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

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Abstrak. Turunan senyawa kukurbitan telah berhasil diisolasi dari ekstrak metanol daun pare *Momordica charatia*. 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 kukurbitan 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 moderatdengan %penghambatan pada konsentrasi 100 µg/mL masing-masing sebesar 27,34 % dan 15,79 %.

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

Abstract. Cucurbitane derivative has been isolated from methanol extract of the leaves of bitter melon (*Momordica charantia).* Purification of methanol extract using vacuum column chromatography method using *n*-hexane: ethyl acetate as the mobile phase was obtained a white powder isolate. This isolate was elucidated to obtain chemical structures based on spectroscopic data (FT-NMR, FT-IR, and LC-MS/MS) and resulted a cucurbitane derivate, namely momordicine I. The MeOH extract and the isolate were evaluated for α-glucosidase inhibitory effect. Both MeOH extract and momordicine I showed moderate activities with %inhibition 27.34% and 15.79 % at 100 µg/mL repectively.

Keyword : Cucubitane, *Momordica charantia*, momordicine I, α-glucosidase

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 antidiabetic compound, momordicinin, 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% H_2SO_4 (v/v) in methanol, silica G_{60} plates, silica gel G_{60} 0.040-0.063 mm. The material used in the enzyme inhibition test were the α-glucosidase enzyme derived from *Saccharomyces cereviceae* recombinant with 125 U/mg activity (Sigma Aldrich, USA), *p*-nitrophenyl-α-D-glucosidase (*p*-NPG) (Sigma Aldrich), USA), phosphate buffers, Na_2CO_3, aquabidest, 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 G_{60} 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 ^oC and reincubated then added 1000 μL of 200 mM Na_2CO_3 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 $C_{30}H_{48}0_4$. ^1H-NMR spectrum data (Table 1) shows an aldehyde (δ_H 9.88 ppm), seven methyl signals, six methyl singlet and methyl doublet at chemical shift (δ_H 0.81, 0.92, 1.08, 1.25 ppm), and three methane

oxygenated at (δ_H 3.5H, 3.50, 3.99, 4.44 ppm).

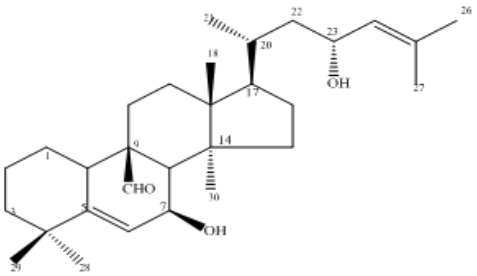

Figure 1. structure of Momordicine I

The ^{13}C -NMR spectrum data (Table 1) showed 30 carbon signals aided by the Distrotionless enhancement by polarization transfer (DEPT) data and compared with previously published data (Yasuda *et al*., 1984 and Zhang *et al*., 2014) showed seven methyl (δ_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_c 77.0, 66.6, 67.0), three carbon quartener (46.6,42.3,46.8), two double bonds (δ_c 124.0, 147.4, 130.5, 133.5) and there are characteristics for an aldehyde (δ_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_C 51.9 ppm), C-9 (δ_C 46.8 ppm), and C-11 (δ_C 23.3 ppm). All these enabled the structure of isolate compound to be assigned as momordicine I.

Table 1. ^1H and ^{13}C NMR spectral data of momordicine I (measured at 500 MHz in $CDCl_3$)

No	δc (ppm)	δ_H (ppm), multiplicity, *J*(Hz)
1	22.2	1.41 (d, 5,4)
2	28.6	1.93 (m)
3	77.0	3.50
4	42.3	-
5	147.4	-
6	124.0	5.90 (d, 4.2)
7	66.6	3.99 (d, 4.2)
8	50.9	1.93
9	46.8	-
10	35.7	2.59 (dd, 4.8, 12.6)
11	23.3	1.59
12	30.3	1.24 (d, 2.4)
13	46.6	-
14	46.8	-
15	40.3	1.36 (m)
16	29.9	2.01
17	52.1	1.48 (m)
18	15.3	0.92 (s)
19	209.8	9.88
20	33.8	1.7 (m)
21	19.3	0.96 (d, 6.6)
22	45.6	1.61 (m)
23	68.6	4.44 (dt, 2.4, 8.4)
24	130.5	5.15 (d,8.4)
25	133.5	-
26	26.0	1.70 (s)
27	18.2	1.67 (s)
28	27.8	1.08 (s)
29	26.1	1.025
30	18.8	0.81

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). *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

Sample	% Enzyme inhibition*)
MeOH extract	27.34
Momordicine I	15.79
Acarbose	47.54

*Percentage of enzyme inhibition at the concentration of 100 µg/mL, acarbosa 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

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