ASM Sc. J., 16, Special Issue 1, 2021 for SCIEMATHIC2019, 76-84

A Potent α-Glucosidase Inhibitor of *Moringa Oleifera* Leaves

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Moringa oleifera (Moringaceae) is an ornamental plant in tropical and subtropical areas. *M. oleifera*, known as kelor, is widely used in Indonesia as an edible plant and health supplement. In our research, the discovery of the bioactivity of various extracts, fractions, and isolated compound of *M. oleifera* leaves has been evaluated by antioxidant and α-glucosidase assay. As our previous report, *M. oleifera* leaves extract has good antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline sulfonic acid) (ABTS) radicals. This study presents α-glucosidase inhibitory activity of *M. oleifera* leaves extracts, fractions, and isolated compound. α-Glucosidase enzyme causes hyperglycemia related with diabetes disease. The result showed the highest α-glucosidase inhibitory activity of *M. oleifera* is methanol extract and a new compound O-butyl, N- {4- [3'-O-β-D-glucopyranosyl) (4'-O-acetyl-α-L-ramnosiloxy)] - benzyl} carbamate **(1)** with IC_{50} value of 38.33 and 102.99 μg/mL respectively, compared with acarbose as a positive control with IC_{50} value of 304.25 μg/mL. This study concluded that *M. oleifera* should be potential as a α-glucosidase inhibitor.

Keywords: chemical constituents; jamu; diabetes mellitus; α-glucosidase; traditional medicines

I. INTRODUCTION

α-Glucosidase enzyme (EC 3.2.1.20, α-D-glucoside glucohydrolase) is an enzyme found in the human intestinal. This enzyme catalyses the production of glucose from a non-reducing sugar substrate by breaking down the glycoside bonds (Fatmawati *et al.*, 2011). The digestive process causes the pancreatic enzyme α-glucosidase to be released into the small intestine (Kim *et al.*, 2008). For diabetes mellitus (DM) patients type II, the inhibition of the α-glucosidase enzyme can be used as a treatment. The presence of an α-glucosidase inhibitor will be slow the absorption of glucose in the small intestine, so glucose levels in the blood for diabetics can decrease.

Antioxidant plays an important role in human immunity to protect against infections, diabetes, and cancer. Synthetic antioxidants are known as butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA) are generally used in the food and beverage and industry. When BHA and BHT used for long-time, it can be promoted to carcinogenic. Therefore, research of natural antioxidants is needed for natural antioxidant sources. Organic materials and food can be used as source of natural antioxidants present. There is increasing interest of natural antioxidant evaluation form many natural products such as plants or marine animal (Wolfie *et. al.*, 2003; Liyana & Shahidi, 2005).

Moringa oleifera is a species of the *Moringaceae* family that is commonly known as *kelor* in Indonesia. People in Indonesia use leaves of *M. oleifera* as vegetable and traditional medicines. The leaves have high nutritional value, consist of important minerals such as Fe, Ca, protein, vitamins, ascorbic acid, β-carotene, and amino acids. They also consist of various phenolic compounds such us flavonoid and carotenoids (Anwar *et al.*, 2007). These compounds

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provide *M. oleifera* leaves as a good source of natural antioxidant (Anwar *et al.*, 2005).

Antioxidant is great importance in preventing stress that may cause several degenerative diseases such as cancer, heart disease, hypertension, and stroke (Helen *et al.*, 2000). On the other hand, antioxidant is a molecule to stabilise free radicals before they attack cells (Kaliora *et al.*, 2006). The frequent consumption of food antioxidants such as fruits and vegetables can prevent the degenerative diseases because of the presence of antioxidant compounds including flavonoids and phenolic compounds (Lako *et al.*, 2007).

For this reason, the discovery of antioxidant and α-glucosidase inhibitor from natural resources has been studied continuously. In this study, we focus on bioassay as antioxidant and α-glucosidase assay guided fractionation of *M. oleifera* leaves extract.

II. MATERIALS AND METHODS

A. Materials

The leaves of *M. oleifera* were collected from Jombang, East Java, Indonesia. Silica gel 60 GF_{254} were used for column chromatography. The spraying reagent used for TLC was cerium sulphate ($Ce(SO_4)_2$). Solvent methanol, ethyl acetate, dichloromethane, *n*-hexane, DMSO, ethanol and trolox from Wako Pure Chemical Industries Japan. The α-glucosidase enzyme, buffer HEPES (4-(2-hydroxymethyl)-1-pyperazynetanasulfonic), DPPH and ABTS from Wako (Japan). Acarbose and trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) from Sigma Aldrich. Incubator EYELA SLI-400 has been used for incubation process of sample. The reaction was monitored by spectrophotometer (UV Jasco V-530) from Japan.

B. Methods

The dried powder of leaves *M. oleifera* were macerated with methanol for 24 hours. The filtrates were evaporated with rotary evaporator to remove the solvent. The crude methanol extracts of *M. oleifera* leaves was obtained.

1. Fractionation of Ethyl acetate Fraction

The ethyl acetate fraction (EtOAc) (40.02 gr) was subjected to silica gel (250 gr) vacuum column chromatography (VCC) eluting with *n*-hexane : ethyl acetate : methanol (100:0:0; 4:1:0; 7:3:0; 3:2:0; 1:1:0; 3:7:0; 1:4:0; 1:9:0; 0:100:0; 0:9:1; 0:4:1; 0:1:1; and 0:0:100, each 500 mL) to yield nine fractions (E1-E9). Fraction E7 (0.428 gr) was fractionated by using HPLC with mobile phase of solvent A and B. Solvent A was 0,1% TFA in water (H_2O) and solvent B was acetonitrile (AcCN). The gradient elution with mixtures of solvent H_2O: AcCN (85:15→65:35) resulted in isolated compound (**1**). The UV detector used 220 nm and the flow rate was 1 mL/min. The injection volume was 10 µL. Repeated purification of Fraction E7 was performed by using RP-HPLC-ODS by Waters. The flow rate was 5 mL/min with the same condition as analytical one. The injection volume was 500 µL. The 1D- and 2D-NMR spectra of compound **(1)** was recorded in CD_3OD by using ^{1}H-NMR (600 MHz) and ^{13}C- NMR (150 MHz) spectroscopy. The compound was then identified by comparison with published data of Marumoside B (Sanakitpichan *et al.*, 2011).

2. Fractionation of n-Hexane Fraction

Crude extract (300 gr) was partitioned by using water and organic solvent into three fractions. The ethyl acetate soluble, *n*-hexane soluble and water-soluble (residue) portions. The *n*-hexane fraction (50.02 gr) was subjected to column chromatography (GCC) using silica gel (150 gr). GCC eluting with *n*-hexane: dichloromethane (100:0→10:90) and n-hexane: ethyl acetate (90:10→ 20:80) to obtain nine fraction (F1-F9). Fraction F8 (2,15 g) was further separated with silica gel column chromatography by using *n*-hexane: dichloromethane (100:0→10:90, each 300 mL) to yield eight sub-fractions (F8a-F8h). Sub-fraction F8b was then recrystallised with methanol to afford compound (1) (35.02 mg). 1D and 2D-NMR spectra compound (2) was recorded in $CDCl_3$ using ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopy.

3. α-Glucosidase Inhibitory Activity Assay

The assay for inhibiting the activity of α-glucosidase enzymes was carried out based on previous research methods (Fatmawati *et al.*, 2011). The mixture consists of methanol or DMSO or water as solvent was put into two tubes. The first tube (A) is without the compound test (control) and the second tube (B) with samples. Both tubes were added 0.1 mL

(5 units/mL) of α-glucosidase enzyme in 0.15 M HEPES buffer. The mixture was added 0.1 M sucrose (in 0.15 M HEPES buffer) then incubated at 37°C for 30 min. After incubation, the mixture was heated to 100°C for 10 minutes. The concentration of glucose can be observed by the glucose oxidation method by using BF-5S biosensors. The percentage of inhibition can be calculated with the Equation (1).

$$\text{Inhibition } (\%) = \left[\frac{\Delta C - \Delta T}{\Delta C}\right] \times 100\% \qquad (1)$$

Where ΔC = glucose concentration without the addition of samples, ΔT = glucose concentration with samples. Acarbose was used as a positive control.

4. DPPH Antioxidant Activity

The radical stabilisation activity of *M. oleifera* extracts was evaluated by DPPH (Fitriana *et al.*, 2016). The method was derived from Dudonn´e *et al.* (2009). First, *M. oleifera* extracts was dissolved with concentration of 33.33 μL of methanol. 1 mL of radical solution DPPH (6×10^{-5} M) was added to methanol solutions of *M. oleifera* extracts (33.33 μL). The mixed solution was mixed with vortex mixer, then incubated at 37°C for 20 min. The decreasing absorbance of the mixture was measured with spectrophotometer UV at 515 nm for *As* value. Thus, the solution colour change from violet to yellow pale during reduction by the samples. A blank sample was prepared with 33.33 μL of methanol and measured by the same wavelength for *Ab* value. Trolox was used as positive control. All the steps were performed in triplicate. IC_{50} value is a required concentration to scavenge 50% of DPPH free radical. The DPPH activity calculated by using the equation (2).

$$\text{DPPH Activity } (\%) = \left[\frac{Ab - As}{Ab}\right] \times 100\% \qquad (2)$$

Where *Ab* is an absorbance value of the blank and *As* is an absorbance value of the samples.

5. ABTS Antioxidant Activity

ABTS antioxidant activity was obtained based on the previous method (Re *et al.*, 1999). The ABTS [2,2′ azino - bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging based on the ability of antioxidant molecules against ABTS·+. The ABTS·+ was generated by peroxydisulfate oxidation. ABTS stock solution was prepared by 5 mL of 7 mM ABTS in ammonium aqueous solution. The ABTS·+ was produced by reaction of the ABTS stock solution with 88 μL of 140 mM potassium persulfate (Na2S2O8). The ABTS·+ solution allowed to stand at refrigerator storage for 12-16 hours at 4°C in darkness to yield a dark blue solution. Then, ethanol 99.5% was added to this solution. The working solution absorbance should be 0.7 ± 0.02 at 734 nm. 1 mL of working solution was mixed with 10 μL of *M. oleifera* extract and shaken well for 10 seconds by hand. The mixture was incubated for 4 min at 30°C. The absorbance of the mixture was measured with spectrophotometer UV at 734 nm (*As* value). Positive control was used Trolox. For a blank solution was used Ethanol 99.5% (*Ab* value). The antioxidant activity of the *M. oleifera* extracts was measured by using Equation (2).

III. RESULTS AND DISCUSSIONS

A. α-Glucosidase Inhibitory Assay

α-Glucosidase enzyme is an enzyme that plays an important role in the catalysis process of disaccharide solving into glucose which is then absorbed by the body (Fatmawati *et al.,* 2011). Testing of inhibition activity of the α-glucosidase enzyme was carried out on *M. oleifera* leaves extract with a variety of solvents. The result of inhibition percentage of α-glucosidase enzymes by several extracts is presented in Figure 1.

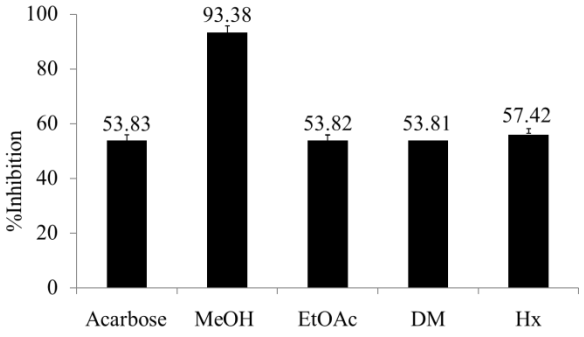

Figure 1. α-Glucosidase inhibitory activity of *M. oleifera* extract at a concentration of 333.33 µg/mL; values are mean ± SD of triplicate experiments. Acarbose is as a positive control; MeOH is methanol extract; EtOAc is ethyl acetate extract; DM is dichloromethane extract; and Hx is n-hexane extract of *M. oleifera*.

The result showed that methanol extract has the largest α-glucosidase inhibitory activity among the other extracts, which was 93.36%. While the percentage inhibition value for ethyl acetate extract was 53.82%, dichloromethane extract 53.81%, and *n*-hexane extract 57.42%. Positive control was acarbose which is commonly used as a medicine in type II diabetics. Acarbose had an inhibitory percentage value of 53.83% with a value of IC_{50} 304.2 μg/mL. Furthermore, the inhibitory activity assay of α-glucosidase of methanol extract with various concentrations to determine an IC_{50} value was shown in Figure 2.

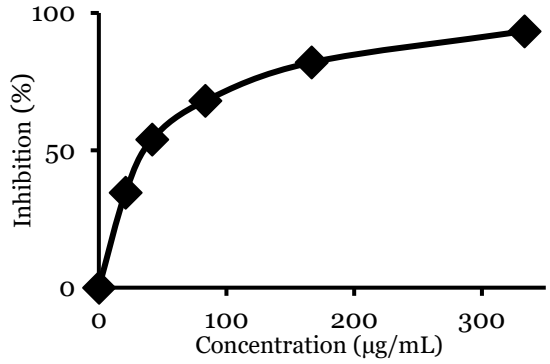

Figure 2. α-Glucosidase inhibitory activity of *M. oleifera* methanol extract

Compound **(1)** has inhibitory activity on the α-glucosidase enzyme of 72.28% at a concentration of 333.33 μg/mL with an IC_{50} value of 102.99 μg/mL presented in Figure 3.

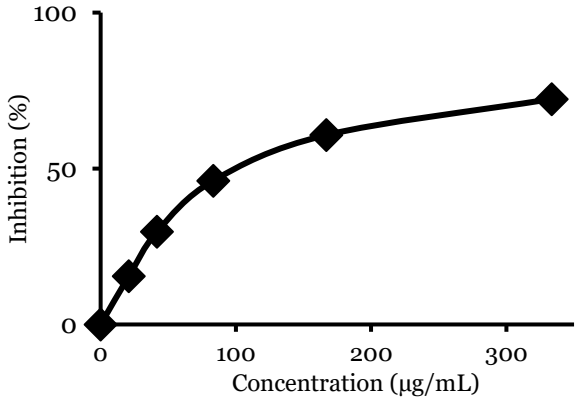

Figure 3. α-Glucosidase inhibitory activity of compound **(1)**.

Compound **(1)** had the potential α-glucosidase inhibition when compared with acarbose. Acarbose had a higher IC_{50} value because acarbose is less sensitive to the α-glucosidase enzyme originating from *Saccharomyces cerevisiae*. Acarbose is highly sensitive to the α-glucosidase enzyme originating from mammals (Kim *et al.,* 2008). One mechanism of α-glucosidase inhibition is the ability of compounds to form hydrogen bonds with residual acids. The mechanism of inhibition of the α-glucosidase enzyme by compound **(1)** had not been determined yet. The presence of hydroxyl groups and C-O ether bonds in the glucopyranosyl group of compounds is one of the factors that can increase the activity of compounds for inhibition of the α-glucosidase enzyme. The possibility of the mechanism that can be occurred because of these groups can bonding hydrogen with acid residues (Hyun *et al.,* 2014). Based on the chemical structure of compound **(1)**, the number of hydroxyl groups and C-O ether bonds in the glucopiranosyl group which can increase the inhibition of the α-glucosidase enzyme is upper than that of acarbose.

Compound **(2)** was also tested for inhibition of α-glucosidase activity. In the α-glucosidase activity test, compound **(2)** had a low activity of 21.03% at a concentration of 333.33 μg/mL.

B. *Isolation of Compound (1) from M. oleifera*

The EtOAc soluble was subjected in column chromatography to yield 9 fractions (E1-E9). Sub fraction E1-E9 have been evaluated for antioxidant activity. The result showed that the E7 have the highest antioxidant activity. The E7 HPLC analysis was shown in Figure 4. The vial collecting based on time retention, and then evaporated to remove the solvent. Furthermore, all samples were freeze dried.

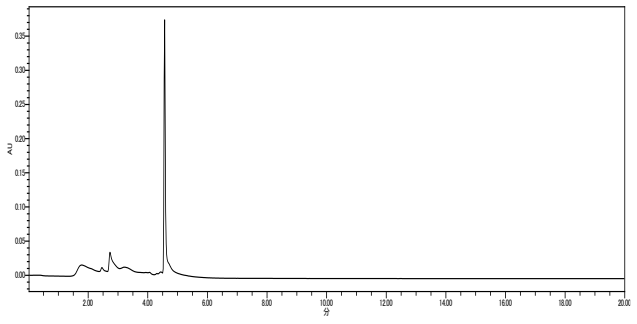

Figure 4. HPLC Chromatogram of **(1)**

Compound **(1)** was oily paste (12.1 mg). The chemical shift (δ) ^{1}H-NMR and ^{13}C-NMR plays a role in determining the chemical structure. Methanol solvent (CD_3OD) was used for ^{1}H-NMR measurements with frequencies of 600 MHz and ^{13}C-NMR with a frequency of 150 MHz. The solvent used for

NMR measurement was based on the solubility of the compound. The shift data (δ) 1H-NMR compound **(1)** (Table 1) indicated the presence of 38 protons, while from the 13C-NMR shift data (δ) informs the presence of 25 carbons.

The ^{1}H-NMR chemical shift data at δH 7.02 (2H, d, J 8.4 Hz) and δH 7.24 (2H, d, J 8.4 Hz) had the same coupling constant value. The coupling constant value of J 8.4 Hz indicated the presence of two aromatic protons located in the ortho AA 'position (Silverstein and Webster, 1998). The presence of two quartz carbon signals at δC 155.6 and δC 131.2 indicated a 1.4 aromatic ring system substituted with the AA'BB system. Based on HMBC data, the correlation between proton δH 3.45 (2H, s) with δC 131,2; δC 177.2 and δC 130.2 indicated that there was a substituted methylene group on the aromatic ring. Based on these data, it was hypothesised that the E7d compound has a benzyl framework with a substituted 1.4 pattern.

2D-NMR measurements such as Heteronuclear Multiple Bond Correlation (HMBC) and Homonuclear Multiple Quantum Correlation (HMQC) were required. Correlation of HMBC to know the correlation of distance (3 carbons) between proton and neighbour carbon. While the HMQC correlation measurement was used to determine the correlation of the carbon chemical shift with the proton chemical shift, which indicated where the proton positions were attached directly to the carbon. Based on HSQC and HMBC data obtained a long-distance correlation between protons with neighbouring carbon as in Table 1 and Figure 5.

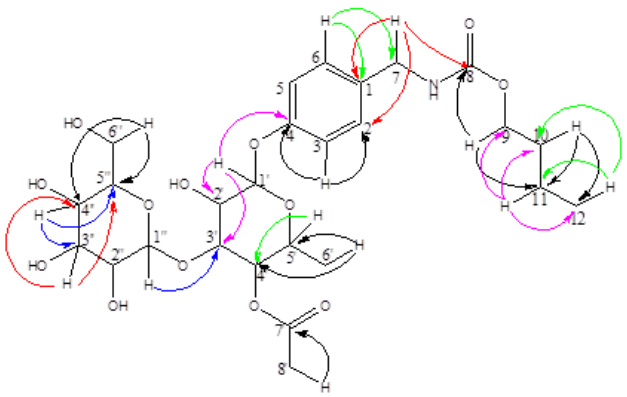

Figure 5. HMBC Corelation of compound **(1)**

Based on HSQC data the existence of proton doublet at δ_H 5,41 (1H, d, *J* 1,9 Hz) bound to δ_C 99.6 indicated the presence of anomeric proton which was characteristic of α-L-ramnopyranosyl group. The coupling constant value of *J* 1.9 Hz in the anomeric proton indicates that the α-L-ramnopyranosyl group was bound to an-glycosidicaglyone (Faizi *et al.,* 1994). Based on HSQC data, a group of signals reinforcing the presence of α-L-ramnopyranosyl groups ie the correlation δH/δC 5.41 (1H, d, J 1.9 Hz)/99.6; 4.28 (1H, br s)/70.2; 3,96 (1H, dd, J 3,1Hz; J 9.0 Hz)/82,7; 3.70 (1H, dd, J 9.0Hz, J 9.0 Hz)/71.4; 3.68 (1H, m)/71.0 and 1.22 (3H, d, J 5.5 Hz)/18.1.

The presence of HMBC secondary methyl proton at δ_H 1.22 with δ_C 71.4 and δ_C 71.0 further strengthened the presence of α-L-ramnopyranosyl group. The singlet peak at a chemical shift of δ_H 2.56(3H) indicates the presence of a methyl group attached to a group having a large electronegativity. This is reinforced by the HMBC correlation between proton δ_H 2.56 and δ_C 176.1 indicating the presence of substituted acetyl groups in the ramnopyranosyl group. The HMBC correlation between proton δ_H 5.41 with δ_C 155.6 and δ_C 70.2 indicates that this ramnopyranosyl group is attached to C-4 in the aromatic ring.

Table 1. NMR spectroscopic data of compound **(1)** (CD_3OD)

Position	δ_H	δ_c	HMBC (H to C)
1		131.2	
2,6	7,24 (2H, d, *J* 8,4 Hz)	130.6	
3,5	7,02 (2H, d, *J* 8,4 Hz)	117.6	1, 2, 4
4		155.6	1, 3, 7
7	3,45 (2H, s)	42.5	
8		177.2	1, 2, 8
9	4,11 (2H, t)	55.5	
10	2,32 (2H, m)	31.6	8, 11
11	2,12 (1H, m), 1,92 (1H, m)	26.3	11, 12
12	1,78 (3H, m)	19.8	9, 10, 12
	Ramnopyranosyl		
1′	5,41 (1H, d, *J* 1,9 Hz)	99.6	4, 2′, 3′
2′	4,28 (1H, br s)	71.3	3′, 4′
3′	3,96 (1H, dd, *J* 3,1Hz; *J* 9,0 Hz)	82.7	2′, 4′, 1″
4′	3,70 (1H, dd, *J* 9,0Hz; *J* 9,0 Hz)	71.4	3′, 5′
5′	3,68 (1H, m)	71.0	4′
6′	1,22 (3H, d, *J* 5,5 Hz)	18.1	4′, 5′
7′		176.1	
8′	2,56 (3H, s)	29.8	7′
	Glucospyranosyl		
1″	4,59 (1H, d, *J* 7,6 Hz)	105.8	3′
2″	3,63 (1H, dd, *J* 3.0 Hz; *J* 9,6 Hz)	73.8	3′, 4″
3″	3,32 (1H, t, *J* 7,4 Hz)	77.7	4″, 5″
4″	3,39 (1H, t)	72.6	3″, 5″
5″	3,19 (1H, m)	75.4	3″
6″	3,83 (1H, dd, *J* 2,4 Hz; *J* 12,0 Hz)	62.2	4″, 5″
	3,70 (1H, dd, *J* 6,0 Hz; *J* 12,0 Hz)		

Based on HSQC data the presence of proton doublets at δH 4.59 (1H, d, J 7.6 Hz) bound to δC 105.8 indicated the presence of anomeric protons characteristic of the β-D-glucopyranosyl group (Sahakitpichan *et al.,* 2011). The presence of HMBC correlation between anomeric proton δH 4.59 (1H, d, J 7.6 Hz) with δC 82.7 indicated if glucopyranosyl group was substituted on the ramnopyranosyl group. This can be seen from more deshielding carbon chemical shift compared to other carbon shifts in the ramnopyranosyl group (Sahakitpichan *et al.,* 2011). Based on HSQC data, a group of signals that reinforce the presence of β-D-glucopyranosyl group was the correlation δH/δC 4,59 (1H, d, J 7.6 Hz)/105.8; 3.63 (1H, dd, J 3.0 Hz, J 9.6 Hz)/73.8; 3.32 (1H, t, J 7.4 Hz)/77.7; 3.39 (1H, t)/72.6; 3.19 (1H, m)/75.4 and 3.83 (1H, dd, J 2.4 Hz, J 12.0 Hz) and 3.70 (1H, dd, J 6.0 Hz; J 12.0 Hz)/62.2.

The protons for N-H and O-H in E7d compounds were not appeared due to the influence of the CD3OD solvents used for NMR. This was because the CD3OD solvent causes proton exchange between protons attached to atoms other than carbon with the solvent through hydrogen bonding (Cheenpracha *et al.,* 2010). HSQC data, indicating that proton δH 4.11 (2H, t) bound to δC 55.5 indicated the condition of the surrounding environment has a high electronegativity. The existence of carbon shifts that deshielding δC 55.5 signifies the presence of a C-O heteroatom bond attached to the carbonyl group. This was reinforced by the HMBC correlation between proton δH 4.11 (2H, t) with δC 177.2 and δC 26.3. HMBC proton methylene

δH 4,11 (2H, t) correlated with 177.2 and δC 26.3 indicated that the group was adjacent to the carbonyl group. Based on HMBC and HSQC data, a suitable set of signals for aliphatic chains substituted on the benzyl framework can be predicted as an O-butyl chain.

The 1H-NMR and 13C-NMR data obtained on carbamate compounds that have similarities with the 1H-NMR and 13C NMR data of Marumoside B compounds found from the *M. oleifera* leaves from Thailand [14]. Based on the correlation data of HMBC and HMQC obtained, the chemical structure of compound **(1)** was new namely O-butyl, N- {4- [3'-O-β-D-glucopyranosyl) (4'-O-acetyl-α-L-ramnosiloxy)] - benzyl} carbamate.

C. Isolation of Compound (2) from M. oleifera

The leaves of *M. oleifera* were extracted with methanol. The extract was successively fractionated with water, *n*-hexane and ethyl acetate. The *n*-hexane, ethyl acetate and water soluble showed the inhibitory activity of 98.9%, 90.19% and 58.5%, respectively at concentration of 333.33 µg/mL. The fraction was further purified by a series of chromatography techniques (silica gel column and reversed phase HPLC).

The *n*-hexane soluble was subjected in silica gel column chromatography and yielded nine sub fractions (H1-H9). Sub fraction H8 was further separated with silica gel column chromatography to yield eight sub-fractions (H8a-H8h). Sub fraction H8b was recrystallised with methanol to yield compound (**2**) (35 mg). The compound was identified as stearic acid by comparison of previous spectroscopic data (Sun *et al.,* 2014). Compound (**2**) was isolated as white an amorphous powder. The shift data (δ) of 1H-NMR of compound (**2**) (Table 2) provided information about 36 protons, while the 13C-NMR shift data (δ) showed 18 carbons. Its molecular formula was determined to be $C_{18}H_{36}O_2$.

Table 2. NMR spectroscopic data of compound (**2**)

Position	δ_H	δ_C
1		179.6
2	2,27 (2H, t)	33.9
3	1,56 (2H, m)	24.7
4		29.0
5		29.3
6		29.6
7		29.6
8		29.6
9		29.6
10		29.6
11	1,39 (24H, br s)	29.7
12		29.6
13		29.5
14		29.3
15		29.1
16		33.9
17		22.8
18	0,81 (3H, t)	14.2

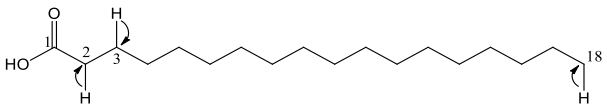

Figure 6. HMQC correlation of compound **(2)**

Based on the ^{1}H-NMR spectrum data, there were four signals. The presence of chemical shifts in the proton δ_H 2.27 (2H, t) indicated deshielding of chemical shift compared to the other protons. This was reinforced by HSCQ data showing that proton δ_H 2.27 was attached to carbon δ_C 33.9. The peak proton broad singlet at δ_H 1.39 (24 H, br s) signifies the presence of protons attached to the carbon with the same chemical environment, the protons appear in the same peak. Peak integrity of NMR protons can be used to calculate the number of protons. HSCQ data reinforce the hypothesis if the protons were bound to carbon with the same chemical environment. The carbon shift in the region of δ_C 179.6 indicated the presence of one carbonyl group. Data of NMR proton and carbon shift of this compound was presented in Table 2. HMQC data (Figure 6) also showed the correlation between protons attached to carbon that confirm that this compound was stearic acid (**2**).

D. Antioxidant Activity of Compound (1) and (2)

M. oleifera is a plant with multipurpose biological activities. Antioxidant from natural source can improve the system antioxidant in body for scavenging free radicals. The interest in antioxidant from natural sources increased rather than synthetic sources. Phenolic compounds which naturally present in *M. oleifera* plant can reduce the risk of many diseases and its effects which correlated with the antioxidant compounds. Recently, there were some reports about *M. oleifera* leaves which rich in phenolic compounds such as flavonoids, gallic acid, quercetin and kaempferol as antioxidant activity (Fitriana *et al.,* 2016). Here in, *in vitro* antioxidant activity of *M. oleifera* extracts were evaluated by using DPPH and ABTS assays.

Antioxidant activity have been testing used DPPH and ABTS methods. The stability of DPPH radicals has been used extensively to determine the antioxidant activity of a compound. This was based on the ability of a compound to capture free radicals by donating a hydrogen atom. The ability of compounds to reduce the radical DPPH can be seen from the colour change of DPPH radical (purple) solution to DPPH (yellow). While the ABTS method has a higher sensitivity than DPPH. ABTS radical cation (light blue) is stabilised by the compound and changes to yellow pale (Fitriana *et al.*, 2018). The antioxidant mechanism of DPPH and ABTS has a difference that is the DPPH test of antioxidant ability based on the ability to donate hydrogen, whereas in ABTS test based on the ability to donate proton radical (Putri *et al.,* 2018).

Based on measurement of absorbance value of blank and sample, it can be calculated the percentage value of inhibition of DPPH of compound **(1)** was 12,31% (IC_{50} >322,54 µg/mL) and ABTS radical cation inhibition value equal to 43,74% (IC_{50} >99,99 µg/mL). In the DPPH activity test, compound **(2)** had low inhibitory activity of 21% at a concentration of 322.54 µg/mL. The result of ABTS antioxidant activity of compound **(2)** has inhibited activity of 26,40% at concentration 99,99 µg/mL. Both compounds had low antioxidant activity when compared to positive control. Trolox was used as Positive control with DPPH inhibition percentage value of 96.61% with IC_{50} value of 5.45 µg/mL, whereas ABTS of 94.99% with IC_{50} value of 3.06 µg/mL.

IV. CONCLUSION

M. oleifera, collected from Jombang, Indonesia, is potent to be a natural α-glucoside inhibitor. Isolation and fractionation of *M. oleifera* extracts yielded two compounds. The structure has elucidated with 1D- and 2D-NMR. Compound **(1)** as O-butyl, N- {4- [3'-O-β-D-glucopyranosyl) (4'-O-acetyl-α-L-ramnosiloxy)] - benzyl} carbamate and compound **(2)** as stearic acid. Antioxidant activity from both of compounds also was determined using DPPH and ABTS methods. This finding provides scientific evidence for the Indonesian traditional people way, which used *M. oleifera* leaves as one of nutrition food to prevent diseases. This study also indicated that *M. oleifera* leaves can be used as α-glucosidase inhibitor and antioxidant source.

V. ACKNOWLEDGEMENT

This research was supported by a grant from Directorate General of Higher Education, Ministry of Education and Culture, Indonesia. The name of grant is "Research Project for National Research Collaboration and Scientific Publication Between Unipdu and ITS year 2019, number of contract 001/K7/KM/PENELITIAN/2019.

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