**α-GLUCOSIDASE INHIBITORY ACTIVITY OF CUCURBITANE DERIVATE ISOLATED FROM METHANOL EXTRACT OF MOMORDICA CHARANTIA L. LEAVES**

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**Abstract.** Turunan senyawa kurkubitan telah berhasil diisolasi dari ekstrak metanol daun pare *Momordica charantia*. Pemurnian ekstrak metanol menggunakan metode kromatografi kolom vakum dengan *n*-heksana:etil asetat sebagai fasa gerak. Senyawa isolat berbentuk serbuk putih dielusidasi untuk mendapatkan struktur kimia berdasarkan data spektroskopi (FT-NMR, FT-IR, dan LC-MS/MS) dan diperoleh senyawa turunan kurkubitan yakni momordisin I. Ekstrak MeOH dan senyawa isolat dievaluasi efek inhibisinya terhadap penghambatan enzim α-glukosidase. Ekstrak MeOH dan senyawa isolat menunjukkan aktivitas penghambatan yang moderat dengan %penghambatan pada konsentrasi 100 µg/mL masing-masing sebesar 27,34 % dan 15,79 %.

**Kata kunci:** Kukurbitan, *Momordica charantia*, momordisin I, α-glukosidase

**INTRODUCTION**

Bitter melon (*Momordica charantia*) is a member of the Cucurbitaceae family which is easily cultivated and spreads to include China, India, and Southeast Asia. In Indonesia, bitter melon is consumed as a vegetable and used as traditional medicine. Utilization of *M. charantia* empirically carried out by the community is a cure for diabetes, heart disease and stomachache (Grover and Yadan, 2002). *M. charantia* has various medicinal potentials as antidiabetic (Joseph and Jini, 2013), antibacterial (Kumar *et al.*, 2010) and antimicrobial (Svobodova *et al.*, 2010). The antidiabetic effect is inseparable from the role of secondary metabolites compounds in the form of triterpenes, proteins, steroids, alkaloids, lipids, and phenolic compounds (Zhanga *et al.*, 2012). *M. charantia* leaves contain momordica, momordine, charantine, trichosanic acid, resin, resinic acid, saponin, vitamins A and C (Tan *et al.*, 2008). Previous studies have shown the compounds momordicoside A and M from *M. charantia* fruit have inhibitory activity...
against rat intestinal α-glucosidase enzymes (Nguyen et al., 2010). Earlier anti-diabetic compound, momordicin, has α-amylase inhibitory activity (Kulkarni et al., 2019). While in this study used α-glucosidase enzyme from saccharomyces cerevisiae recombinant.

The enzyme α-glucosidase plays an important role in the hydrolysis of complex carbohydrates into glucose which can be absorbed through the intestine. Inhibition of α-glucosidase work can reduce glucose absorption in patients with hyperglycemia (Ben et al., 2017). One of the α-glucosidase inhibiting agents is acarbose which has been reported to reduce the intestinal absorption of sugar in humans (Jenkins et al., 1981). The results of this previous study encouraged this research to explore metabolite compounds contained in M. charantia leaves used methanol as extractor and tested their inhibitory activity against the α-glucosidase enzyme.

MATERIALS AND METHODS

Materials
The materials used were bitter melon leaves (Momordica charantia), organic solvents (ethyl acetate, n-hexane, methanol, 10% H2SO4 (v/v) in methanol, silica G60 plates, silica gel G60 0.040-0.063 mm. The material used in the enzyme inhibition test were the α-glucosidase enzyme derived from Saccharomyces cerevisiae recombinant with 125 U/mg activity (Sigma Aldrich, USA), p-nitrophenyl-α-D-glucosidase (p-NPG) (Sigma Aldrich, USA), phosphate buffers, Na2CO3, aquabides, dimethyl sulfoxide (DMSO), and acarbose.

Methods
1. Extraction and Isolation
M. charantia (1.5 kg) dried leaves were extracted using the maceration method with 15 L of methanol solvent and re-extracted four times. Maserate was concentrated with a rotary vacuum evaporator obtained a green residue (68 g). MeOH extract (30 g) was further separated using a column of vacuum chromatography with silica G60 as a stationary phase and a mixture of n-hexane: ethyl acetate: methanol gradient to increase polarity, obtained 30 fractions. Fraction 17 has been recrystallized using n-hexane: ethyl acetate and obtained white crystal (0.8 g). The isolated compound was identified using FT-NMR, FT-IR, and LC-MS/MS.

2. Inhibitory Activity Of α-Glucosidase Enzyme
Testing the inhibitory activity of α-glucosidase enzymes adapting the procedure from Fajriah et al (2018). The α-glucosidase enzyme solution containing 200 mg of albumin was dissolved into a phosphate buffer solution (pH 7). The extract was prepared by dissolving 5µL in DMSO, and then a reaction mixture was added consisting of 250 µL p-nitrophenyl α-D-glucopyranose 20 mM as substrate and 490 µL phosphate buffer 100 mM (pH 7). Enzyme solution (250 µL) was added after the reaction mixture was incubated 5 minutes at 37 °C and reincubated then added 1000 µL of 200 mM Na2CO3 solution to stop the reaction. The sample concentration for activity evaluation was 100 µg/mL. The results of the reaction in the form of p-nitrophenol were measured at λ 400 nm and compared with acarbose as a positive control at a concentration of 50 µg/mL.

RESULT AND DISCUSSION

1. Structural elucidation
MeOH extract of M. charantia leaves obtained white powder with molecular weight m/z 495.73 [M + Na]+. The IR absorption bands suggested the presence of various functional groups such as an aldehyde and hydroxyl at 1705 cm⁻¹ and 33092 cm⁻¹. FT-NMR data indicated the molecular formula was C30H48O4. 1H-NMR spectrum data (Table 1) shows an aldehyde (δH 9.88 ppm), seven methyl signals, six methyl singlet and methyl doublet at chemical shift (δH 0.81, 0.92, 1.08, 1.25 ppm), and three methane
oxygenated at (δ\textsubscript{H} 3.5H, 3.50, 3.99, 4.44 ppm).

![Figure 1. structure of Momordicine I](image)

The \textsuperscript{13}C -NMR spectrum data (Table 1) showed 30 carbon signals aided by the Distortionless enhancement by polarization transfer (DEPT) data and compared with previously published data (Yasuda \textit{et al.}, 1984 and Zhang \textit{et al.}, 2014) showed seven methyl (δ\textsubscript{c} 15.3, 18.8, 27.8, 18.2, 19.3, 26.1, 26.0 ppm), seven methylene, seven methines and three of them are hydroxymetin (δ\textsubscript{c} 77.0, 66.6, 67.0), three carbon quartener (46.6, 42.3, 46.8), two double bonds (δ\textsubscript{c} 124.0, 147.4, 130.5, 133.5) and there are characteristics for an aldehyde (δ\textsubscript{c} 209.8 ppm). This is confirmed by HMBC (Figure 1) that the aldehyde group is located in C-9 and the proton aldehyde H-19 correlation C-8 (δ\textsubscript{c} 51.9 ppm), C-9 (δ\textsubscript{c} 46.8 ppm), and C-11 (δ\textsubscript{c} 23.5 ppm). All these enabled the structure of isolate compound to be assigned as momordicine I.

<table>
<thead>
<tr>
<th>No</th>
<th>δ\textsubscript{c} (ppm)</th>
<th>δ\textsubscript{H} (ppm), multiplicity, J(Hz)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>22.2</td>
<td>1.41 (d, 5.4)</td>
</tr>
<tr>
<td>2</td>
<td>28.6</td>
<td>1.93 (m)</td>
</tr>
<tr>
<td>3</td>
<td>77.0</td>
<td>3.50</td>
</tr>
<tr>
<td>4</td>
<td>42.3</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>147.4</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>124.0</td>
<td>5.90 (d, 4.2)</td>
</tr>
<tr>
<td>7</td>
<td>66.6</td>
<td>3.99 (d, 4.2)</td>
</tr>
</tbody>
</table>

2. Inhibitory Activity of alpha glucosidase

MeOH extract and momordicine I was tested for their inhibitory activity against the α-glucosidase enzyme. The results were presented in percent inhibition of the concentration of the test material (Table 2). \textit{M. charantia} methanol extract showed inhibition activity of 27.34 % at a concentration of 100 µg/mL, the low value is probably due to the role of the synergy of the active substance inside the extract. The value of extract was greater than the inhibitory activity of momordicine I 15.79% with the same concentration. Likewise, the activity of the acarbose as a positive control is greater than momordisin I with an inhibitory value of 47.54 % at a concentration of 50 µg/mL. This difference in percent inhibition is based on active
substituents that are bound to acarbose. Based on this data, it is possible to explore compounds that have better activity as an α-glucosidase enzyme inhibitor agents than the extract of MeOH M. charantia leaves in the future.

Table 2. α -Glucosidase inhibitory activity of isolate compound

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Enzyme inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>27.34</td>
</tr>
<tr>
<td>Momordicine I</td>
<td>15.79</td>
</tr>
<tr>
<td>Acarbose</td>
<td>47.54</td>
</tr>
</tbody>
</table>

*Percentage of enzyme inhibition at the concentration of 100 µg/mL, acarbose at 50 µg/mL.

CONCLUSION
The cucurbitane compound derivative that has been isolated from the MeOH extract of M. charantia leaves is momordicine I and its inhibitory activity on the α-glucosidase enzyme gives a value of 27.34 and 15.79 % at a concentration of 100 µg/mL which is quite inhibiting.

CONFLICT OF INTEREST
This research is supported by the 2019 grant for the master thesis research of Directorate of Research and Development of the KEMENRISTEKDIKTI Of Indonesia, with the contract number of 1739/UN4.2/PL.01.10/2019.

ACKNOWLEDGMENTS
The authors thank the staff of the Laboratory of Natural Product Chemical Laboratory at the LIPI Research Centre for facilities and instruments that support this research.

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