry Pharmacy Bulletin*. 2016;64(5):497–501.
9. Huang Y, Shen C, Shen Y, Chiou W, Chen C. Anti-inflammatory and Antiosteoporosis Flavonoids from the Rhizomes of *Helminthostachys zeylanica*. *Journal of Natural Products*. 2017;80:246–253.
10. Fitrya, Muharni. An Antihyperuricemia Effect of Ethanol Extract of Tunjuk Langit Rhizome (*Helminthostachys Zeylanica* Linn Hook) on Swiss Male Mice. *Traditional Medicine Journal*. 2014;19(January):14–18.
11. Li KB, Chan KY. Production and properties of  $\alpha$ -glucosidase from *Lactobacillus acidophilus*. *Applied Environmental Microbiology*. 1983;46(6):1380–1387.
12. Lee S, Lin H, Chen C. Acylated Flavonol Monorhamnosides,  $\alpha$ -glucosidase Inhibitors, from *Machilus philippinensis*.

- Phytochemistry*. 2008;69:2347–2353.
13. Wang H, Du Y, Song H.  $\alpha$ -Glucosidase and  $\alpha$ -amylase inhibitory activities of guava leaves. *Food Chemistry*. 2010;123(1):6–13.
  14. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of  $\alpha$ -Glucosidase and  $\alpha$ -Amylase by Flavonoids. *Journal of Nutritional and Vitaminology*. 2006;52(2):149–153.