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# INVESTIGATION OF ANTIMICROBIAL ACTIVITY AND CHEMICAL CONSTITUENTS OF *MOMORDICA CHARANTIA* L. VAR. *ABBREVIATA* SER.

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**Abstract.** The antimicrobial potential of the four extracts of *Momordica charantia* L. var. *abbreviata* Ser., including hexane, chloroform, ethyl acetate and methanol-water 80:20 (*v/v*) extracts was screened against four bacterial and four fungal strains, using microbroth dilution assay. The chloroform extract showed the highest growth inhibitory activity with MIC = 200 µg/mL on both *Escherichia coli* and *Bacillus subtilis* and MIC = 100 µg/mL on *Aspergillus niger*. Phytochemical study on the bioactive chloroform extract led to the isolation of four known compounds as octadecan-1-ol (**1**), (23*E*)-5*β*,19-epoxycucurbita-6,23,25-trien-3*β*-ol (**2**), 5*α*-poriferasta-7,25-dien-3*β*-ol (**3**) and 3-*O*-(6′-*O*-palmitoyl-*β*-D-glucopyranosyl)-clerosterol (**4**). Their structures were elucidated by spectroscopic methods including 1D- and 2D-NMR, MS and in comparison with literature data. Here, such metabolites were reported for the first time from *M. charantia* L. var. *abbreviata* Ser.

*Keywords:* 5*α*-poriferasta-7,25-dien-3*β*-ol, antibacterial, antifungal, clerosterol, *Momordica charantia* L. var. *abbreviata* Ser.

*Classification numbers:* 1.1.1, 1.1.6

## 1. INTRODUCTION

In recent years, antibiotics are becoming less effective against certain illnesses, not only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is necessary to discover new drugs. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. About 80 % of the world's population used plant extracts or their active constituents as folk medicine in traditional therapies. In fact, plants which are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., were demonstrated having antimicrobial properties *in vitro* [1]. In an effort to expand the spectrum of antimicrobial agents

from natural resources, *Momordica charantia* L. var. *abbreviata* Ser (MCA) has been selected. The investigated species is normally smaller than cultivated bitter gourd *M. charantia* L. both belonging to the family Cucurbitaceae. Scientific information published on chemical and biological properties of MCA remains limited, although both species have been consumed as a vegetable and folk medicine. Preliminary studies on chemical constituents of MCA showed the presence of polyphenols, triterpenoids, saponins and flavonoid glycosides [2]. Several triacylglycerols which contain two different fatty acyl chains such as palmitic, stearic, oleic, linoleic, and conjugated linolenic acid were also reported in the seed oil of MCA [3]. So far, its fruit extracts and components have been shown to possess some pharmacological actions including the antioxidant and anti-inflammatory activities, and hepatoprotection against alcoholic fatty liver [4]. The purpose of this study was to investigate the phytochemical compositions, antimicrobial activities of the extracts of MCA against some pathogenic microorganisms that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The fruits of *Momordica charantia* L. var. *abbreviata* Ser. were collected in Vinh Long province, Vietnam on September 2016. This plant was identified by Dr. Dang Minh Quan, School of Education, Can Tho University, Vietnam. A voucher specimen (No MCA-0916) was deposited in the herbarium of the Department of Chemistry, School of Education, Can Tho University.

### 2.2. General experimental procedures

The NMR experiments were performed on a Bruker DMX 500 spectrometer. MS were carried out on a Agilent 6310 spectrometer. Column chromatography was performed on normal phase silica gel (40-63 $\mu$m, Keselgel 60, Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on Kieselgel $60F_{254}$ plates (Merck, Darmstadt, Germany) and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity $\geq$ 99.0 %.

### 2.3. Antimicrobial activity test

Antimicrobial activity test was carried out at the Department of Experimental Biology, Institute of Natural Products Chemistry, VAST, using the method described by Vanden Berghe, Vlietinck, and Mckane, Kandel [5, 6]. This experiment was performed by microdilution technique on 96-well microtiter plate. Two gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* subsp. *aureus*) and four fungal strains (*Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces cerevisiae*, *Candida albicans*) were employed to determine antimicrobial activity and minimum inhibitory concentration (MIC). The reference antibiotics were streptomycin, tetracycline and nystatin. Fungi and bacteria were cultured in nutrient media. The test microorganisms were activated before the testing in fluid nutrient media. MIC is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria.

### 2.4. Extraction and isolation

Dried and powdered fruits of MCA (3.0 kg) were macerated for 12 hours with 30 L hexane to furnish 45.0 g of hexane extract (yield 1.50 %). The dried resulting powdered material was extracted exhaustively with 30 L of chloroform, ethyl acetate and methanol-water 80:20 (*v/v*) to give chloroform extract (72.0 g, yield 2.40 %), ethyl acetate extract (43.5 g, yield 1.45 %), and methanol-water 80:20 (*v/v*) extract (90.0 g, yield 3.00 %), respectively. Since the chloroform extract was the most effective against the test organisms than the other extracting solvents, a phytochemistry study was performed on this extract. The chloroform extract was subjected to a silica gel column and eluted with hexane:acetone with increasing acetone ratios to obtain fourteen fractions (C1 to C14). Fraction C2 (12.5 g) was applied on normal phase silica gel column chromatography, eluted with the solvent system of hexane:chloroform (95:5) to afford compound (**1)** (430 mg). Fraction C4 (8.3 g) was subjected to column chromatography using normal phase silica gel, eluted with hexane:ethyl acetate with increasing ethyl acetate ratios to obtain thirteen sub-fractions (C4.1 to C4.13). Then, sub-fraction C4.8 (2.1 g) was rechromatographed with normal-phase column chromatography of silica gel and eluted with the solvent system of hexane:ethyl acetate (95:5) to give compound (**2)** (500 mg). The sub-fraction C4.12 (1.7 g) was purified by another normal-phase column chromatography and eluted with *n*-hexane:ethyl acetate (85:15) to give compound (**3**) (200 mg). Fraction C10 (16.8 g) was applied to silica gel column chromatography, eluted with chloroform:acetone (80:20) to obtain compound (**4)** (175 mg).

Octadecan-1-ol (**1**): colorless paraffin; $^{1}$H-NMR ($CDCl_3$, 500 MHz) $\delta_{H\ ppm}$: 3.64 (2H, *t*, 7.5, H-1), 1.56-1.53 (32H, *m*, H-2 - H-17), 0.88 (3H, *t*, 7.0, H-18); $^{13}$C-NMR ($CDCl_3$, 125 MHz) $\delta_{C\ ppm}$: 63.1 (C-1), 32.8 (C-2), 31.9 (C-16), 29.4 – 29.7 (C×12, (C-4)-(C-15)), 25.8 (C-3), 22.7 (C-17), 14.1 (C-18); ESI-MS *m/z* 269.28 $[M]^-$ calcd. for $C_{18}H_{37}O$. Found 269.38.

(23*E*)-5*β*,19-Epoxycucurbita-6,23,25-trien-3*β*-ol (**2**): white amorphous power; $^{1}$H-NMR ($CDCl_3$, 500 MHz) $\delta_{H\ ppm}$: 6.14 (1H, *d*, 15.5, H-24), 6.05 (1H, *dd*, 10.0, 2.5, H-6), 5.62 (1H, *dd*, 10.0, 4.0, H-7), 5.61 (1H, *ddd*, 15.5, 7.0, 6.5, H-23), 4.86 (2H, *s*, H-26), 3.94 (1H, *dd*, 9.5, 3.0, H-3), 3.67 (1H, *d*, 8.5, H-19a), 3.51 (1H, *d*, 8.5, H-19b), 1.84 (3H, *s*, H-27), 1.02 (3H, *s*, H-29), 0.93 (3H, *s*, H-28), 0.91 (3H, *d*, 6.5, H-21), 0.87 (3H, *s*, H-30), 0.78 (3H, *s*, H-18); $^{13}$C-NMR ($CDCl_3$, 125 MHz) $\delta_{C\ ppm}$: 17.6 (C-1), 26.7 (C-2), 76.2 (C-3), 37.2 (C-4), 87.5 (C-5), 132.8 (C-6), 131.5 (C-7), 52.0 (C-8), 45.3 (C-9), 38.9 (C-10), 23.6 (C-11), 30.8 (C-12), 45.5 (C-13), 48.6 (C-14), 83.2 (C-15), 28.1 (C-16), 50.3 (C-17), 14.9 (C-18), 79.9 (C-19), 36.6 (C-20), 18.8 (C-21), 39.8 (C-22), 129.2 (C-23), 134.2 (C-24), 142.2 (C-25), 114.1 (C-26), 18.6 (C-27), 24.6 (C-28), 20.5 (C-29), 20.1 (C-30); ESI-MS *m/z* 439.35 $[M+H]^+$ calcd. for $C_{30}H_{47}O_2$. Found 439.40.

5*α*-Poriferasta-7,25-dien-3*β*-ol (**3**): white amorphous power; $^{1}$H-NMR ($CDCl_3$, 500 MHz) $\delta_{H\ ppm}$: 5.15 (1H, *m*, H-7), 4.73 (1H, *dd*, 2.5, 1.5, H-27a), 4.64 (1H, *d*, 2.5, H-27b), 3.59 (1H, *m*, H-3), 1.57 (3H, *s*, H-26), 0.91 (3H, *d*, 6.5 Hz, H-21), 0.80 (3H, *t*, 7.5 Hz, H-29), 0.79 (3H, *s*, H-19), 0.53 (3H, *s*, H-18); $^{13}$C-NMR ($CDCl_3$, 125 MHz) $\delta_{C\ ppm}$: 37.2 (C-1), 31.5 (C-2), 71.1 (C-3), 38.0 (C-4), 40.3 (C-5), 29.7 (C-6), 117.5 (C-7), 139.6 (C-8), 49.5 (C-9), 34.2 (C-10), 21.6 (C-11), 39.6 (C-12), 43.4 (C-13), 55.1 (C-14), 23.0 (C-15), 27.9 (C-16), 56.1 (C-17), 11.8 (C-18), 13.0 (C-19), 36.0 (C-20), 18.8 (C-21), 33.6 (C-22), 29.5 (C-23), 49.5 (C-24), 147.6 (C-25), 17.8 (C-26), 111.4 (C-27), 26.5 (C-28), 12.1 (C-29); ESI-MS *m/z* 413.37 $[M+H]^+$ calcd. for $C_{29}H_{49}O$. Found 413.45.

3-*O*-(6′-*O*-Palmitoyl-*β*-D-glucopyranosyl)-clerosterol (**4**): colorless paraffin; $^{1}$H-NMR ($CDCl_3$, 500 MHz) $\delta_{H\ ppm}$: 3.47 (1H, *m*, H-3), 5.35 (1H, *brs*, H-6), 0.68 (3H, *s*, H-18), 1.02 (3H, *s*, H-19), 0.89 (3H, *d*, 7.0, H-21), 1.57 (3H, *s*, H-26), 4.69 (2H, *brs*, H-27), 0.81 (3H, *t*, 7.5, H-

29), 4.37 (1H, *d*, 7.5, H-1'), 3.34 (1H, *t*, 8.0, H-2'), 3.53 (1H, *t*, 9.0, H-3'), 3.37 (1H, *t*, 9.0, H-4'), 3.51 (1H, *m*, H-5'), 4.33 (2H, *brs*, H-6'), 2.32 (2H, *t*, 7.5, H-2"), 0.88 (3H, *t*, 6.5, H-16"); $^{13}$C-NMR ($CDCl_3$, 125 MHz) $\delta_{C\ ppm}$: 36.0 (C-1), 29.3 (C-2), 73.8 (C-3), 38.9 (C-4), 140.4 (C-5), 122.1 (C-6), 31.9 (C-7), 31.9 (C-8), 50.2 (C-9), 36.7 (C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 56.8 (C-14), 24.3 (C-15), 29.2 (C-16), 56.2 (C-17), 11.8 (C-18), 19.4 (C-19), 35.5 (C-20), 18.7 (C-21), 34.3 (C-22), 29.4 (C-23), 49.5 (C-24), 147.5 (C-25), 17.8 (C-26), 111.4 (C-27), 26.5 (C-28), 12.0 (C-29), 101.3 (C-1'), 73.4 (C-2'), 76.2 (C-3'), 70.4 (C-4'), 79.7 (C-5'), 63.6 (C-6'), 174.3 (C-1"), 34.3 (C-2"), 25.0 (C-3"), 22.7-29.8 (C-4" – C-15"), 14.1 (C-16"); APCI-MS *m/z* 255.49 $[C_{15}H_{31}COO]^-$, 610.98 $[M–H+HCOOH–H_2O–C_{15}H_{31}CO]^-$, 812.65 $[M]^-$ calcd. for $C_{51}H_{88}O_7$. Found 812.33.

## 3. RESULTS AND DISCUSSION

The antimicrobial activity of the four extracts, including hexane, chloroform, ethyl acetate and methanol-water 80:20 (*v/v*) were studied against four pathogenic bacterial and four fungal strains. Antibacterial and antifungal potential of the extracts were assessed in MIC values (Table 1). The results revealed that the chloroform extract was more effective against the test organisms than the other extracting solvents with MIC 200 µg/ml for both *E. coli* and *B. subtilis* and MIC 100 µg/mL for *A. niger*. Moreover, *A. niger* showed the high susceptibility with MIC 100 µg/mL to almost the extracts, except methanol-water 80:20 (*v/v*) extract. All the investigated extracts were not effective against *P. aeruginosa*, *S. aureus*, *F. oxysporum*, *S. cerevisiae* and *C. albicans*. Our obtained results were good agreement with the previous data as water and methanolic extracts of MCA collected in Taiwan were non-inhibitory activity against methicillin-resistant *S. aureus* or *P. aeruginosa* but these extracts were activity against *E. coli* and *S. enterica* [7].

*Table 1.* Minimum inhibitory concentration (MIC) of the extracts of MCA against some microorganisms.

| **Extracts** | **MIC (µg/ml)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Gr (-) bacteria** | | **Gr (+) bacteria** | | **Fungi** | | | |
| | *E. coli* | *P. aeruginosa* | *B. subtilis* | *S. aureus* | *A. niger* | *F. oxysporum* | *S. cerevisiae* | *C. albicans* |
| Hexane | - | - | - | - | 100 | - | - | - |
| Chloroform | 200 | - | 200 | - | 100 | - | - | - |
| Ethyl acetate | - | - | - | - | 100 | - | - | - |
| Methanol-Water 80:20 (*v/v*) | - | - | - | - | - | - | - | - |

(-): Non-detected.

The presence of antimicrobial activities compounds in the chloroform extract is perhaps the reason for its antimicrobial effects therefore a phytochemical constituents investigation on such extract was performed. In this study, four known compounds were isolated for the first time in the chloroform extract of MCA (Figure 1).

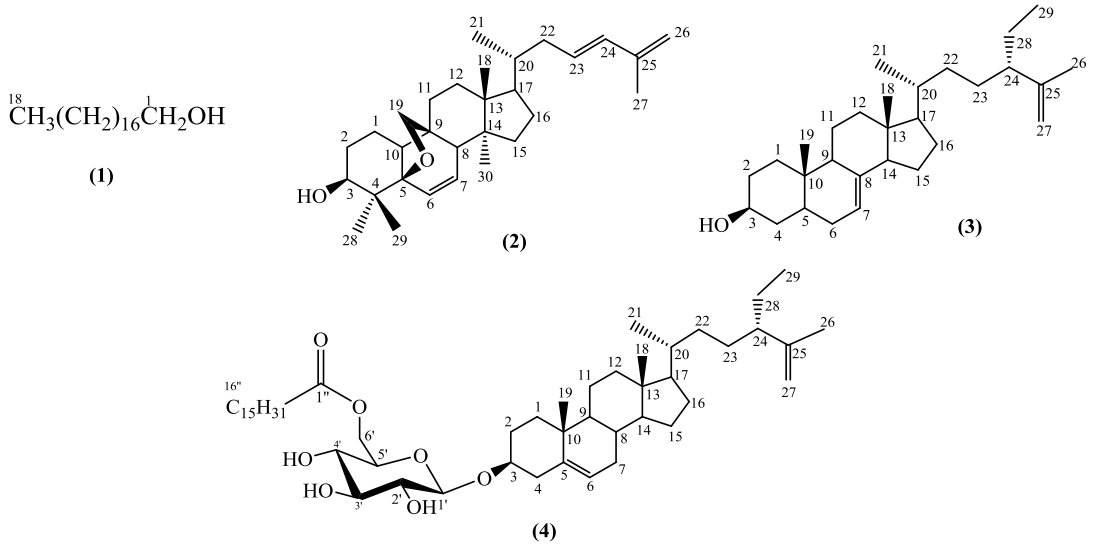


*Figure 1.* Structures of isolated compounds from MCA.

Compound (**1)** appeared as colorless paraffin and the ESI-MS showed an ion peak at *m/z* 269.28 $[M]^-$ corresponding the molecular formula of $C_{18}H_{38}O$. The $^1$H-NMR spectrum displayed one oxymethylene group at $\delta_{H\ ppm}$ 3.64 (2H, *t*, *J* = 7.5 Hz, H-1), one methyl group at $\delta_{H\ ppm}$ 0.88 (3H, *t*, *J* = 7.0 Hz, H-18), sixteen methylene groups [$\delta_{H\ ppm}$ 1.56-1.53 (32H, *m*, 16×$CH_2$). $^{13}$C-NMR spectrum showed the resonances of 18 carbons including one oxymethylene group at $\delta_{C\ ppm}$ 63.1 (C-1), one methyl group at $\delta_{C\ ppm}$ 14.1 (C-18), and sixteen –$CH_2$– group at $\delta_{C\ ppm}$ 22.7 – 32.8 (C-2 – C-17). Comparison with previously reported data [8], compound (**1)** was elucidated as octadecan-1-ol.

Compound (**2**) obtained as white amorphous powder. The compound possessed the molecular formula $C_{30}H_{46}O_2$ as determined from the ESI-MS ($[M + H]^+$ *m/z* 439.40). The $^{13}$C-NMR and DEPT spectra suggested that (**2**) were a triterpenoid with eight degrees of unsaturation. The $^{13}$C- and $^1$H-NMR spectra of (**2**) showed the presence of four tertiary methyls [[$\delta_{H\ ppm}$ 0.78 (H-18)/$\delta_{C\ ppm}$ 14.9 (C-18), $\delta_{H\ ppm}$ 0.90 (H-28)/$\delta_{C\ ppm}$ 24.6 (C-28), $\delta_{H\ ppm}$ 1.02 (H-29)/$\delta_{C\ ppm}$ 20.5 (C-29) and $\delta_{H\ ppm}$ 0.87 (H-30)/$\delta_{C\ ppm}$ 20.1 (C-30)], a secondary methyl [$\delta_{H\ ppm}$ 0.91 (*d*, *J* = 6.5 Hz, H-21)/$\delta_{C\ ppm}$ 18.8 (C-21)], a vinylic methyl [$\delta_{H\ ppm}$ 1.84 (H-28)/$\delta_{C\ ppm}$ 18.6 (C-27)], a secondary hydroxyl [$\delta_{H\ ppm}$ 3.94 (*dd*, *J* = 9.5, 3.0 Hz, H-3)/$\delta_{C\ ppm}$ 76.2 (C-3)], two disubstituted double bonds [$\delta_{H\ ppm}$ 6.05 (1H, *dd*, *J* = 10.0, 2.5 Hz, H-6)/$\delta_{C\ ppm}$ 132.8 (C-6), $\delta_{H\ ppm}$ 5.62 (*dd*, *J* = 10.0, 4.0 Hz, H-7)/$\delta_{C\ ppm}$ 131.5 (C-7), $\delta_{H\ ppm}$ 5.61 (*ddd*, *J* = 15.5, 7.0, 6.5 Hz, H-23)/$\delta_{C\ ppm}$ 129.2 (C-23) and $\delta_{H\ ppm}$ 6.14 (*d*, *J* = 15.5, H-24)/$\delta_{C\ ppm}$ 134.2 (C-24)], a terminal methylene [$\delta_{H\ ppm}$ 4.86 (H-26)/$\delta_{C\ ppm}$ 114.1 (C-26)] and an oxymethylene group [$\delta_{H\ ppm}$ 3.67 (*d*, *J* = 8.5 Hz, H-19a), 3.51 (*d*, *J* = 8.5 Hz, H-19b)/$\delta_{C\ ppm}$ 79.9 (C-19)]. The above evidence coupled with comparison of the NMR data of (**2**) to those in the literature [9] indicating that (**2**) was (23*E*)-5$\beta$,19-epoxycucurbita-6,23,25-trien-3$\beta$-ol.

Compound (**3**) appeared as white amorphous powder. The molecular formula of **3** was assigned as $C_{29}H_{48}O$ based on an ion peak at *m/z* 413.45 $[M+H]^+$ in ESI-MS. The $^1$H-NMR spectrum exhibited the presence of two tertiary methyls [$\delta_{H\ ppm}$ 0.79 (3H, *s*, H-19) and 0.53 (3H, *s*, H-18)], an allylic methyl at $\delta_{H\ ppm}$ 1.57 (3H, *s*, H-26), a secondary methyl at $\delta_{H\ ppm}$ 0.91 (3H, *d*,

*J* = 6.5 Hz, H-21), a primary methyl at $\delta_{H\ ppm}$ 0.80 (3H, *t*, *J* = 7.5 Hz, H-29), an oxygenated proton at $\delta_{H\ ppm}$ 3.59 (1H, *m*, H-3), terminal methylene protons [$\delta_H$ 4.73 (1H, *dd*, *J* = 2.5, 1.5 Hz, H-27a) and 4.64 (1H, *d*, *J* = 2.5 Hz, H-27b)], and an olefinic proton at $\delta_{H\ ppm}$ 5.15 (1H, *m*, H-7). The $^{13}$C-NMR spectrum of **3** showed the presence of an oxygenated carbon at $\delta_{C\ ppm}$ 71.1 (C-3) and four olefinic carbons [$\delta_{C\ ppm}$ 111.4 (C-27), 117.5 (C-7), 139.6 (C-8) and 147.6 (C-25)]. The spectral data were similar to those in literature [10] suggesting that compound (**3**) was 5*α*-poriferasta-7,25-dien-3*β*-ol.

Compound (**4**) obtained as colorless paraffin. The characteristic signals of a fatty acid chain were observed with a terminal methyl group at $\delta_{H\ ppm}$ 0.88 (3H, *t*, *J* = 6.5 Hz, H-16), a methylene linking carbonyl at $\delta_{H\ ppm}$ 2.32 (2H, *t*, *J* = 7.5 Hz, H-2"), and multiple methylene groups in the $^1$H-NMR spectrum. The $^1$H-NMR spectrum also showed the signals corresponding to a sugar moiety [$\delta_{H\ ppm}$ 4.37 (1H, *d*, *J* = 7.5 Hz, H-1'), 3.34 (1H, *t*, *J* = 8.0 Hz, H-2'), 3.53 (1H, *t*, *J* = 9.0 Hz, H-3'), 3.37 (1H, *t*, *J* = 9.0 Hz, H-4'), 3.51 (1H, *m*, H-5') and 4.33 (2H, *brs*, H-6')]. The $^{13}$C-NMR spectrum showed the signals for a sugar moiety at $\delta_{C\ ppm}$ 101.3 (C-1'), 73.4 (C-2'), 76.2 (C-3'), 70.4 (C-4'), 79.7 (C-5') and 63.6 (C-6'). The glucose unit was linked to fatty acid chain at position C-6' due to HMBC correlation between H-6' and C-1" as well as the downfield shift of H-6' in glucose unit. The NMR data of an aglycone unit in (**4**) were very similar to those of clerosterol [11]. The major differences were the downfield shift of C-3 ($\delta_{C\ ppm}$ 73.8) and the upfield shift of C-2 ($\delta_{C\ ppm}$ 29.3) and C-4 ($\delta_{C\ ppm}$ 38.9) in the $^{13}$C-NMR spectrum of (**4**) indicating the sugar unit was bonded at C-3 of clerosterol. Moreover, the APCI-MS spectrum showed an ion peak at *m/z* 812.65 $[M]^-$, suggesting the molecular formula of $C_{51}H_{88}O_7$, together with the observed fragmentation patterns at *m/z* 255.49 $[C_{15}H_{31}COO]^-$ and *m/z* 610.98 $[M–H+HCOOH–H_2O–C_{15}H_{31}CO]^-$, indicating the fatty acid chain as palmitic acid (Figure 2). On the basis of above evidences, the structure of (**4**) was determined as 3-*O*-(6'-*O*-palmitoyl-*β*-D-glucopyranosyl)-clerosterol [11].

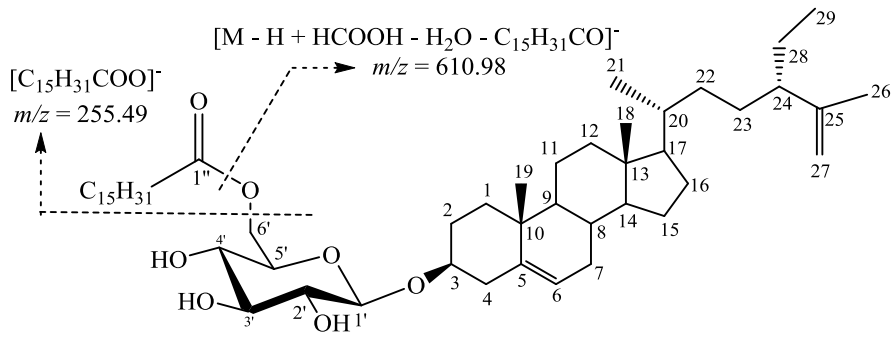


*Figure 2.* The fragmentation patterns in MS spectrum of (**4**).

## 4. CONCLUSIONS

Primary screening of antimicrobial activity of four extracts (hexane, chloroform, ethyl acetate and methanol-water) from the fruits of *Momordica charantia* L. var. *abbreviata* Ser. showed that the chloroform extract possessed the highest activity with the MIC value 100 µg/mL against *Aspergillus niger*, and 200 µg/mL against two microorganisms *Escherichia coli* and *Bacillus subtilis*. In this paper, four compounds were isolated and structures elucidated for the first time in such species including octadecan-1-ol (**1**), (23*E*)-5*β*,19-epoxycucurbita-6,23,25-

trien-3$\beta$-ol (**2**), 5$\alpha$-poriferasta-7,25-dien-3$\beta$-ol (**3**) and 3-*O*-(6'-*O*-palmitoyl-$\beta$-D-glucopyranosyl)-clerosterol (**4**).

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