Cytotoxic Isothiocyanates from *Moringa oleifera* Lam Seeds

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The dichloromethane extract of the freeze-dried seeds of *Moringa oleifera* Lam afforded 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**1**), 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**), squalene (**3**) and sitosterol (**4**). The dichloromethane extract of the air-dried leaves of *M. oleifera* afforded polyprenol (**5**), phytyl fatty acid ester (**6**) and lutein (**7**). The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **3**-**7** were identified by comparison of their ^{13}C NMR data with those reported in the literature.

Isothiocyanates **1** and **2** were evaluated for cytotoxicity against the human cancer cell lines, non-small cell lung adenocarcinoma (A549) and colon carcinoma (HCT 116), and the non-cancer cell line Chinese hamster ovary cells (AA8) using the MTT cytotoxicity assay. Compounds **1** and **2** were cytotoxic against A549 with IC_{50} values of 10 and 12 μM, respectively, while the positive control, Doxorubicin gave an IC_{50} value of 4 μM. Isothiocyanates **1** and **2** were also cytotoxic against HCT 116 with IC_{50} values of 12 and 11 μM, respectively while Doxorubicin exhibited an IC_{50} value of 3 μM. Compounds **1**, **2** and Doxorubicin were also cytotoxic against AA8 with IC_{50} values of 22, 15 and 4 μM, respectively.

KEYWORDS

Moringa oleifera, Moringaceae, 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate, 4-(α-L-rhamnosyloxy)benzyl isothiocyanate, squalene, sitosterol, polyprenol, cytotoxic

INTRODUCTION

Moringa oleifera Lam commonly known as malunggay has been used to combat malnutrition, specially among infants and nursing mothers (Fahey 2005). Specific components of *M. oleifera* preparations which include the isothiocyanate compounds have been reported to have hypotensive, anticancer and antibacterial activity (Fuglie 1999). A review of the medical evidence of the nutritional, therapeutic and prophylactic properties of *M. oleifera* has been provided (Fahey 2005).

The ethanol extract of the seeds of *M. oleifera* afforded 4-(α-L-rhamnosyloxy)benzyl isothiocyanate, niazimicin and *O*-ethyl-4-(α-L-rhamnosyloxy)benzyl carbamate. The first two compounds showed very significant potential antitumor promoting activity using an *in vitro* assay which tested their inhibitory effects on Epstein-Barr virus-early antigen (EBV-EA) activation in Raji cells induced by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Guevara et al. 1999). Niazimicin was also found to have potent antitumor promoting

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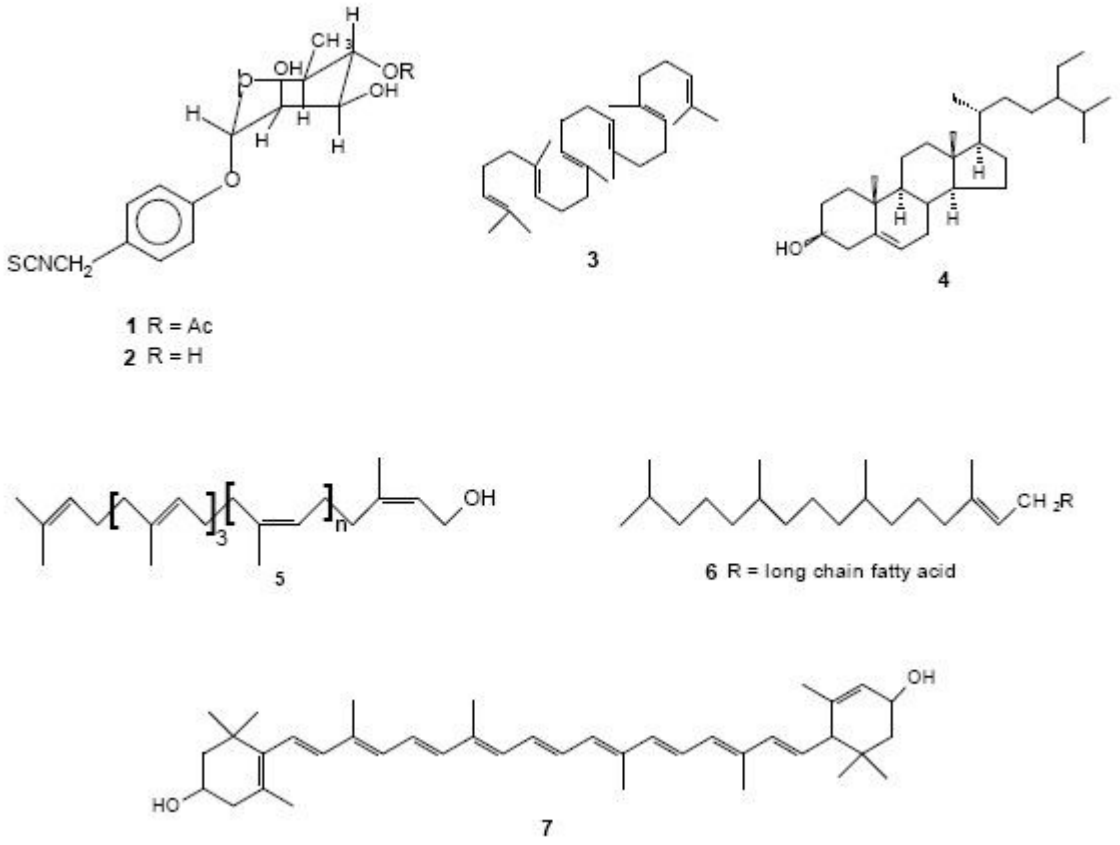

Figure 1. Chemical constituents of *Moringa oleifera* seeds: 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**1**), 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**), squalene (**3**), sitosterol (**4**); and leaves: polyprenol (**5**), phytyl fatty acid ester (**6**), lutein (**7**).

activity in the two-stage carcinogenesis in mouse skin using 7,12-dimethylbenz[a]anthracene (DMBA) as initiator and TPA as tumor promoter (Guevara et al. 1999). The seeds of *Moringa oleifera* yielded 4-(α-L-rhamnosyloxy)benzyl isothiocyanate which exhibited minimum bactericidal concentration of 40 µmol/l for *Mycobacterium phlei* and 56 µmol/l for *Bacillus subtilis* (Eilert et al. 1981). A new nitrile, 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate has also been reported from *M. oleifera* (Faizi et al. 1994) which is of relevance to our present report.

We report herein the isolation of 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**1**), 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**), squalene (**3**), sitosterol (**4**) (Figure 1), triacylglycerol and long chain unsaturated hydrocarbons from the dichloromethane extract of the freeze-dried seeds of *M. oleifera* and polyprenol (**5**), phytyl fatty acid ester (**6**), lutein (**7**) (Figure 1) and long chain unsaturated hydrocarbons from the air-dried leaves of *M. oleifera*. The cytotoxicity of **1** and **2** against the human cancer cell lines, non-small cell lung adenocarcinoma (A549) and colon carcinoma (HCT 116), and the non-cancer cell line Chinese hamster ovary cells (AA8) is likewise reported.

MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were taken with a Jasco DIP-370 digital polarimeter. NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H-NMR and at 150 MHz for ^{13}C-NMR spectra. Two-Dimensional (2D) NMR data (COSY, HSQC and HMBC) were obtained for **1** and **2**, while NOESY was obtained for **1**. EI- and ESI-MS were recorded on Finnigan GCQ and LCQ spectrometers, respectively. Column chromatography was performed with silica gel 60 (70-230 mesh); TLC was performed with plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin sulfuric acid and warming.

Sample Collection

Fresh leaves and fruits of *Moringa oleifera* Lam were collected from Ibaan, Batangas and Tanauan City, Batangas, respectively in May 2010. The sample was authenticated at the Jose Vera Santos Herbarium, Institute of Biology of the University of the Philippines Diliman.

Isolation of Constituents from the Seeds of *M. oleifera*

Fresh seeds of *M. oleifera* were ground in an osterizer and then freeze-dried. The freeze-dried seeds (103.5 g) were soaked in dichloromethane for three days, and then filtered. The filtrate was concentrated *in vacuo* to afford a crude extract (7.9 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment as eluents. The 30% acetone in DCM fraction was rechromatographed (5x) using 2.5% ethyl acetate in petroleum ether to afford **3** (15 mg). The 50% acetone in DCM was rechromatographed (3x) using 15% ethyl acetate in petroleum ether to afford **4** (18 mg). The 60%-80% acetone in DCM were combined and rechromatographed with DCM:diethyl ether:acetonitrile (2:2:6) as eluent. The less polar fractions were rechromatographed (3x) with the same solvent to afford **1** (95 mg) after washing with diethyl ether. The more polar fractions were rechromatographed (3x) with DCM:diethyl ether:acetonitrile (2.5:2.5:5) to afford **2** (125 mg) after washing with diethyl ether.

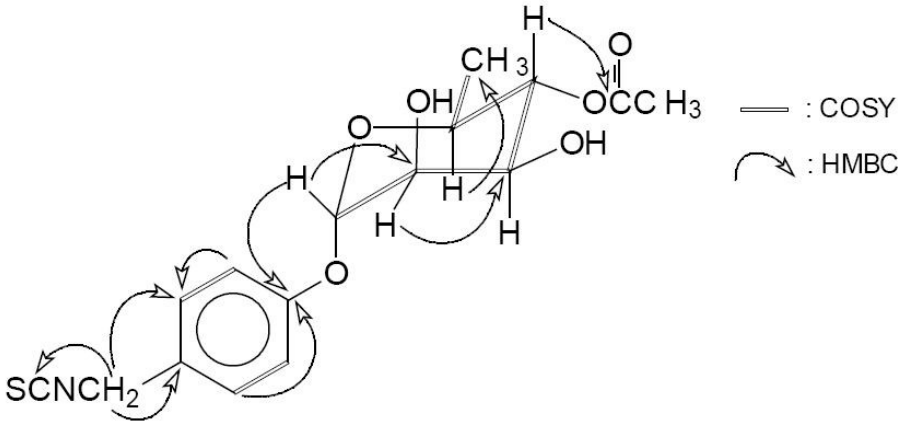

Figure 2. ^{1}H-^{1}H COSY and key ^{1}H-^{13}C long-range correlations of **1**.

Isolation of Constituents from the Leaves of *M. oleifera*

Air-dried leaves of *M. oleifera* (712 g) were ground in an osterizer and then soaked in dichloromethane for three days and filtered. The filtrate was concentrated *in vacuo* to afford a crude extract (52 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment. The 70% acetone in DCM was rechromatographed with 10% ethyl acetate in petroleum ether as eluent. The more polar fractions were rechromatographed (3x) with 10% ethyl acetate in petroleum ether to afford **5** (55 mg), while the less polar fractions were rechromatographed (3x) with 5% ethyl acetate in petroleum ether as eluent to afford a mixture of **6** (18 mg) after washing with petroleum ether. The 80% acetone in DCM was rechromatographed (4x) in diethyl ether:acetonitrile: dichloromethane (1:1:8) as eluent to afford **7** (14 mg) after washing with diethyl ether.

4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**1**): -125.6 (*c* = 0.9, $CHCl_3$); ^{1}H NMR: δ 7.05 (H-2, H-6, d, *J* = 9 Hz), 7.25 (H-3, H-5, d, *J* = 9 Hz), 4.64 (H_2-7, s), 5.54 (H-1′, d, *J* = 1.2 Hz), 4.13 (H-2′, br s), 4.08 (H-3′, d, *J* = 9 Hz), 4.85 (H-4′, t, *J* = 9 Hz), 3.85 (H-5′, m), 1.17 (H-6′, d, *J* = 6 Hz), 2.13 (s, OAc), 3.10, 2.36 (2′-OH, 3′-OH, br s); ^{13}C NMR: δ 156.0 (C-1), 116.6 (C-2, C-6), 128.4 (C-3, C-5), 128.2 (C-4), 48.2 (C-7), 132.2 (C-8), 97.2 (C-1′), 70.1 (C-2′), 70.6 (C-3′), 75.4 (C-4′), 66.4 (C-5′), 17.5 (C-6′), 172.3 and 21.0 (OAc). ESI-MS: *m/z* = 376.03 ($C_{16}H_{19}O_6NSNa$).

4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**): -106.5 (*c* = 1.3, $CHCl_3$); ^{1}H NMR: δ 7.03 (H-2, H-6, d, *J* = 9 Hz), 7.20 (H-3, H-5, d, *J* = 9 Hz), 4.62 (H_2-7, s), 5.54 (H-1′, d, *J* = 1.2 Hz), 4.16 (H-2′, br s), 4.00 (H-3′, dd, *J* = 3.0, 9.6 Hz), 3.58 (H-4′, t, *J* = 9.6 Hz), 3.73 (H-5′, m), 1.26 (H-6′, d, *J* = 6 Hz), 4.35 (OH, br s), 2.50 (2OH, br s); ^{13}C NMR: δ 156.0 (C-1), 116.7 (C-2, C-6), 128.4 (C-3, C-5), 128.2 (C-4), 48.2 (C-7), 132.2 (C-8), 97.7 (C-1′), 70.8 (C-2′), 71.5 (C-3′), 73.1 (C-4′), 68.8 (C-5′), 17.5 (C-6′). EIMS: *m/z* = 310.9 ($C_{14}H_{17}O_5NS$).

Squalene (**3**): ^{13}C NMR: δ 25.7 (C-1), 131.3 (C-2), 124.3 (C-3), 26.7 (C-4), 39.7 (C-5), 134.9 (C-6), 124.3 (C-7), 26.7 (C-8), 39.7 (C-9), 134.9 (C-10), 124.3 (C-11), 28.3 (C-12), 17.7 (C-2′), 16.0 (C-6′), 16.0 (C-10′).

Sitosterol (**4**): ^{13}C NMR: δ 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 19.4 (C-19), 36.5 (C-20), 19.0 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.0 (C-26), 19.8 (C-27), 23.1 (C-28), 11.9 (C-29).

Polyprenol (**5**): ^{13}C NMR: δ 59.02 (CH_2OH), 139.90, 136.08, 135.37, 135.28, 135.23, 135.20, 134.97, 134.89, 131.25, 125.01, 124.98, 124.93, 124.87 124.51, 124.44, 124.39, 124.25, 124.22, 124.12, 39.76, 39.72, 32.22, 32.20, 32.17, 31.98, 26.76, 26.67, 26.63, 26.39, 26.35, 26.30, 25.69, 23.45, 23.43, 23.36, 17.68, 16.00, 15.99.

Phytyl fatty acid ester (**6**): ^{13}C NMR: δ 61.2 (C-1), 118.1 (C-2), 142.6 (C-3), 39.9 (C-4), 25.0 (C-5), 36.6 (C-6), 32.7 (C-7), 37.35 (C-8), 24.5 (C-9), 37.4 (C-10), 32.8 (C-11), 37.29 (C-12), 24.8 (C-13), 39.4 (C-14), 28.0 (C-15), 22.7 (C-16), 16.4 (C-17), 19.70 (C-18), 19.74 (C-19), 22.6 (C-20), 174.0 (C-1′), 34.4

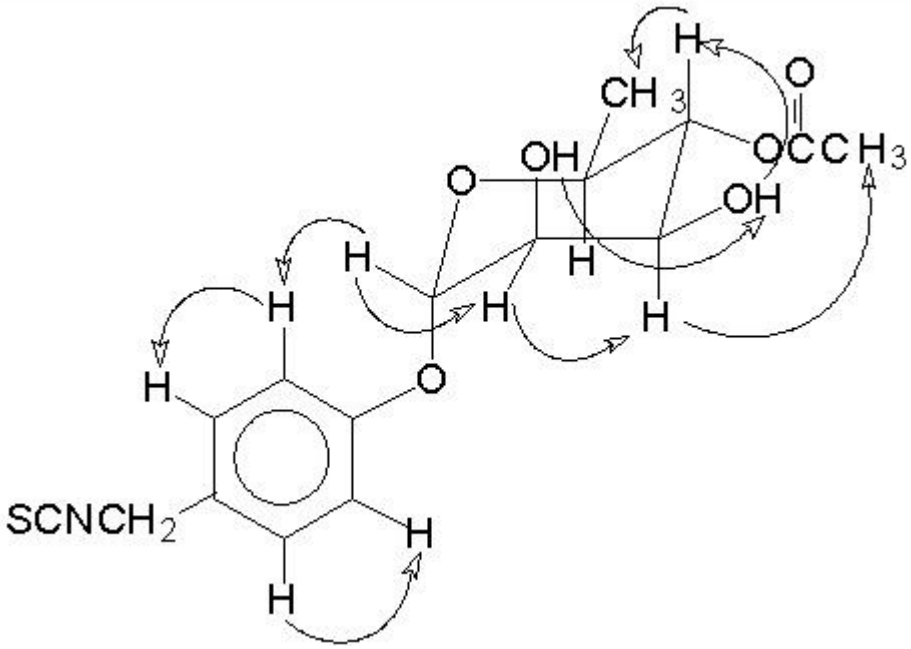

Figure 3. NOESY correlations of **1**.

(C-2′), 25.0 (C-3′), 29.5 (C-4′), 29.2-29.7 (C-5′-C-17′), 14.1 (C-18′).

Lutein (**7**): ^{13}C NMR: δ 37.1 (C-1), 48.4 (C-2), 65.1 (C-3), 42.5 (C-4), 126.2 (C-5), 138.0 (C-6), 125.6 (C-7), 138.5 (C-8), 135.7 (C-9), 131.3 (C-10), 124.9 (C-11), 137.5 (C-12), 136.4 (C-13), 132.6 (C-14), 130.1 (C-15), 28.7 (C-16), 30.3 (C-17), 21.6 (C-18), 12.7 (C-19 and C-20), 34.0 (C-1′), 44.6 (C-2′), 65.9 (C-3′), 124.5 (C-4′), 137.7 (C-5′), 55.0 (C-6′), 128.7 (C-7′), 130.8 (C-8′), 135.1 (C-9′), 137.6 (C-10′), 124.8 (C-11′), 137.7 (C-12′), 136.5 (C-13′), 132.6 (C-14′), 130.1 (C-15′), 24.7 (C-16′), 29.5 (C-17′), 22.9 (C-18′), 13.1 (C-19′), 12.8 (C-20′).

Cytotoxicity Tests

Compounds **1** and **2** were tested for cytotoxic activity against a human colon carcinoma (HCT 116) cell line, a human lung non-small cell adenocarcinoma (A549) cell line and the non-cancer cell line Chinese hamster ovary cells (AA8) at the Institute of Biology, University of the Philippines Diliman, Quezon City. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Doxorubicin was used as the positive control, while dimethylsulfoxide (DMSO) was used as the negative control. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay reported in the literature was employed (Mossman 1983, Freshney 1994, Jacinto et al. 2011, Ragasa et al. 2008).

The cells were plated into a 96-well microtiter plate. The cell concentration was counted using a hemocytometer. Twenty mL of cell medium suspension with a concentration of 4 x 10^4 cells/mL was used for a microtiter plate. One hundred ninety µL of the suspension was transferred to each well. The microtiter plate was incubated in a humidifier incubator with 5% CO_2 for 24h at 37^0C.

Four milligrams each of **1** and **2** were dissolved in 1 milliliter each of DMSO to make 4 mg/mL solutions. Compounds **1** and **2**, doxorubicin and DMSO were serially diluted.

After 24h of incubation, the cells were treated with 10 µL of **1** and **2** at 50, 25, 12.5 and 6.25 µg/mL. Three replicate wells for each concentration were used. The microtiter plate containing the treated cells was incubated for 72h, after which the media from the wells were discarded and replaced with 20 µL of MTT dissolved in PBS (5 mg/mL) under low light intensity conditions. The plates were wrapped in aluminum foil and incubated at 37^0C in 5% CO_2 for 24h. After incubation, 100 µL of DMSO was added into each well to dissolve the MTT-formazan crystals. Using an ELISA reader, the absorbances were measured at wavelengths of 570 nm (measuring filter) and 620 nm (reference filter). Three trial assays were conducted with 3 replicate wells per concentration. The purple MTT-formazan crystals produced from the experiment indicated the presence of live cells, since the yellow MTT-tetrazolium dye can be reduced by the mitochondria of living cells. Hence, a yellow product indicated cell death through either necrosis or apoptosis of the cancer cells. Absorbance readings were used to calculate the IC_{50} (concentration which resulted in a 50% reduction in cell viability) values of the samples using simple linear regression. The linear intrapolation/extrapolation method for sublethal toxicity: the inhibition concentration ICp approach (Version 2) from a toxtat software program was used.

RESULTS AND DISCUSSION

The dichloromethane extract of the freeze-dried seeds of *M. oleifera* afforded **1-4**, triacylglycerol and long chain unsaturated hydrocarbons by silica gel chromatography. The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR data of **1** (see experimental) gave resonances for two aromatic proton doublets at δ 7.05 which were ortho coupled to the proton doublets at δ 7.25 by 9 Hz; benzylic methylene protons at δ 4.64 (br s); and an acetate at δ 2.13 (s).

These resonances suggested a 1,4-disubstituted benzene and an acetate in **1**. The coupled protons in **1** were verified from the COSY spectrum (Figure 2). A glycoside was deduced from the anomeric proton at δ 5.54 (H-1′, d, J = 1.2 Hz) which was coupled to the oxymethine proton at δ 4.13 (H-2′, br s), which was in turn coupled to another oxymethine proton at δ 4.08 (H-3′, d, J = 9 Hz). This proton was further coupled to the oxymethine proton at δ 4.85 (H-4′, t, J = 9 Hz), which was also coupled to another oxymethine proton at δ 3.85 (H-5′, m), which was finally coupled to the methyl doublet at δ 1.17 (H_3-6′, d, J = 9 Hz). The H-2′ broad singlet indicated that it is in the equatorial position, while the large coupling constant, J = 9 Hz for H-3′(d) and H-4′(t) suggested that H-3′, and H-4′ are in the axial position. The H-4′ triplet indicated that it is also coupled to H-5′ by 9 Hz, suggesting that H-5′ is also in the axial position. Two broad resonances at δ 3.10 and 2.36 indicated two hydroxyl protons in the glycoside.

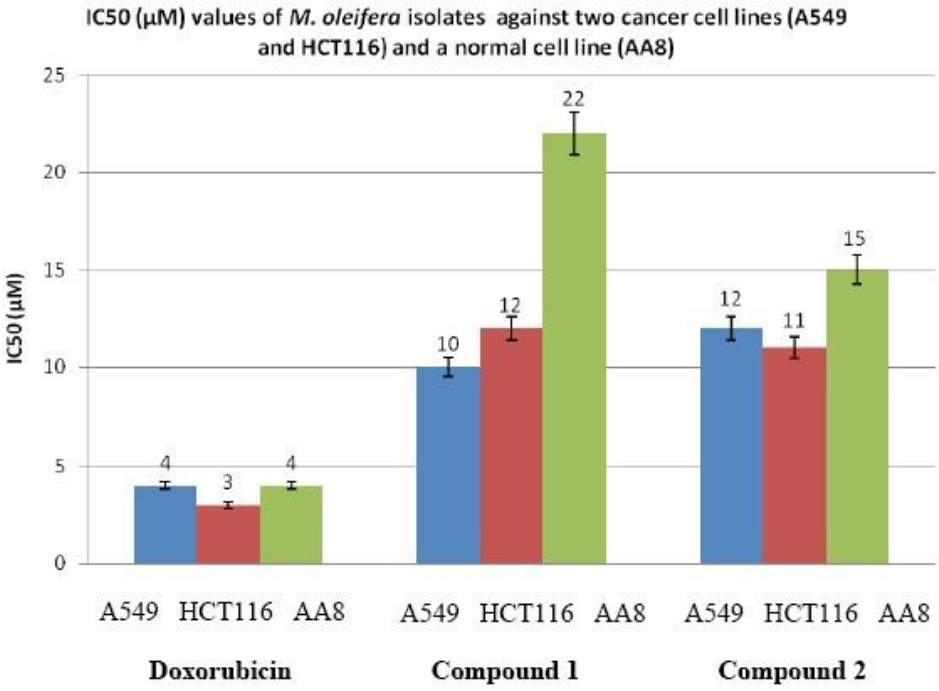

Figure 4. Inhibitory Concentrations at 50% (IC_{50}) of compounds **1** and **2** from *M. oleifera* tested against human cancer cell lines: lung adenocarcinoma A549, colon carcinoma HCT 116 and the non-cancer Chinese hamster ovary AA8 using the MTT assay. Each value is the mean of three trials with three replicates.

The ^{13}C NMR data of **1** (see experimental) confirmed the presence of the 1,4-disubstituted benzene ring by the resonances at δ 116.6 (2C), 128.4 (2C), 128.2 and 156.0. The deshielded resonance at δ 156.0 suggested that the anomeric carbon of the glycoside was attached to the benzene ring through this carbon. Based on chemical shifts, the anomeric carbon was attributed to the resonance δ 97.2; the oxymethine protons were assigned to the resonances at δ 70.1, 70.6, 75.4 and 66.4; the methyl carbon at δ 17.5; the acetate at δ 21.0 and 172.3; the benzylic carbon at δ 48.2; and the thiocyanate carbon at δ 132.2.

Protons attached to carbons were assigned (see experimental) from HSQC 2D NMR data and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Figure 2. Thus, the isothiocyanate was attached to C-7 on the basis of long-range correlation between H_2-7 and C-8. The benzylic methylene protons were attached to C-4 due to long-range correlations between H_2-7 and C-3, C-4 and C-5. The glycoside was attached to the benzene ring at C-1 based on the long-range correlation observed between H-1′ and this carbon. The acetate was attached to C-4′ since long-range correlation was observed between H-4′ and the carbonyl carbon of the acetate. All long-range correlations are consistent with the structure of **1.**

The relative configuration of **1** was deduced by NOESY. The aromatic proton (H-2) was close in space to the anomeric proton (H-1′), which was close to H-2′, which was in turn close to H-3′, which was finally close to the acetate methyl. This indicated that they are located on the same face of the molecule. On the opposite face of **1**, H-4′ was close to H_3-6′ and the hydroxyl proton at δ 2.36, which was in turn close to another hydroxyl proton at δ 3.10. All NOESY correlations were consistent with the relative configuration of **1** (Figure 3).

The ESI-MS of **1** gave a pseudomolecular ion at m/z = 376.03 [M + Na^+], which corresponded to a molecular formula of $C_{16}H_{19}O_6NS$. The structure of **1** was confirmed by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literature for 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate **1** (Faizi et al. 1994).

The ^{1}H NMR data of **2** (see experimental) did not show the acetate methyl (δ 2.13) and the shielding of the H-4′ resonance from 4.85 in **1** to 3.58 in **2**, suggesting that the acetate in **1** was replaced by a hydroxyl in **2**. This was supported by the ^{13}C NMR data of **2** (see experimental) which indicated the non-appearance of the ^{13}C NMR resonances of the acetate at δ 172.3 and 21.0 and the shielding of the oxymethine carbon (C-4′) from δ 75.4 in **1** to δ 73.1 in **2**. The EI-MS of **2** gave a molecular ion at m/z = 310.9 [M^+], which corresponded to a molecular formula

of $C_{14}H_{17}O_5NS$. Literature search revealed that **2** is 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**) (Eilert et al. 1981).

The *M. oleifera* seeds also afforded **3** and **4**. The structures of **3** and **4** were confirmed by comparison of their ^{13}C NMR data (see experimental) with those found in the literature for squalene (Brown and Martens 1997) and sitosterol (Kojima et al. 1990), respectively.

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *Moringa oleifera* afforded **5-7.** The structures of these compounds were confirmed by comparison of their ^{13}C NMR data (see experimental) with those found in the literature for polyprenol (**5**) (Rideout et al. 2003), phytyl fatty acid ester (**6**) (Ragasa et al. 2004), and lutein (**7**) (Lee et al. 2003).

Cytotoxicity tests were conducted on **1** and **2** against the human cancer cell lines, non-small cell lung adenocarcinoma (A549) and colon carcinoma (HCT 116) and the non-cancer cell line Chinese hamster ovary cells (AA8) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. Results of the study (Figure 2) indicated that **1** and **2** showed cytotoxicity against A549 with IC_{50} values of 10 and 12 μM, respectively, while the positive control, the commercial anticancer drug, Doxorubicin gave an IC_{50} value of 4 μM. Isothiocyanates **1** and **2** were also cytotoxic against HCT 116 with IC_{50} values of 12 and 11 μM, respectively, while Doxorubicin exhibited an IC_{50} value of 3 μM. Compounds **1**, **2** and Doxorubicin also exhibited cytotoxicity against AA8 with IC_{50} values of 22, 15 and 4 μM, respectively. It is noted that **2** is slightly more cytotoxic than **1** against all the cell lines tested, while the positive control, Doxorubicin is more cytotoxic than the isothiocyanates tested.

The selectivity index (SI) (Al-Qubaisi et al. 2011) was obtained by dividing the IC_{50} value of the non-cancer cell line Chinese hamster ovary cells AA8 by the IC_{50} value of the human non-small cell lung adenocarcinoma A549. The SI of **1** for AA8 is 2.10 times higher than A549, while the SI of **2** for AA8 is 1.26 times higher than A549. On the other hand, the SI of the positive control, Doxorubicin for AA8 is 1.10 times higher than A549. Therefore, all the compounds tested gave higher SI for AA8 than A549. Furthermore, compared with the test compounds, the positive control showed higher cytotoxicity towards normal AA8 cell line with SI of 1.10.

The SI of **1** for AA8 is 1.90 times higher than the human colon carcinoma HCT 116, while the SI of **2** for AA8 is 1.31 times higher than HCT 116. On the other hand, the SI of the positive control, Doxorubicin for AA8 is 1.23 times higher than HCT 116. Therefore, all the compounds tested gave higher SI for AA8 than HCT 116. Furthermore, compared with the test compounds, the positive control showed higher cytotoxicity towards normal AA8 cell line with SI of 1.23.

The suggested effective doses for IC_{50} values for plant extracts and pure compounds to be considered active according to National Cancer Institute (NCI) guidelines should be less than 20 and 4 μg/mL, respectively (Geran et al. 1972). The equivalent IC_{50} values of **1** and **2** in μg/ml against the non-small cell lung adenocarcinoma (A549) are 3.69 and 3.64 μg/ml, respectively and colon carcinoma (HCT 116) with IC_{50} values of 4.06 and 3.50 μg/ml, respectively. These are within the effective doses of pure compounds to be considered active according to NCI guidelines.

CONCLUSION

4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate (**1**) and 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2**) isolated from the freeze-dried seeds of *Moringa oleifera* Lam were cytotoxic against the non-small cell lung adenocarcinoma (A549) with IC_{50} values of 10.45 and 11.71 μM, respectively and colon carcinoma (HCT 116) with IC_{50} values of 11.50 and 11.26 μM, respectively. According to NCI guidelines **1** and **2** are within the effective doses to be considered active.

CONFLICTS OF INTEREST

None

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Mr. Ruel M. Levida worked on the isolation and purification of the compounds from *M. oleifera.* Dr. Consolacion Y. Ragasa purified and identified the compounds and wrote the manuscript. Dr. Chien-Chang Shen obtained the NMR spectra, identified the compounds and reviewed the manuscript. Dr. Ming-Jaw Don obtained the mass spectra.

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