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Research Article

α-Glucosidase Inhibitory Activity of Triglycerides and Methyl Linoleate of *Murraya koenigii* Spreng from Malaysia

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Abstract

This study was designed to examine the α-glucosidase inhibitory activity of triglycerides and methyl linoleate isolated from the root extract of *Murraya koenigii* Spreng in Malaysia. Although *M. koenigii* has been widely reported for its medicinal properties, few studies have examined the α-glucosidase inhibitory activity of its isolated lipid compounds, particularly triglycerides and methyl linoleate. The chemical compounds were obtained by cold extraction with various solvents and purified by chromatographic techniques, including NMR, MS, and IR analyses. Antidiabetic activity was evaluated using an α-glucosidase inhibitory assay. The results of these studies indicated that triglyceride **(1)** (IC_{50} = 33.6 ± 0.05 µg/mL) from the hexane crude extract and methyl linoleate **(2)** (IC_{50} = 13.9 ± 0.19 µg/mL) from the methanol crude extract exhibited potent inhibitory activity in the α-glucosidase inhibitory assay. The results showed that root extracts of *M. koenigii* exhibit significant antidiabetic activity, which may help identify new chemical classes of natural antidiabetic substances.

Keywords: α-glucosidase inhibitory activity, antidiabetic activity, methyl linoleate, *Murraya koenigii,* triglycerides

1. INTRODUCTION

Diabetes mellitus, one of the most common metabolic disorders, commonly known as diabetes, is caused by a deficiency of the pancreatic hormone insulin, leading to elevated blood glucose levels and disturbances in carbohydrate, fat, and protein metabolism [1-2]. Effective management of diabetes requires controlling postprandial blood glucose levels, and one therapeutic strategy is to inhibit α-glucosidase, an enzyme responsible for disaccharide hydrolysis during carbohydrate digestion. Inhibition of α-glucosidase reduces disaccharide hydrolysis and delays glucose absorption, thereby lowering postprandial hyperglycemia. α-Glucosidase inhibitors, such as acarbose, miglitol, and voglibose, have been used in the treatment of diabetes; however, they may cause side effects, including liver disorders, abdominal pain, and diarrhea [3]. Therefore, increasing attention has been directed toward natural products as alternative sources of antidiabetic agents.

Several natural compounds, including alkaloids, flavonoids, terpenoids, and glycosides, have been isolated from medicinal plants and reported to possess antidiabetic activities [4]. Hence, the isolation of active compounds from traditional antidiabetic plants is important for the discovery of potential α-glucosidase inhibitors. *Murraya koenigii* belongs to the Rutaceae family, a large plant family comprising approximately 150 genera and 1600 species. This species has been widely used as a natural flavoring in curries and as an ingredient in traditional medicine formulations [5-6].

The leaves of *M. koenigii* are traditionally used to treat headaches, stomach aches, vomiting, and diarrhea [7-8]. In addition, curry leaves have been reported to exhibit antioxidant and antidiabetic properties [9-10]. Several compounds have been found in *M. koenigii*, including mahanimbine, mahanimbicine, mahanine, pheophorbide a ethyl ester, pheophorbide b ethyl ester, and murrayazolinol [11-12]. In addition, other compounds such as quercetin, apigenin, rutin, kaempferol, myrcetin, girinibine, and muconal have also been detected in *M. koenigii* [13]. Although several carbazole alkaloids and flavonoids have been reported from *M. koenigii*, information regarding the specific compounds responsible for α-glucosidase inhibitory activity remains limited. Furthermore, studies focusing on the isolation and characterization of active constituents from hexane and methanol extracts are scarce. Therefore, this study aimed to isolate compounds from hexane and methanol extracts of *M. koenigii* and evaluate their α-glucosidase inhibitory activity.

2. RESEARCH METHODS

Plant Materials

The roots of *M. koenigii* were collected from Pendang, Kedah, Darul Aman. Malaysia in 2012. The specimen was identified by the Herbarium Group of the Chemistry Department, Faculty of Sciences and Mathematics, Sultan Idris Education University.

Extraction and Isolation

Plant materials were extracted using a cold extraction process. The dried roots of *M. koenigii* (1.83 kg) were ground into a fine powder and successively soaked in *n*-hexane at room temperature for 72 h until the plant material was fully immersed in the solvent. Each extraction step was repeated three times. The residue was subsequently wetted with 10% NH_3 and extracted with dichloromethane for 120 h under the same conditions, then extracted with methanol for 72 h. The filtrates obtained from each extraction were combined and concentrated under reduced pressure using a rotary evaporator to afford brown syrups of the *n*-hexane crude extract (25.50 g), dichloromethane crude extract (24.60 g), and methanol crude extract (46.43 g). The *n*-hexane (20 g) and methanol (45 g) crude extracts were subsequently subjected to silica gel column chromatography using a gradient of *n*-hexane, dichloromethane, and methanol, affording triglyceride (**1**) and methyl linoleate (**2**), respectively. The structures of the isolated compounds were confirmed based on NMR analyses (^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, and HMBC) and comparison with literature data.

Inhibitions Assay for alpha-glucosidase Activity

α-glucosidase inhibition was performed in a 96-well microplate according to the method reported [14], with minor modifications. Briefly, the enzyme reaction was performed using 4-nitrophenyl-α-D-glucopyranosidase (pNPG) as the substrate in 0.1 M phosphate buffer (pH 7.0). A volume of 20 µL of sample solution at various concentrations, 80 µL of 0.1 mM phosphate buffer (pH 7.0) and 40 µL α-glucosidase enzyme (0.2 units/mL) were incubated in plates at 37°C for 15 minutes. After 15 minutes of incubation, 40 µL of 2.5 mM pNPG solution in 0.1 M phosphate buffer (pH 7.0) was added to each well to start the reaction, and the wells were incubated at 37 °C for another 30 minutes. The reaction was terminated by adding 100 µL of 0.2 M Na_2CO_3 to the mixture. The absorbance was measured using a microplate reader at 405 nm. Acarbose was used as a positive control, and IC_{50} values were calculated by the method. The concentration range for the sample and control was 1.12–71.43 µg/mL. The inhibitions of the test sample on α-glucosidase were calculated as:

$$\text{Inhibition} = 1 - \text{Inhibition}\ (\%) = [1 - (A_S/A_C)] \times 100 \quad (1)$$

Where A_S, A_C were the absorbances of sample and control, respectively. The measurement was carried out in triplicate [15].

3. RESULTS AND DISCUSSION

Inhibition of α-glucosidase activity

The α-glucosidase inhibitory activities of the two isolated compounds are shown in **Table 1**. The effectiveness of enzymatic inhibition of the test samples was determined by calculating the IC_{50} value. A lower IC_{50} value indicates stronger enzymatic inhibitory activity. According to Annapandian and Sundaram (2017) [16], the polarity of the solvent used during plant extraction may influence antidiabetic activity because solvents with different polarities extract different classes of chemical compounds. The inhibitory effects of triglycerides and methyl linoleate isolated from *M. koenigii* roots were evaluated using acarbose (IC_{50} = 51.7±0.05 µg/mL) as the positive control. The IC_{50} values for triglycerides and methyl linoleate were 33.6 ± 0.05 µg/mL and 13.9 ± 0.19 µg/mL, respectively. The lower IC_{50} values of triglycerides and methyl linoleate compared with acarbose indicate stronger α-glucosidase inhibitory activity of the isolated compounds, as a lower IC_{50} value reflects a smaller concentration required to inhibit 50% of enzyme activity. In this study, methyl linoleate (13.9 ± 0.19 µg/mL) and triglycerides (33.6 ± 0.05 µg/mL) demonstrated greater inhibitory potency than acarbose (51.7 ± 0.05 µg/mL).

Table 1. IC_{50} values of triglycerides and methyl linoleate in α-glucosidase inhibition

Compounds	IC_{50} (µg/mL)
Triglycerides	33.6 ± 0.05
Methyl linoleate	13.9 ± 0.19
[a]Acarbose	51.7 ± 0.05

[a]Positive control

Based on **Figure 1**, the α-glucosidase inhibitory activity increased with increasing concentration for all tested samples. By comparing the percentage inhibition at the same concentration, methyl linoleate generally exhibited higher inhibitory activity than triglycerides and acarbose, particularly at higher concentrations. At 71.43 µg/mL, methyl linoleate showed the highest inhibition (74.65%), followed by triglycerides (70.05%) and acarbose (56.90%). Observation of the inhibition curves demonstrated that methyl linoleate showed a steeper increase in inhibitory activity with increasing concentration, indicating stronger inhibitory potency, whereas triglycerides and acarbose exhibited a more gradual increase. These observations are consistent with the IC_{50} value (13.9 ± 0.19 µg/mL), followed by triglycerides (33.6 ± 0.05 µg/mL) and acarbose (51.7 ± 0.05 µg/mL). Since lower IC_{50} values indicate stronger enzyme inhibition, the findings suggest that methyl linoleate exhibited the strongest α-glucosidase inhibitory activity among the tested compounds, as supported by its highest maximum percentage inhibition in **Figure 1**.

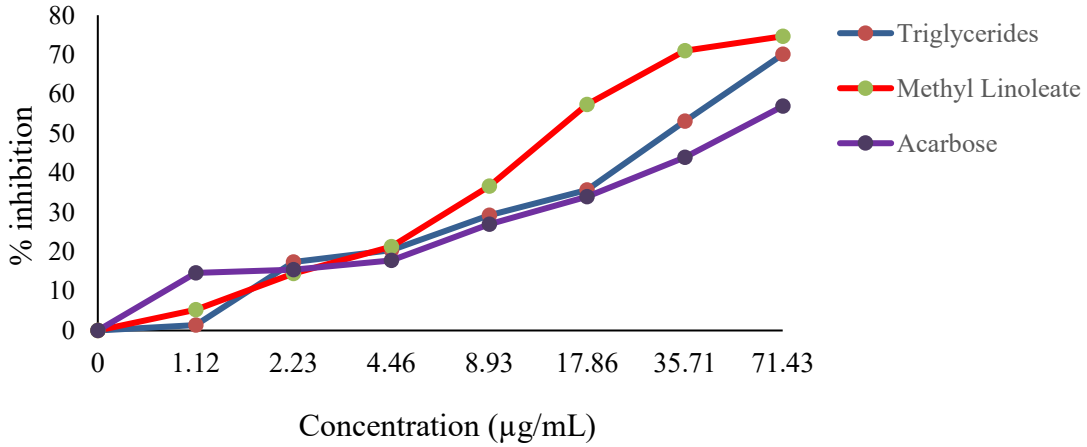
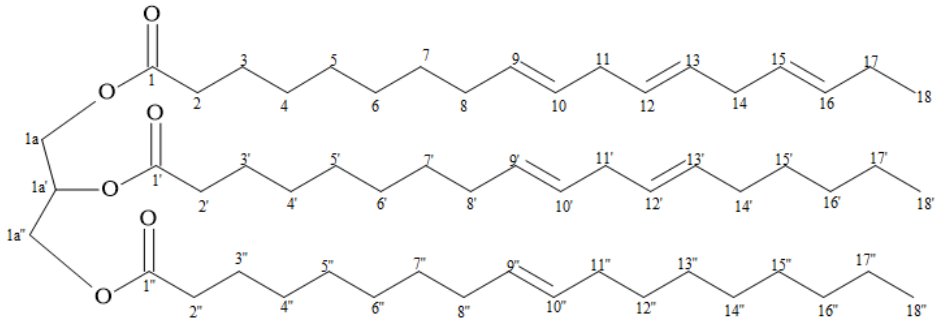


Figure 1. α-glucosidase inhibitory activity of isolated compounds



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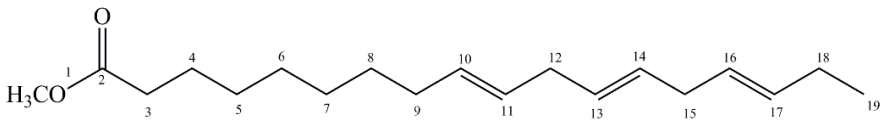

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Figure 2. Chemical structure of compound **1-2**

Separation and Structural Elucidation

Compounds **1** and **2** were isolated from crude *n*-hexane and methanol extracts of the roots of *M. koenigii* by column chromatography and identified as triglycerides and methyl linoleate, respectively, by comparing their ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopic data with those previously reported in the literature. The chemical structures of these compounds are shown in **Figure 2**.

Compound **1** was isolated as a brownish viscous oil from *n*-hexane crude extract and identified as a mixture of three fatty acids, namely linolenic, linoleic, and oleic acids, present as triglycerides. The molecular formula was deduced as $C_{57}H_{98}O_6$ by the molecular ion peak at *m/z* 879.0 $[M+H]^+$. The IR spectrum indicated the presence of the C-O group (1025.46 cm^{-1}) and C=O (1695.88 cm^{-1})[17]. The UV spectrum showed maximum absorption at 231 nm, suggesting the presence of C=C, while a band at 271 nm corresponds to C=O[18]. ^{1}H and ^{13}C-NMR spectral data suggested that the compound was a mixture of three fatty acids: linolenic, linoleic, and oleic acids.

The ^{1}H-NMR spectrum (**Table 2**) showed two multiplet signals at δ_H 5.36 ppm (*m*, H-10, H-10′, H-10″, H-12, H-12′, H-15) and δ_H 5.32 ppm (*m*, H-9, H-9′, H-9″, H-13, H-13′, H-16) corresponding to the olefinic proton from the fatty acid. The two multiplets are also observed at δ_H 2.32 (*m*, H-2) and δ_H 2.31 (*m*, H-2′, H-2″) from methylenic groups in the three fatty acids. The signals shown at δ_H 4.30 (*d*, 4.60 Hz, H-1a″), δ_H 4.28 (*d*, 4.55 Hz, H-1a), δ_H 4.15 (*d*, 6.30 Hz, H-1a″), δ_H 4.13 (*d*, 5.70 Hz, H-1a), and δ_H 5.25 (*m*, H-1a) proved the presence of the glycerol part of triglycerides. The terminal methyl group that appeared at δ_H 0.99 (*t*, 7.45 Hz, H-18) showed the presence of a signal of linolenic acid, and an overlapping multiplet signal at δ_H 0.88 (*m*, H-18′, H-18″) belongs to linoleic and oleic acids. The overlapping triplet signals methylenic groups between olefinic protons at δ_H 2.77 (*t*, 6.85 Hz) are assigned to H-11, H-14, and H-11″. The signal between δ_H 1.25 and 2.31 ppm was attributed to methylene groups in the fatty acid chains. The resonances of H-7, H-7', and H-7" were highly overlapped with neighboring methylene protons and thus appeared as unresolved multiplets around δ_H 1.25 ppm.

Table 2: ^{1}H NMR and ^{13}C NMR data of compound **1** [19-20]

Position	^{1}H NMR [500 MHz, δ_H (*J*, Hz)]			^{13}C NMR [125 MHz, δ_C]		
	Linolenic acid	Linoleic acid	Oleic acid	Linolenic acid	Linoleic acid	Oleic acid
1, 1′, 1″				173.4	173.0	173.4
1a, 1a′, 1a″	4.13 (*d*, 5.70) 4.28 (*d*, 4.55)	5.25 (*m*)	4.15 (*d*, 6.30) 4.30 (*d*, 4.60)	62.2	68.9	62.2
2, 2′, 2″	2.32 (*m*)	2.31 (*m*)	2.31 (*m*)	34.3	34.1	34.1
3, 3′, 3″	1.60 (*m*)	1.56 (*m*)	1.56 (*m*)	24.9	24.9	24.9
4, 4′, 4″	1.29 (*m*)	1.29 (*m*)	1.29 (*m*)	29.1	29.1	29.1
5, 5′, 5″	1.29 (*m*)	1.29 (*m*)	1.29 (*m*)	29.4	29.4	29.4
6, 6′, 6″	1.29 (*m*)	1.25 (*s*)	1.25 (*s*)	29.7	29.7	29.7
7, 7′, 7″	1.25 (*m*)	1.25 (*m*)	1.25 (*m*)	29.8	29.8	29.8
8, 8′, 8″	2.04(*m*)	2.04 (*m*)	2.04 (*m*)	27.3	27.3	27.3
9, 9′, 9″	5.32 (*m*)	5.32 (*m*)	5.32 (*m*)	130.1	130.1	130.3
10, 10′, 10″	5.36 (*m*)	5.36 (*m*)	5.36 (*m*)	128.0	128.1	128.3
11, 11′, 11″	2.77 (*t*, 6.85)	2.77 (*t*, 6.85)	1.29 (*m*)	25.7	25.6	39.4
12, 12′, 12″	5.36 (*m*)	5.36 (*m*)	1.29 (*m*)	127.8	127.8	29.3
13, 13′, 13″	5.32 (*m*)	5.32 (*m*)	1.29 (*m*)	130.3	130.8	29.2
14, 14′, 14″	2.77 (*t*, 6.85)	2.04 (*m*)	1.29 (*m*)	25.6	27.3	29.1
15, 15′, 15″	5.36 (*m*)	1.25 (*m*)	1.25 (*m*)	128.0	29.6	29.6
16, 16′, 16″	5.32 (*m*)	1.29 (*m*)	1.25 (*s*)	128.4	31.6	32.0
17, 17′, 17″	2.04 (*m*)	1.29 (*m*)	2.04 (*m*)	20.6	22.7	20.6
18, 18′, 18″	0.99 (*t*, 7.45)	0.89 (*m*)	0.89 (*m*)	14.4	14.2	14.4

The ^{13}C-NMR (**Table 2**) spectrum and DEPT of this compound consists of 57 carbons, comprising three quaternary carbons at δ_C 173.4 (C-1 and C-1″) and 173.0 (C-1′) and three methyl groups; δ_C 14.4 (C-18 and C-18″) and 14.2 (C-18′). The spectrum also showed the esterified glycerol signal at δ_C 62.2 (C-1a and C-1a″) and 68.9 (C-1a′). The olefinic carbons were showed at δ_C 130.1 (C-9), 130.3 (C-13), 128.4 (C-16), 128.0 (C-15) and 127.8 (C-12) (linolenic acid), 130.8 (C-13'), 130.1 (C-9′), 128.1 (C-10′) and 127.8 (C-12′) (linoleic acid) and 130.3 (C-9″), 128.3 (C-10″) (oleic acid).

The geometry of the double bonds was tentatively assigned as *cis* (*Z*) based on comparison with published spectroscopic data for naturally occurring oleic, linoleic, and linolenic acid residues, which predominantly exist in the cis configuration. The olefinic proton signals observed at δ_H 5.32 – 5.36 ppm together with allylic methylene signals were consistent with unsaturated *cis* fatty acid chains [21-22]. The remaining signals observed between δ_C 22.7 – 34.2 ppm were attributed to the long-chain methylene carbons of the fatty acid chains. These methylene resonances are characteristic of triglyceride structures containing unsaturated fatty acids and support the presence of an extended aliphatic chain in Compound **1**.

The structure assignment was further supported by 2D NMR experiments. In the COSY spectrum, correlations were observed between the olefinic protons H-9/H-10 and H-12/H-13, confirming the connectivity of the unsaturated fatty acid chains. HMQC analysis established direct one-bond correlations between proton and carbon resonances, while HMBC correlations from glycerol methylene protons (H-1a/H-1a') to the ester carbonyl carbons (C-1/C-1') confirmed the triglyceride backbone structure. The complete assignment of carbon signals was accomplished through ^{1}H-^{1}H COSY, HMQC, and HMBC experiments (**Figure 3**).

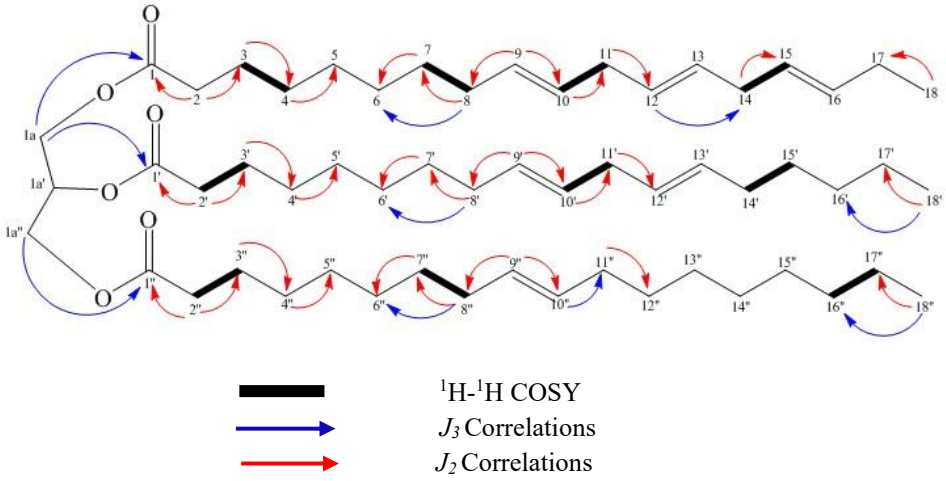

Figure 3. Selected ^{1}H-^{1}H COSY, HMBC and HMQC of compound **1**

Compound **2** is a carboxylic acid ester and it was isolated as a yellowish oil from the methanol crude extract known as methyl linoleate. The IR spectrum indicated the presence of C=O absorption at 1696 cm^{-1} and C-O absorption at 1025 cm^{-1}. The mass spectrum showed a molecular ion peak at *m/z* 292 $[M+H]^+$ corresponding to the molecular formula of $C_{19}H_{32}O_2$. The ^{1}H-NMR (**Table 3**) showed a *singlet* at δ_H 3.66 (*s*, 3H), corresponding to the methoxy group of the ester functionality. Meanwhile, the multiplet signals at δ_H 5.34 (*m,* H-11) and δ_H 5.35 (*m,* H-10, H-14) represented the olefinic methine protons of the fatty acid chain. The overlapping triplet at δ_H 2.76 (*t*, 6.30 Hz, H-12) was assigned to the bis-allylic methylene group located between the olefinic protons. In addition, the signals appearing at δ_H 2.30 (*t*, 8.00 Hz, C-3) and δ_H 1.61 (*m,* C-4) were attributed to methylene groups adjacent to the ester carbonyl group. The signals at δ_H 1.24 (*s,* H-8) and δ_H 1.30 (*m,* H-5, H-6, H-7) corresponded to the backbone methylene protons of the long fatty acid chain. The multiplet signal at δ_H 2.03 (*m*) was assigned to the allylic methylene protons attached to C-9 and C-18. The terminal methyl group appeared at δ 0.88 (*m*, H-9).

Table 3. ^{1}H-NMR and ^{13}C-NMR data of compound **2** (23-24)

Position	^{1}H-NMR [500 MHz, δ_H (*J*, Hz)]	^{13}C-NMR [125 MHz, δ_C]
1-OCH$_3$	3.66 (*m*)	51.2
2		174.4
3	2.30 (*t*, 8.00)	34.3
4	1.61 (*m*)	25.0
5	1.30 (*m*)	29.2
6	1.30 (*m*)	31.6
7	1.30 (*m*)	22.7
8	1.24 (*s*)	29.8
9	2.03 (*m*)	27.3
10	5.35 (*m*)	130.6
11	5.34 (*m*)	128.5
12	2.76 (*t*, 6.30)	25.7
13	5.34 (*m*)	128.5
14	5.35 (*m*)	130.6
15	2.76 (*t*, 6.30)	25.7
16	1.24 (*s*)	130.6
17	1.30 (*m*)	128.5
18	2.03 (*m*)	27.3
19	0.88 (*m*)	14.2

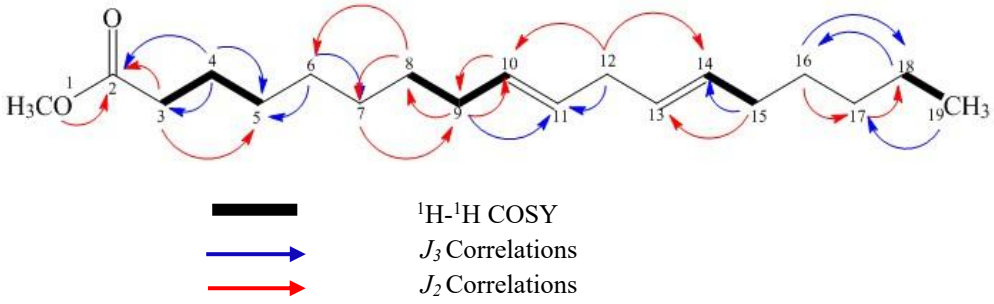

Figure 4. Selected ^{1}H-^{1}H COSY, HMBC and HMQC of compound **2**

The ^{13}C-NMR spectrum (**Table 3**) showed the ester carbonyl carbon resonance at δ_C 174.4 (C-2), while the signal at δ_C 51.2 (C-1) corresponded to the methoxy carbon of the ester group. The olefinic carbons appeared at δ_C 130.6 (C-10 and C-14) and δ_C 128.5 (C-11 and C-13), confirming the presence of unsaturation in the fatty acid chain. The terminal methyl carbon resonance was observed at δ_C 14.2 (C-19). The remaining methylene carbons of the fatty acid chain resonated between δ_C 22.5 and 34.5 ppm. The complete structural assignment was further supported by ^{1}H-^{1}H COSY, HMQC, and HMBC spectral analyses (**Figure 4**).

4. CONCLUSIONS

Phytochemical study of *n*-hexane and methanol crude extract of the roots of *M. koenigii* afforded triglycerides (**1**) and methyl linoleate (**2**). The present study demonstrated α-glucosidase inhibitory activity of the isolated compounds, with triglycerides and methyl linoleate showing IC_{50} values of 33.6 ± 0.05 µg/mL and 13.9 ± 0.19 µg/mL, respectively.

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