Antidiabetic Activity of Triterpenoids from *Anisophyllea disticha*

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**Abstract.** Diabetes and its complication are strongly associated with mortality and morbidity rate in Indonesia. The number of diabetes patients is expected to increase every year. $\alpha$-Glucosidase enzyme inhibitor widely used for treatment of diabetes. It may be obtained by isolation of Indonesian medicinal plants. As medicinal plant which is used by Talang Mamak tribe, Rakit Kulim, Indragiri Hulu, Riau, *Anisophyllea disticha* has its own attraction to study because its potential secondary metabolites have never been reported scientifically. Therefore, this study aims to determine antidiabetic activity by $\alpha$-glucosidase inhibition method of metabolites this plant. The isolation of metabolites was based on solvent polarity fractionation by vacuum liquid chromatography method on ethyl acetate extract. Chemical structures of the metabolites were elucidated based on spectroscopic characteristics, included FTIR and 1D H-NMR. The isolated compounds were assumed to be two triterpene derivatives. The IC$_{50}$ of compound 1, compound 2, and acarbose as a positive control were $46.246 \pm 0.166$, $80.273 \pm 0.08$ and $19.737 \pm 0.046$, respectively. Therefore it can be concluded that both compounds have potential as antidiabetic.

1. **Introduction**

Diabetes is a chronic metabolic and a serious complex multifactorial disorder characterized by chronic hyperglycemia. Symptoms of its complication leads to vascular, nephropathy, and cardiovascular disease. Moreover, diabetes and chronic hyperglycemia are strongly associated with insulin resistance and would lead to oxidative stress [1-3].

Participants with diabetes tended to suffered excess mortality due to its complication. Death rates in men and women increased significantly with age [4, 5]. Number of adults with diabetes in the world has increased from 108 million in 1980 to 422 million in 2017, with an estimate of a 48% increase to 628.6 million people by 2045 [6].

Treatment of diabetes can be managed by using $\alpha$-glucosidase inhibitors which has a beneficial effect on glycemic index control for diabetes patients because its ability to reduce glucose absorption in blood. $\alpha$-Glucosidase inhibitor which usually consist of active compounds from alkaloids, triterpenoids and flavonoids groups can be obtained from natural medicinal plants [7-9].

*Anisophyllea disticha* is a medicinal plant species from Talang Mamak tribe whose potential activity has never been reported scientifically. The plant is used ethnomedically to treat headache, diarrhea, dysentery and fatigue [10, 11]. The scientific report of genus of *Anisophyllea* is limited, but the ethanol extract of leaves and roots from *A. laurina* has been reported to have antidiabetic activity. [12] Therefore, this study aims to determine antidiabetic activity of isolated secondary metabolites from *A. disticha*. The results of this report can be useful in further antidiabetes investigation.
2. Methodology

2.1. Collection and Preparation Material
The leaves of A. disticha was collected from Rakit Kulim District, Indragiri Hulu Regency, Riau Province. They were dried and ground into powder.

2.2. Extraction and Isolation
Some 5 kg of the powder was macerated using n-hexane and ethyl acetate, respectively. The ethyl acetate extract was dried in vacuo and subjected to vacuum liquid chromatography (VLC), based on solvent polarity fractionation (n-hexane, n-hexane: ethyl acetate, ethyl acetate, and ethyl acetate: methanol) to obtain 12 fractions. The fractions 5 and 6 were recrystallized by acetone and afforded as white powder compounds. Structures of the compounds were determined by using spectroscopy methods including infrared (Shimadzu Prestige 21 FT-IR) and NMR (Bruker Ascends 600 MHz for 1H-NMR). NMR spectra was recorded at Department of Chemistry ITB, Bandung.

2.3. Antidiabetic Assay
Antidiabetic activity was determined by inhibition of α-glucosidase enzyme with p-nitrophenyl-α-D-glucopyranoside as substrate. In this study, in vitro α-glucosidase inhibition assay has followed previous journal method.[13] Samples of the compounds were diluted by two fold dilution method at 1000 - 31.25 µg / mL concentration. The sample in each well accompanied by phosphate buffer (pH 7), 25 µl p-NPG 20 mM and 25 µL α-glucosidase (0.2 U / mL) (S1). Non-substrate-sample is tested by adding the amount of phosphate buffer into 75 µl (S0). As comparison, measurement of blank (B1) and control blank (B0) were carried out using DMSO as a substitute for samples of the compound. In this test, acarbose is used as positive control. The test was carried out in a 96-well microplate and incubated for 30 minutes at 37°C. % inhibition can be calculated using the following formula:

\[
% \text{Inhibition} = \frac{(B_0 - B_1) - (S_1 - S_0)}{(B_1 - B_0)} \times 100
\]  (1)

IC_{50} values were calculated using logarithmic equation \((Y = a \ln X + b)\) from calibration curve by plotting sample concentration (x-axis) with % inhibition value (y-axis).

3. Results and Discussion

3.1. Compound Structure
Spectroscopy of compounds 1 and 2 showed similar result. It means the both compounds are derivative each other. Infrared spectroscopy of compounds 1 showed maximum absorption at 1693 cm\(^{-1}\) wave numbers indicating the presence of C=O groups, 2967, 2951 and 2877 cm\(^{-1}\) indicating the presence of CH groups, and at 1242 cm\(^{-1}\) indicating the presence of C=O groups while compound 2 only has maximum absorption at 1693 cm\(^{-1}\) indicating the presence of C=O groups and 2945, 2877, and 2850 cm\(^{-1}\) indicating the presence of CH groups. Spectrum analysis of 1H-NMR compound 1 showed six methyls at 1.17 (3H, s); 1.05(3H, s), 1.02(6H, s); 0.94(3H, s); 0.87(3H, s) chemical shift (figure 1) while compound 2 showed eight methyls at 1.30 (3H, s); 1.17 (3H, s); 1.12 (3H, s); 1.05(3H, s); 1.02(6H, s); 0.94(3H, s); 0.87(3H, s) chemical shift (figure 2). Therefore, these compounds were assumed as triterpene derivatives. H-NMR spectra of methyls from both compounds are showed in Figure 1 and 2, both figure showed that significant difference of compounds 1 and 2 can be seen in addition methyl of compound 2 at 1.3 ppm (3H, s) chemical shift.

3.2. Antidiabetic Activity
As seen in table 1, compounds 1 and 2 have relative small IC_{50} values. The differences in antidiabetic activity of the compounds can be due to structural differences. However, IR and H-NMR data could not determine overall of the differences and structure of both compounds. Important position for
antidiabetic activity in triterpenoid including carbonyl group at C-28, carboxyl group at C-17 and methyl group at C-29 of oleanane triterpenoid have important role on alpha glucosidase inhibition.[14] Compared with acarbose as a positive control, both isolated compounds from A. disticha leaves can be concluded that they have potential as antidiabetic.

Figure 1. Isolated Compound 1 H-NMR Spectra

Figure 2. Isolated Compound 2 H-NMR Spectra
Table 1. α-Glucosidase Inhibition by Compound 1 and Compound 2

| Concentration (μg/mL) | % Inhibition | IC₅₀ (ppm) |
|-----------------------|-------------|-----------|
|                       |             |           |
| Compound 1            |             |           |
| 1000                  | 80,447      |           |
| 500                   | 72,516      |           |
| 250                   | 64,722      |           |
| 125                   | 60,757      |           |
| 62.5                  | 53,646      |           |
| 31.25                 | 45,579      | 46,246±0,166 |
| Compound 2            |             |           |
| 1000                  | 78,532      |           |
| 500                   | 71,285      |           |
| 250                   | 63,218      |           |
| 125                   | 55,697      |           |
| 62.5                  | 48,177      |           |
| 31.25                 | 37,785      | 80,273±0,080 |
| Acarbose              |             |           |
| 100                   | 81,951      |           |
| 50                    | 69,371      |           |
| 25                    | 54,603      |           |
| 12.5                  | 39,152      |           |
| 6.25                  | 25,478      |           |
| 3,125                 | 16,180      | 19,737±0,046 |

4. Conclusion
Isolation of secondary metabolites from leaves of medicinal plants *Anisophylla disticha* produced two triterpenoids which have strong inhibition in inhibiting of α-glucosidase performance. IC₅₀ of compound 1 and compound 2 were 46.246 ± 0.166 ppm and 80.273 ± 0.08 ppm, respectively. IC₅₀ values of both compounds have not significant different with acarbose IC₅₀ value as positive control. Therefore, it can be concluded that triterpenoids from *A.disticha* have potential as antidiabetic.

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