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Original Article

Antidiabetic Constituents from *Helminthostachys zeylanica* (L) Hook (Ophioglossaceae)

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ABSTRACT

Background: The roots of tunjuk langit (*Helminthostacshys zeylanica*) have been used traditionally in some villages in Indonesia, particularly in Riau Province. **Objective:** In this study we reported two flavonoids and their antidiabetic activity. **Material and methods:** Isolation of the metabolites was based on polarity fractionation method. Purification processes were conducted by vacuum liquid chromatography (VLC). Chemical structures were elucidated based on spectroscopy characteristics, including FTIR and 1D/2D NMR. **Results:** The isolated compounds were identified as ugonin J and K. The antidiabetic activity was measured by α-glucosidase inhibitor assay. The antidiabetic activity of ugonin J was found at IC_{50} 273,13±0,402 ppm and Ugonin K was found at IC_{50} 138,21±0,263 ppm (moderately active). **Conclusion:** Therefore this plant can be used traditionally as antidiabetic medicine.
Key words: *Helminthostacys zeylanica*; Ophioglossaceae; Antidiabetic; α-glucosidase.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder due to insulin resistance or decreased production of insulin by the beta cells of the pancreas. The World Health Organization reported that the worldwide prevalence of diabetes was expected to increase to 642 million by the year 2040, with many new cases of diabetes occurring in developing countries, especially in Asia. Type 2 diabetes mellitus (T2DM) accounts for more than 90% of total diabetes cases. α-Glucosidase is an enzyme that plays an important role in the absorption of glucose in the gastrointestinal tract. Inhibiton of this enzyme activity can reduce blood glucose levels and preventing postprandial hyperglycemia in patients with T2DM.[1] Antidiabetic medications can improve the health of T2DM patients. Currently, most of available antidiabetic drugs have side effects and are associated with the enhancement of diabetes related complications. Some α-glucosidase inhibitors have been successfully isolated from medicinal plants for the development of new drugs with increased potency and lower side effects than the existing drugs.

The roots of *Helminthostachys zeylanica* (L) Hook (Ophioglossaceae) have been used traditionally as a medicine in some tribes in Indonesia. In Riau Province, this plant is called tunjuk langit. It is also called rawu berkubang (Malay), paku pacar bumi (South Sumatran tribe), jajalakan (Sundanese), pakis kaler or manonor paku urang or manon (Javanese) and bute-bute (South Sulawesi).[2] In India, this fern is called kamraj and in Taiwan, it is called daodi-ugon.[3,4] Traditionally the roots have been used for cough, dysentery, antipyretics, nose and lung diseases.

Secondary metabolites of this plant have been reported to contain prenylated flavonoids. Some eight compounds were isolated *i.e.* ugonin E-L.[4] These compounds have been isolated from the roots. It was also found some stilben derivatives isolated from the roots, along with some other prenylated flavonoids.[5] Yamauchi *et al* reported two flavonoid glycosides.[6] The roots was reported to have antihyperuricemia and cytotoxic against P-388 murine cells.[7,8] Recent study showed that a water extract of *H. zeylanica* could reduce inflammation in lung epithelial cells.[9] Ugonin J and K have been reported to have extracellular melanogenesis inhibitory activity.[6] Ugonin U possessed immunomodulator effect on human neutrophils.[10] Nevertheless, the antidiabetic activity from *H. zeylanica* has never been reported previously. Therefore, the objective of this study was to assess the α-glucosidase inhibitory activity of the secondary metabolites from *H. zeylanica* roots.

MATERIAL AND METHOD

General experimental procedure

IR spectra were recorded on a spectrophotometer IR Prestige-21 (Shimadzu) using potassium bromide pellets. NMR spectral analyses were carried out with NMR Bruker Avance spectrometer with 500 MHz (^{1}H) and 125 MHz (^{13}C). TMS was used as internal standard material. The absorbance of the enzyme assay reaction mixture was measured by TriStar LB 941 multimode microplate reader (Berthold Technologies) at 410 nm.

Collection of plant material

The roots of tunjuk langit were collected in Kelayang District, Indragiri Hulu Regency, Riau Province. Plant determination was conducted at Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau.

Extraction and isolation

Isolation of the metabolites was based on polarity fractionation method. As much as 18 kg of the

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fresh roots was macerated with 17 L methanol for 24 h, followed by ultrasonication for 1 h. This process was repeated two times. Approximately 100 g of dried extract was dissolved in methanol then partitioned with *n*-hexane (2:1). Methanol fraction was dried and subjected to vacuum liquid chromatography (VLC) to give 12 fractions (a mixture of *n*-hexane, ethyl acetate and methanol). The compounds were obtained from fractions 4 and 5. They were afforded as yellow crystalline compound in fraction 4 and fraction 5. The purity of this compound was analysed by HPLC (Shimadzu UFLC) using ODS column (RP-18). Structures were determined by using spectroscopy methods including infrared (Shimadzu Prestige 21 FT-IR) and NMR (Bruker Ascends 600 MHz for ^{1}H-NMR, ^{13}C- NMR, COSY, HSQC, and HMBC analyses). NMR spectra were recorded at Department of Chemistry ITB, Bandung. Inhibition of glucosidase with *p*-nitrophenyl-α-D-glucopyranoside was employed as antidiabetic assay.

In vitro α-glucosidase inhibition assay

The *α*-glucosidase inhibitory activity was assessed by the standard method, with some modifications. Enzyme solution was prepared by dissolving 1 mg of *α*-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 µl of 20 mM *p*-nitrophenyl-*D*-glucopyranose as substrate and 50 µl of 100 mM phosphate buffer (pH 7). Briefly, each extracts was dissolved in DMSO and aliquots of samples (10 µL) was added to the reaction mixture to final concentrations of: 31,25 µg/mL, 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/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 µL) was taken and added into the reaction mixture at final concentration of 0,0625 µg/mL; 0,125 µg/mL; 0,25 µg/mL; 0,5 µg/mL ; and 1 µg/ mL. Blanks, controls and each concentration of samples were done in triplicate. Following incubation at 37°C for 5 minutes, 25 µl of enzyme solution was added into the reaction mixture and incubated further for 15 minutes. Enzyme reaction was stopped by adding 100 µl of 0,1M Na_2CO_3. Blanks, controls, and samples absorbance of the *p*-nitrophenol product was measured by microplate reader spectrophotometer at 410 nm wavelength.

RESULT AND DISCUSSION

Characterization of compounds

IR spectra of compound **1** showed some bands at, 3447 (broad) (O-H stretch), 3084 (aromatic C-H stretch), 2972 (aliphatic C-H stretch), 1650 (C=O stretch), 1602 (C=C stretch) and 1223 (C-O bending). This spectrum indicated that compound **1** was a flavonoid-typed compound. In ^{1}H NMR spectrum, five aromatic or methylenic protons were shown δ_H 7.41, 7.39, 6.91, 6.57 and 6.64, as well as a methoxy unit shown δ_H 3.91. Some aliphatic protons were shown in ^{1}H NMR spectrum. It was found that compound **1** possessed 22 protons. There were 25 carbons found in ^{13}C spectra, including aromatic, *sp*3 aliphatic and *sp*2 aliphatic carbons. Therefore, it was assumed that this compound is prenylated flavonoid, a monoterpene attached to a flavonoid ring. COSY and HMBC spectra the monoterpene was attached to C-6 of the flavone. This confirmed that compound **1** was a prenylated flavone. Published data indicated that this compound was ugonin type flavone, as reported by Huang.[4] The results of structure characterization indicated that the compound **1** was comparable to ugonin K (Table 1).[4]

IR spectrum of compound **2** showed similarity from compound **1** which indicated the compound was have similar structure. In ^{1}H NMR spectrum, five aromatic or methylenic protons were shown δ_H 7.36, 7.35, 6.89, 6.49, and 6.41. This a typical a four-substituted flavonoid. Some aliphatic protons were shown in ^{1}H NMR spectrum. It was found that possessed 22 protons. There were 25 carbons found in ^{13}C spectra, aromatic, *sp*3 aliphatic and *sp*2 aliphatic carbons. Therefore, it was assumed that this compound is prenylated flavonoid, a monoterpene attached to a flavonoid ring. COSY and HMBC spectra the monoterpene was attached to C-6 of the flavone. This confirmed that compound **2** was prenylated flavone. Published data indicated that this compound was also ugonin type flavone, as reported by Huang.[4] The results of structure characterization indicated that the compound **2** was similar to ugonin J (Table 1) (Figures 1 and 2).[4]

Antidiabetic activity

Antidiabetic analysis was carried out on **1** and **2** by examining the inhibition of α-glucosidase (*in vitro*) with *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG). Reaction was terminated with an addition of 100 µL Na_2CO_3 0,1 M. Then, the absorbance was measure with a 96-well plate reader. **(1)** revealed a moderately activity of *α*-glucosidase enzyme with an IC_{50} value 138,21 ppm, however the activity of ugonin J **(2)** with an IC_{50} value 273,13 ppm. In this study, acarbose was also used as a standard drug for *α*-glucosidase inhibitor. Acarbose at a concentration of 0,0625-1 ppm was used a standard (Table 2).

The interaction of acarbose on the surface of microvilli can be seen in (Figure 3).

From the Figure 3, the α-glucosidase inhibition activity was conducted based on the basic principle of enzymatic reaction, the hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside (PNPG) substrate by the α-glucosidase enzyme to *p*-nitrophenol (yellow color) and glucose.[11,12] Drugs from this class can inhibit the α-glucosidase enzyme in the small intestine so that it will inhibit the disaccharide compounds into glucose.

Table 1: ^{1}H NMR and ^{13}C NMR spectroscopic data (500 MHz) for 1 and 2.

Position	1		2	
	δH (ppm)	δC(ppm)	δH (ppm)	δC(ppm)
2	-	164,87	-	164,44
3	6,57 (s)	102,68	6,49 (s)	101,70
4	-	182,61	-	182,48
4a	-	104,41	-	103,49
5	-	164,05	-	162,75
6	-	112,96	-	112,01
7	-	157,98	-	158,96
8	6,64 (s)	89,33	6,41 (s)	91,85
8a	-	156,13	-	155,65
9	2,75 (dd), 2,95 (dd)	20,74	2,72 (dd), 2,69 (dd)	20,88
10	2,27 (dd)	52,81	2,40 (dd)	52,08
11	-	149,55	-	149,37
12	1,68 (td), 1,28 (m)	34,40	1,69 (td), 1,25 (m)	34,49
13	2,49 (dt), 1,97 (td)	30,73	2,54 (dt), 1,94 (td)	30,85
14	1,60 (m), 1,53 (m)	23,06	1,60 (m), 1,48 (m)	23,08
15	-	31,22	-	32,42
16	0,93 (s)	27,20	0,94 (s)	27,27
17	1,07 (s)	27,20	0,88 (s)	26,18
18	4,41 (t), 4,11 (brs)	108,26	4,42 (t), 4,20 (brs)	108,23
1'	-	122,35	-	122,48
2'	7,39 (s)	112,78	7,36 (s)	112,72
3'	-	145,64	-	145,57
4'	-	149,55	-	149,66
5'	6,91 (dd)	115,36	6,89 (dd)	115,31
6'	7,41 (s)	118,87	7,35 (s)	118,75

Table 2: *α*-glukosidase inhibition by Ugonin K and Ugonin J.

Sample	IC_{50} (ppm)
Ugonin K (compound 1)	138,21±0,263
Ugonin J (compound 2)	273,13±0,402
Acarbose	19,73±0,342

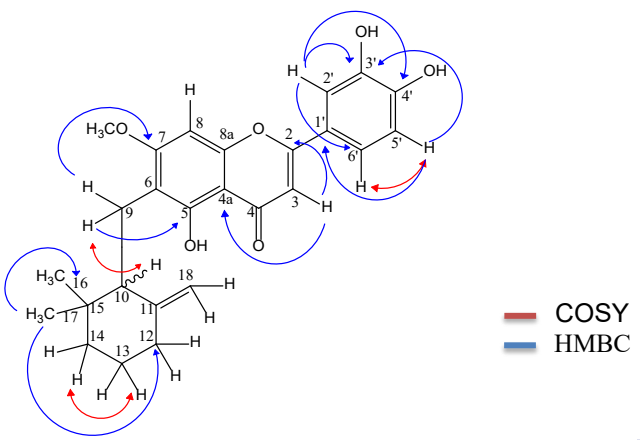

Figure 1: COSY and HMBC correlation of Ugonin K (Compound **1**).

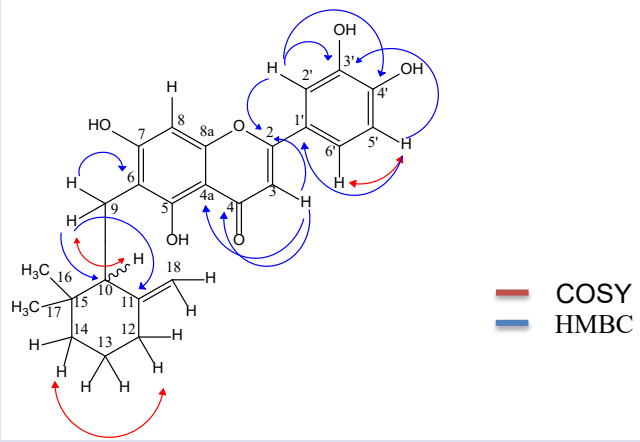

Figure 2: COSY and HMBC correlation of Ugonin J (Compound **2**).

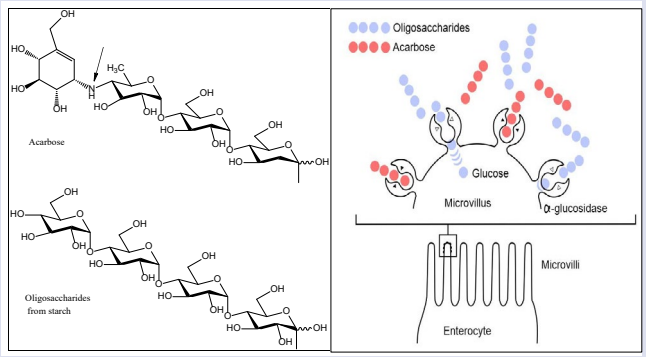

Figure 3: The mechanism of α-glucosidase inhibition by acarbose (*source*: Rosak, 2012)[11].

Therefore, reducing the breakdown of disaccharide compounds into glucose in the small intestine can reduce the absorption of glucose in the blood through the surface of microvilli in the small intestine.[13]

Based on Huang *et al* research that *H. zeylanica* contains various flavonoid compounds such as ugonin E-L.[4] These results agree with Wang *et al* the phytochemical compounds have the ability to inhibit α-glucosidase enzymes such as compounds from the flavonoid.[14] Ugonin J and K contain hydroxy group (-OH) which is a pharmacophore group of acarbose. The group in these compounds interact with the active side of the α-glucosidase enzyme through hydrogen bonds by involving O, N, and H atoms on the active side of the α-glucosidase enzyme.

CONCLUSION

The root of *H. zeylanica* collected in Riau Province contained prenylated flavons ugonin J and K. These compounds possess weak to moderate antidiabetic activity against α-glucosidase (*in vitro*). Therefore this plant can be used traditionally as antidiabetic medicine.

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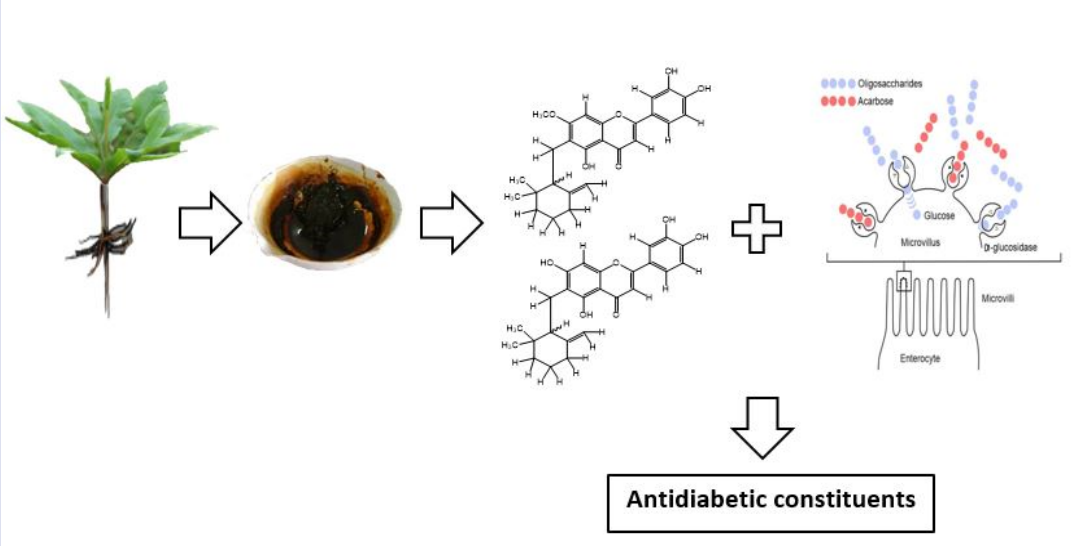
GRAPHICAL ABSTRACT



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