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 *n*-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), (23E)-5β, 19-epoxycucurbita-6, 23, 25-trien-3β-ol (2), 5α-poriferasta-7,25-dien-3β-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 in *M. charantia* L. var. *abbreviata* Ser.

**Key words**: Antibacterial, antifungal, clerosterol, Cucurbitaceae, *Momordica charantia*.

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 use 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 and glycosides, etc, were demonstrated having antimicrobial properties *in vitro* (Gebregiorgis and Nigussie, 2018).

In an effort to expand the spectrum of antimicrobial agents from natural resources, *Momordica charantia* L. var. *abbreviata* Ser. (MCA) was selected. The investigated species is normally smaller than the cultivated bitter gourd *M. charantia* L. (MC) both belonging to the family Cucurbitaceae. Many pharmacological activities including anti-bacterial, anti-inflammatory, anti-viral, cytotoxic, hypoglycemic, and triglyceride-lowering activities have been reported on MC (Lu et al., 2011; Sagor et al., 2015). MC is also known to contain several compounds such as momorcharins, momordenol, momordin, momordicine, momordinol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacin, cucurbitane, cycloartenols, diosgenin, elaeeostearic acids, erythrodial, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, caffeic acid, ferulic acid, fisetin, isorhamnetin, 3β,25-dihydroxy-7β-methoxy-5,23(E)-diene, 3β-hydroxy-7,25-dimethoxy-5,23(E)-diene and 3-O-β-D-allopyranosyl-7β,25-dihydroxy-5,23(E)-dien-19-ol (Wu and Ng, 2008).

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 (Nguyen et al., 2014). 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. 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 (Huang et al., 2015).

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.

**MATERIALS AND METHODS**

**Plant material**

The fruits of MCA 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.

**General experimental procedures**

The NMR experiments were performed on a Bruker DMX 500 spectrometer. MS were carried out on an Agilent 6310 spectrometer. Column chromatography was performed on normal phase silica gel (40-63 μm, Kieselgel 60, Merck 7667). Thin layer chromatography (TLC) was performed on Kieselgel 60F254 plates (Merck) and spots visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 ml sulfuric acid and 20 ml ethanol) and thereafter, heated.

All solvents used were purchased from Chemsol, purity ≥ 99.0%.

**Antimicrobial activity test**

Antimicrobial activity test was carried out at the Department of Experimental Biology, Institute of Natural Products Chemistry, VAST, using the method previously described (Vanden and Vlietinck, 1991; McKane and Kandel, 1996). This experiment was performed by microdilution technique on 96-well microtiter plate. Two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus subsp. aureus*) and four fungal strains (*Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces cerevisiae* and *Candida albicans*) were employed to determine antimicrobial activity and minimum inhibitory concentration (MIC). The reference antibiotics were streptomycin, tetracyclin and nystatin. Fungi and bacteria were cultured in nutrient media. The test micro-organisms were activated before testing in fluid nutrient media. MIC is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria.

**Extraction and isolation**

Dried and powdered fruits of MCA (3.0 kg) were macerated for 12 h with 30 L n-hexane to furnish 45.0 g of n-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 n-hexane: acetone with increasing acetone ratios to obtain fourteen fractions from C1 to C14. Fraction C2 (12.5 g) was applied on normal phase silica gel column chromatography and eluted with the solvent system of n-hexane: chloroform (95:5) to afford compound 1 (430 mg). Fraction C4 was chromatographed and eluted with n-hexane: ethyl acetate with increasing ethyl acetate ratios to obtain thirteen sub-fractions from C4.1 to C4.13. Subfraction C4.8 was rechromatographed and eluted with the solvent system of n-hexane: ethyl acetate (95:5) to give compound 2 (500 mg). Sub-fraction C4.12 was rechromatographed and eluted with n-hexane: ethyl acetate (85:15) to give compound 3 (200 mg). Fraction C10 was applied to silica gel column chromatography and eluted with chloroform: acetone (80: 20) to obtain compound 4 (175 mg).

Octadecan-1-ol (1): colorless paraffin; 1H-NMR (CDCl3, 500 MHz): δHppm 3.64 (2H, t, 7.5), 1.56-1.53 (32H, m), 0.88 (3H, t, 7.0); 13C-NMR (CDCl3, 125 MHz): δCppm 63.1, 32.8, 31.9, 29.4 - 29.7 (C12), 25.8, 22.7, 14.1; ESI-MS m/z 269.28 [M-H]· calculated for C18H32O. Found: 269.38.

23-E)-5β,19-Epoxycurbita-6,23,25-trien-3β-ol (2): white amorphous powder; 1H-NMR (CDCl3, 500 MHz): δHppm 6.14 (1H, d, 15.5, H-24), 6.05 (1H, dd, 10.0, 2.5, H-6), 5.62 (1H, m, H-7), 5.61 (1H, m, 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, 8.0, 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); 13C-NMR (CDCl3, 125 MHz): δCppm 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-
Table 1: Antimicrobial activity of the extracts of MCA.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>200</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>-</td>
</tr>
<tr>
<td>Methanol-Water 80:20 (v/v)</td>
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</table>

:: not determined.

RESULTS AND DISCUSSION

General extraction yields varied between 1.45% for ethyl acetate extract and 3.00% for methanol-water 80:20 (v/v) extract. The antimicrobial activity of the four extracts, including n-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 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 in line with the previous data as water and methanolic extracts of MCA collected in Taiwan showed non-inhibitory activity against methicillin-resistant S. aureus or P. aeruginosa but these extracts exhibited activity against E. coli and S. enterica (Lu et al., 2011).

The presence of antimicrobial activities in compounds in the chloroform extract is perhaps the reason for its antimicrobial effects and as a result, a phytochemical constituent’s 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). Compound 1 appeared as colorless paraffin and the ESI-MS showed an ion peak at 543.37 [M+H]+ corresponding to the molecular formula of C26H49O. The 1H-NMR spectrum displayed one oxymethylene group at δppm 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'''). 13C-NMR (CDCl3, 125 MHz): δppm 30.0 (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 812.65 [M]+ calculated for C53H86O7. Found: 812.33.

3-O(6'-O-Palmitoyl-β-D-glucopyranosyl)-cerosterol (4): colorless paraffin; 1H-NMR (CDCl3, 500 MHz): δppm 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''). 13C-NMR (CDCl3, 125 MHz): δppm 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 812.65 [M]+ calculated for C53H86O7. Found: 812.33.
Compound 3 appeared as white amorphous powder and an ion peak at m/z 413.45 [M + H]^+ in ESI-MS. The molecular formula was assigned as C_{29}H_{48}O based on $^{13}$C-NMR data (C×29) and the molecular ion peak in ESI-MS. $^1$H-NMR exhibited the presence of two tertiary methyls at $\delta$ 0.79 (3H, s) and 0.53 (3H, s), an allylic methyl at $\delta$ 1.57 (3H, s), a secondary methyl at $\delta$ 0.91 (3H, d, 6.5), a primary methyl at $\delta$ 0.80 (3H, t, 7.5), an oxygenated proton at $\delta$ 3.59 (1H, m), terminal methylene protons at $\delta$ 4.73 (1H, m) and 4.64 (1H, d, 2.5), and an olefinic proton at $\delta$ 5.15 (1H, m). The $^{13}$C-NMR spectra of 3 showed the presence an oxygenated carbon at $\delta$ 71.1 and four olefinic carbons at $\delta$ 111.4, 117.5, 139.6 and 147.6. The spectral data were similar to those in literature suggesting that compound 3 was 5$\alpha$-poriferasta-7,25-dien-3$\beta$-ol (Garg and Nes, 1986).

Compound 4 was obtained as colorless paraffin. The characteristic signals of a fatty acid chain were observed with a terminal methyl group at $\delta$ 0.88 (3H, t, 6.5), a methylene linking carbonyl at $\delta$ 2.32 (2H, t, 7.5), and multiple methylene groups in the $^1$H-NMR spectrum. The $^1$H-NMR spectrum also showed the signals corresponding to a sugar moiety at $\delta$ 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') and 4.33 (2H, brs, H-6'). The $^{13}$C-NMR spectrum showed the signals for a sugar moiety at $\delta$ 101.3, 73.4, 76.2, 70.4, 79.7 and 63.6.

The glucose unit was linked to fatty acid chain at position 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 aglycon unit in 4 were very similar to those of clerosterol (Kwon et al., 2003) but major differences were the downfield shift of C-3 (73.8) and the upfield shift of C-2 (29.3) and C-4 (38.9) in the $^{13}$C-NMR spectrum of 4 indicating the sugar unit was bonded at C-3 of clerosterol. Moreover, the observed fragmentation patterns at m/z 255.49 and 610.98 in APCI-MS spectrum indicated the long chain fatty acid and glucoside part of 4, respectively (Figure 2). On the basis of the aforementioned evidences, the structure of 4 was determined as 3-O-(6'-O-Palmitoyl-$\beta$D-glucopyranosyl)-clerosterol.

**Conclusions**

Primary screening of antimicrobial activity of four extracts (n-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 *A. niger*, and 200 μg/ml against two microorganisms *E. coli* and *B. subtilis*. In this paper, four compounds were isolated and structures elucidated for the first time in such species including octadecan-1-ol (1), (23E)-5$\beta$, 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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Figure 2: The fragmentation patterns in MS spectrum of 4

REFERENCES


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