Insulin Secretagogues from *Moringa oleifera* with Cyclooxygenase Enzyme and Lipid Peroxidation Inhibitory Activities

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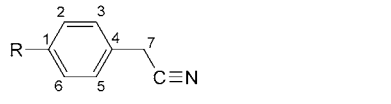
Bioassay-directed isolation and purification of the methanol extract of *Moringa oleifera* fruits yielded bioactive *N*-benzyl thiocarbamates, *N*-benzyl carbamates, benzyl nitriles, and a benzyl ester. Among these, methyl 2-[4-(α-L-rhamnopyranosyl)phenyl]acetate (**2**), *N*-[4-(β-L-rhamnopyranosyl)benzyl]-1-*O*-α-D-glucopyranosylthiocarboxamide (**3**), 1-*O*-phenyl-α-L-rhamnopyranoside (**5**), and 4-[(β-D-glucopyranosyl)-(1 → 3)-(α-L-rhamnopyranosyl)]phenylacetonitrile (**6**) are novel, and their structures were determined by spectroscopic methods. The known compounds isolated and characterized from the MeOH extract were niazirin (= 4-(α-L-rhamnopyranosyl)phenylacetonitrile; **1**), niazicin A (= methyl *N*-{4-[(4′-*O*-acetyl-α-L-rhamnopyranosyl)benzyl]}thiocarbamate; **4**), methyl *N*-{4-[(α-L-rhamnopyranosyl)benzyl]}carbamate (**7**), and methyl *N*-{4-[(4′-*O*-acetyl-α-L-rhamnopyranosyl)benzyl]}carbamate (**8**). The combined yield of these compounds from dried *M. oleifera* fruits was 1.63%. In rodent pancreatic β-cells (INS-1), compounds **4**, **5**, **6**, **7**, and **8** at 100 ppm significantly stimulated insulin release. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme inhibition assays revealed that **5** and **6** were most active at 83 ppm. Compound **6**, however, demonstrated greater specificity for inhibition of COX-2 enzyme (46%) than COX-1 enzyme. Lipid peroxidation assays revealed that **4** and **6** at 50 ppm inhibited peroxidation reactions by 80 and 95%, respectively, while **3** and **8** inhibited lipid peroxidation by 35%. These compounds did not inhibit the cell growth when tested with human breast (MCF-7), central nervous system (CNS, SF-268), lung (NCI-H460), or colon (HCT-116) cancer cell lines. Moreover, these compounds were not cytotoxic at the concentrations tested.

Introduction. – *Moringa oleifera* Lam. belongs to the family Moringaceae. A native of the Himalayan regions of India, it is now grown in Africa, Arabia, Southeast Asia, the Pacific Islands, the Caribbean Islands, and South America. Leaves, flowers, fruits, and bark of this plant have been used in traditional medicine [1][2]. Anecdotally, this plant has been used to treat conditions such as inflammation, paralysis, and hypertension [2]. In India, the leaves and fruits of *M. oleifera* are used as a vegetable and its roots as a substitute for horseradish. The fruits were reported to contain various amino acids, fatty acids, vitamins, and nutrient elements [1][2]. Several reports have appeared on the antimicrobial [2–5], antihypertensive [6–8], and antitumor [9] activities of *M. oleifera* plant parts. The seeds were reported to have strong coagulative and antimicrobial properties [1][3]. Among other compounds, isothiocyanates with antimicrobial activity were identified in *M. oleifera* seeds extract [3]. The presence of several peptides in the seed was attributed to the growth inhibition of *Gram*-positive and -negative bacteria [4]. Carbamates and thiocarbamates isolated from the leaves of *M. oleifera* were implicated for its anecdotal antihypertensive activity [6]. Traditionally, the fruits of *M. oleifera* were used to control or regulate blood sugar, but such claims are unsubstantiated. The anecdotal use of *M. oleifera* for regulating blood sugar was of

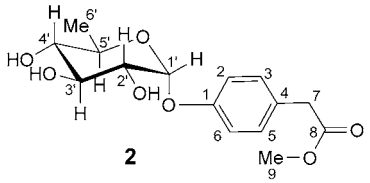
great interest to us, since few plant materials are known for such use. The death toll from atherosclerosis arising from diabetes is significant. The role of coronary inflammation in the initiation and progression of atherothrombosis has been reported [10]. Recent studies connecting inflammation and antioxidant activity to diabetes [11–13], prompted us to determine the *in vitro* phytomedicinal activities of *M. oleifera* fruits. In this paper, we report insulin secretion, COX enzymes, and lipid-peroxidation-inhibitory activities of compounds isolated from *M. oleifera* fruits.

Results and Discussion. – *Chemistry.* Dried and powdered *M. oleifera* fruits were sequentially extracted with hexane, EtOAc, and MeOH. These extracts were evaluated for COX-1 and COX-2 enzyme inhibition activity. The EtOAc and MeOH extracts showed selective inhibition of COX-2 enzyme. These extracts also inhibited lipid peroxidation by 50% at 250 ppm. The hexane extract was intensely green and contained substantial quantities of chlorophyll, which interfered with the lipid peroxidation assay. In addition, it contained large quantities of oleic and palmitic acids as determined by TLC and GC/MS upon comparison with standards. Therefore, this extract was not further investigated. Similarly, the COX inhibitory activity of the EtOAc extract was primarily due to fatty acids. We have previously reported COX enzyme inhibition by saturated and unsaturated fatty acids of varying chain lengths [14]. Therefore, the EtOAc extract was also not studied further. Initial fractionation of the MeOH extract by silica-gel MPLC gave fractions *A*–*G*. These fractions induced insulin secretion from INS-cells at 250 ppm or inhibited COX enzymes and lipid peroxidation.

Fraction *G*, purified by preparative HPLC with MeCN/H_2O as the mobile phase on an ODS column, yielded compounds **1** (R_t = 36.1 min) and **6** (R_t = 19.7 min) as amorphous powders. Compound **1** gave a characteristic band at 2220 cm^{-1}, suggesting the presence of a CN moiety. The bands observed at 1021, 2940, and 1511 cm^{-1} in its IR spectrum were assigned to C–O and aromatic C–H functionalities in the molecule. Analysis of ^1H- and ^{13}C-NMR chemical shifts confirmed that **1** is niazirin (= 4-(α-L-rhamnopyranosyl)phenylacetonitrile) [15].



1 R = α-rhamnopyranoside
6 R = β-glucopyranosyl-(1→3)-α-rhamnopyranoside



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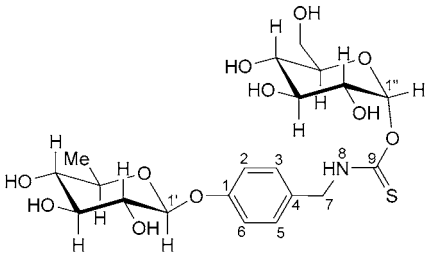

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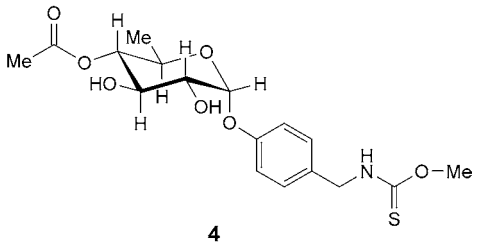

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The IR and NMR spectral data of **6** show strong similarities to those of **1**. The IR spectrum of **6** showed a characteristic band at 2253 cm^{-1}, which was assigned to a CN moiety. There were strong absorptions between 1023 and 1122 cm^{-1} that indicate the presence of several C–O stretching vibrations in the molecule. The UV/VIS spectrum of **6** shows an absorption maximum at 220 nm. Two *doublets* at 5.35 and 4.31 ppm in the ^{1}H-NMR spectrum were assigned to anomeric protons, respectively. The *doublet* at 1.08 ppm corresponds to the Me group of a rhamnose moiety. The signals at 90.73 and 99.09 ppm in the ^{13}C-NMR spectrum confirmed the presence of two anomeric centers in **6**. There were eight other C-atom signals observed in the region between 70.19 and 82.0 ppm and four signals in the aromatic region. The signal at 125.08 ppm was assigned to the CN moiety [15]. The DEPT spectrum of **6** showed the presence of two CH_2 and one Me group at 22.27, 61.82, and 18.57 ppm, respectively. In the HMBC spectrum, the anomeric proton at 5.35 ppm was correlated to a substituted aromatic C-atom at 156.16 ppm (*Fig. 1*). HMBC Correlations were also observed for *ortho*-coupled aromatic protons at 7.25 ppm to the benzylic protons at 3.93 ppm, and the proton signal at 4.31 ppm to the C-atom at 70.84 ppm (*Fig. 1*). The high-resolution fast-atom-bombardment MS (HRFABMS) of **6** gave a molecular-ion peak at *m/z* 442.1630 and fragments with significant intensities at *m/z* 93 $[C_6H_4O+H]^+$ and 307 $[C_{12}O_9H_{19}]^+$. Therefore, the structure of **6** is assigned as 4-[(β-D-glucopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)]phenylacetonitrile.

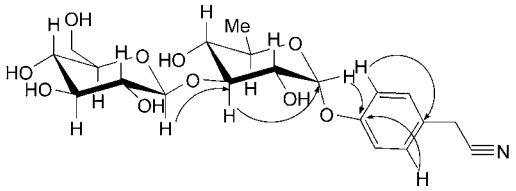


Fig. 1. *Selected HMBC (→) correlations in* **6**

Purification of fraction *D* by preparative HPLC yielded **2** (R_t = 49.2 min). The IR spectrum of **2**, recorded in KBr, show frequencies at 3379, 2932, 1736, 1511, 1230, and 1021 cm^{-1} and were assigned to O–H (stretch), C–H (aromatic, stretch), C=O (stretch), C–C (aromatic, bend), C–O–C (stretch, asymmetric), and C–O–C (stretch, symmetric) vibrations, respectively. The UV spectrum of **2** exhibit maxima at 310 and 360 nm. A weakly coupled *doublet* at 5.39 ppm (J = 2 Hz) in the ^{1}H-NMR spectrum correlates to the C-atom signal at 100.05 ppm, as confirmed by HMQC experiments. Another *doublet* was assigned to the Me group of an α-rhamnose moiety. The HMBC correlations for protons at 3.58 and 5.39 ppm to the C-atom signals at 131.39 and 117.67 ppm, respectively, along with *ortho*-coupled protons at 7.0 and 8.5 ppm, indicate *para* substitution in the molecule. The *singlets* at 3.58 and 3.66 ppm, integrated for two and three protons and correlated (by HMQC) to C-atom signals at 40.89 and 52.39, respectively, were assigned to a CH_2 and Me group in the molecule. Two *doublets* at 8.5 and 7.0 ppm, integrated for two protons each, were assigned to two *ortho*-coupled aromatic protons in the molecule. The ^{13}C-NMR spectrum of **2** showed signals for six aromatic C-atoms, one carbonyl C-atom, one anomeric C-atom (100.05 ppm) and four signals between 70 and 74 ppm. The DEPT spectrum confirmed that the signals at 157.01 and 129.36 ppm correspond to substituted aromatic C-atoms. Further, these C-atoms did not correlate to any protons in the molecule by HMQC. The HRFABMS of **2** gave a molecular-ion peak at *m/z* 313.1288. The MS fragments observed for **2** are at *m/z* 147.12 ($C_6O_4H_{11}$), 166.12 ($[M+H]^+ - 147$), 239.19 ($C_{12}O_5H_{15}$). Based on the spectral evidence, the structure of **2** is confirmed as methyl 2-[4-(α-L-rhamnopyranosyl)phenyl]acetate.

HPLC Purification of fraction *D* yielded **3** (R_t = 35 min) as a pale yellow amorphous powder. The IR spectrum, recorded in KBr, showed several characteristic bands at 3410.1 (NH), 1698.7 (C=S), and 1612.9 (N–H bend) cm^{-1}, indicating the presence of a thiocarbamide linkage in the molecule. The UV spectrum of the compound showed a strong absorption maximum at 285 nm. The ^{1}H-NMR spectrum of **3** showed two signals at 1.11 and 3.70 ppm, integrating for three and two protons, respectively, which were assigned to a Me and $-CH_2$ in the rhamnose and glucose moieties, respectively. Two *doublets* at 6.99 and 7.23 ppm, both integrated for two protons each, suggested the presence of a *para*-substituted aromatic moiety in **3**. Six distinct peaks that exchanged with D_2O were assigned to OH and NH protons. The *doublets* at 5.32 (J = 6.5 Hz) and 4.32 (J = 2.0 Hz) ppm were indicative of two anomeric protons in the molecule. The ^{13}C-NMR spectrum of **3** gave two signals at 99.2 and 110.8 ppm correlated to proton signals at 5.32 and 4.32 ppm, respectively, in HMQC that were assigned to anomeric C-atoms. The analysis of the DEPT spectrum showed the presence of four protonated and

two quaternary C-atoms in the aromatic region. The anomeric proton at 5.32 was also correlated to the quaternary C-atom at 155.75 ppm in the HMBC spectrum. The HMBC correlations observed (*Fig. 2*) in **3** confirmed that the rhamnose moiety is attached to C(1). The NH proton that appears as a *triplet* at 4.33 ppm disappears after shaking with D_2O. Similarly, a *doublet* at 4.03 ppm, assigned to the CH_2 adjacent to the NH, becomes a *singlet* after shaking with D_2O. The signal at 157.71 ppm, assigned to C=S, is correlated to the benzylic and methine protons at 4.03 and 4.32 ppm, respectively, in the HMBC spectrum. This confirmed the position of the glucose moiety as shown in **3** (*Fig. 2*). The HRFABMS of **3** gave a molecular ion at *m*/*z* 492.1505 in addition to fragment peaks at *m*/*z* 93.0, 185.2, and 369.0. Based on the fragmentation pattern and NMR spectral experiments, the structure of **3** is confirmed as *N*-[(4-*β*-L-rhamnopyranosyl)benzyl]-1-*O*-*α*-D-glucopyranosyl-thiocarboxamide.

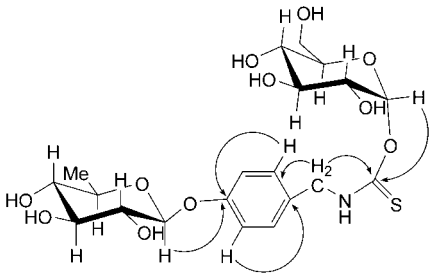
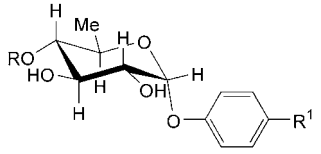


Fig. 2. *Selected HMBC* (→) *correlations in* **3**

Fraction *C* was purified by HPLC and yielded **4**. Spectral studies reveal that **4** is niazicin A (= methyl *N*-{4-[(4′-*O*-acetyl-*α*-L-rhamnopyranosyl)benzyl]}thiocarbamate) [6]. A fraction at 50.4 min was also collected during the purification of **4** and further purification of it by TLC yielded **5**. The UV spectrum of **5** shows only one peak at 330 nm. The ^{1}H-NMR of **5** gave signals for a rhamnose moiety in the molecule in addition to aromatic signals observed. The ^{13}C-NMR of compound **5** showed the presence of four low-field signals at 98.5, 154.84, 128.46, and 116.18 ppm. The signal at 98.5 ppm was assigned to an anomeric C-atom since it was correlated to the anomeric proton at 5.30 ppm, as confirmed by its HMQC spectrum. The DEPT spectrum of **5** showed the presence of one substituted and two unsubstituted C-atoms in the aromatic region. In the HMBC spectrum, the anomeric proton was correlated to the quaternary C-atom at 154.84 ppm. The anomeric proton at 5.30 ppm, along with the 3-H *doublet* at 1.08 ppm, confirmed the presence of the *α*-rhamnose moiety in **5**. The molecular ion observed in its HRFABMS at *m*/*z* 241.1074 further confirms the structure of compound **5** as 1-*O*-phenyl *α*-L-rhamnopyranoside.



	R	R^1
5	H	H
7	H	7 8 9 10 CH_2-NH-CO-OMe
8	CO-Me	CH_2-NH-CO-OMe

The structures of compounds **7** and **8** were established as methyl *N*-{4-[(*α*-L-rhamnopyranosyl)benzyl]}carbamate and methyl *N*-{4-[(4′-*O*-acetyl-*α*-L-rhamnopyra-

nosyl)benzyl]}carbamate by spectral studies and comparison of the spectral data to the reported spectral values [6][16].

Biology. Purified compounds **1**–**8** from *M. oleifera* were tested for their efficacy in stimulating acute insulin release from the pancreatic β-cell line, INS-1. Incubation of INS-1 cells in 4.0 mM glucose for 1 h led to the release of 4 ng insulin/mg of protein. The addition of 100 ppm of **1** or **3** did not significantly affect insulin release (4 to 6 ng insulin/mg protein, *Fig. 3*). In contrast, 100 ppm of **4**, **5**, **6, 7**, or **8** stimulated the release of 15–33 ng insulin/mg of protein. Of particular interest, compounds **5** and **7** led to the most-significant release of insulin at *ca.* 30 ng/mg protein. The ability of these compounds to stimulate insulin release and their overall yield (1.63%) in the dried fruit indicates that *M. oleifera* might be an excellent source of insulin secretagogues. A relatively high proportion of type-2 diabetese patients can regulate their blood glucose concentrations through the use of agents such as sulfonylureas that stimulate insulin release.

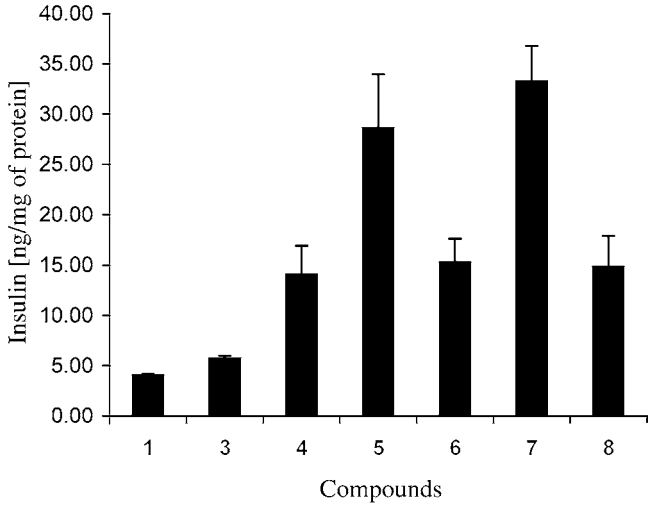


Fig. 3. *Effects of* **1** and **3**–**8** *on insulin release by INS-1 cells.* Static insulin release was determined by incubating cells for 20 min at 37° in KRB buffer containing 4.0 mM glucose and 0.05% DMSO or 100 ppm of the indicated compounds. Insulin released into the medium was determined as described in the *Exper. Part*. Data points represent the mean ± std. dev. ($n=4$).

The conversion of arachidonic acid to prostaglandins, mediated by cyclooxygenase enzymes, is a prerequisite for any inflammation stimulus. In the present investigation, *M. oleifera* compounds were analyzed for their inhibitory effect on COX enzymes. The inhibitory effect of *M. oleifera* compounds on COX enzymes were determined by measuring the O_2 uptake during the endoperoxide formation by arachidonic acid and COX enzymes. Compounds **4** and **6** inhibited the COX-2 enzyme by 20 and 50%, respectively. The COX-1 and COX-2 inhibitory activities of *M. oleifera* compounds tested at 83 ppm are shown in *Fig. 4*. Compounds **1** and **2** did not inhibit COX-1 and COX-2 enzymes at the concentrations tested.

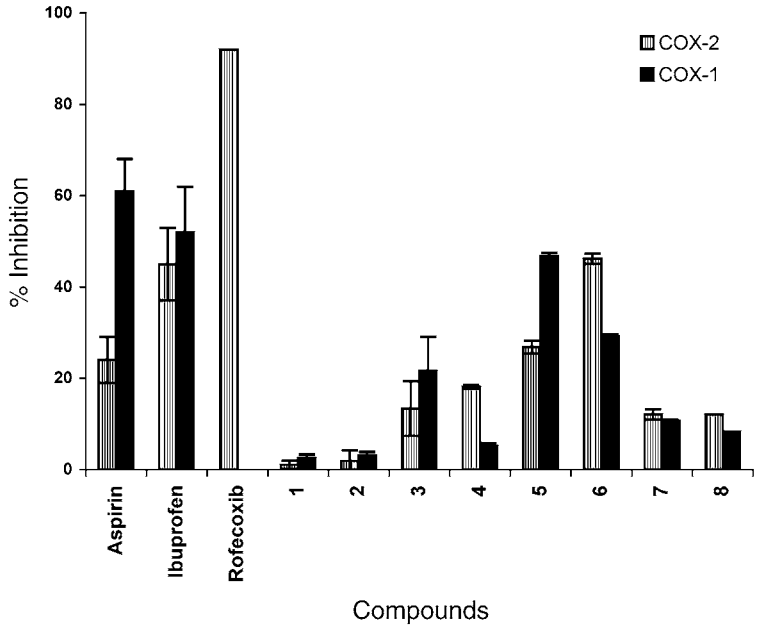


Fig. 4. *COX-1 and COX-2 Enzymes: inhibitory activity of* **1**–**8** *isolated from fruits of* M. oleifera *along with commercial NSAIDs rofecoxib* (*Vioxx*), *aspirin, and ibuprofen* (racemate) at 1.67, 180 and 2.06 ppm, resp. The isolated compounds were all tested at 83 ppm. The vertical bars represent the std. dev. for each data point ($n=2$).

The results of a lipid-peroxidation-inhibition assay of **1**–**8** at 50 ppm, as measured by fluorescence spectroscopy, are shown in *Fig. 5*. Compounds **4** and **6** inhibited lipid peroxidation by 80 and 95% compared to the solvent (DMSO) control. This activity was comparable to the antioxidant standards tested at their respective concentrations (*Fig. 5*). Compounds **3** and **8**, at 50 ppm, inhibited the peroxidation reaction by 35%. In contrast, 50 ppm of **1**, **2**, **5**, and **7** did not affect the peroxidation reaction. In a dose–response study, 1 ppm of **6** inhibited lipid peroxidation by 14%, whereas 1 ppm of **4** showed little or no activity. It was clear that **4** and **6** were better lipid-peroxidation inhibitors than the other *M. oleifera* compounds tested in our laboratory. It was strange, however, that **1** did not exhibit lipid-peroxidation inhibition, since **1** and **6** contain identical aglycones. Compound **1** is less polar than **6**, and, hence, its solubility in the assay buffer could be a factor for its low antioxidant activity. Similarly, **3** showed little or no activity compared to **4**, which could be attributed to the bulkier sugar group attached to the thiocarbamate aglycone in **3**.

Antiproliferative activity of **1**–**8** was evaluated in human breast (MCF-7), central nervous system (CNS, SF-268), lung (NCI-H460), and colon (HCT-116) tumor cell lines [17]. Interestingly, **1**–**8** at 30 ppm did not inhibit the cell growth in any of the cancer cell lines tested.

Diabetes affects children and adults in alarming proportions. The WHO estimates that *ca.* 150 million people around the world are suffering from this disease. Type-2 diabetes has reached epidemic proportions in the US. With its present rate of increase,

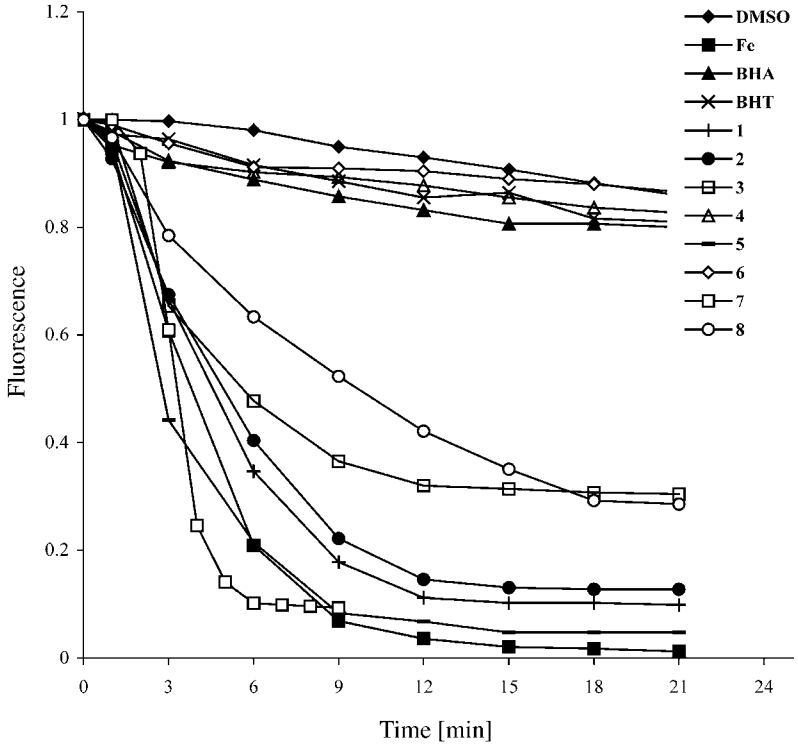


Fig. 5. *Lipid-peroxidation-inhibitory activity of* **1**–**8** (50 ppm). Commercial antioxidants BHA, BHT, and TBHQ were tested at 1.66, 2.2, and 1.8 ppm, resp. The maintenance of fluorescence with time is indicative of the inhibition of peroxidation initiated by Fe^{2+}. The average relative fluorescence was calculated from duplicate experiments by dividing the fluorescence value at a given time by that at $t = 0$ min

diabetes is poised to become one of the world's major diseases affecting public healthcare [18][19]. The prevalence of type-2 diabetes is on the rise in children as well. The ability of *M. oleifera* compounds, which have little or no cytotoxic effects, to induce insulin secretion in INS-1 cells, supports the anecdotal claims that this plant might ameliorate diabetes. For the compounds to be useful for increasing insulin release, the mode of action needs to be defined. It will be of particular interest to determine whether these compounds can potentiate glucose-induced insulin release.

Experimental Part

General. Silica gel (particle size 40–63 μm) was obtained from *Fischer Scientific* (Pittsburgh, PA) and silica-gel TLC plates (20 × 20 cm; 250, 500 and 100 μm thickness) were acquired from *Analtech Inc.* (Newark, DE). All org. solvents and standards used were ACS reagent grade, and solvents were purchased from *Spectrum Laboratory Products, Inc.* (New Brunswick, NJ). Compounds were detected at 210 and 254 nm with a *JA1 UV-310* detector. Preparative HPLC was performed on a recycling preparative *Japan* Analytical Industry Co. LC-20 system with a *Jaigel* C_{18} column (10 μm, 20 × 250 mm; MeCN/H_2O; appropriate flow rates). UV/VIS Spectra: *Shimadzu UV-260* spectrometer; MeOH soln.; λ_{max} in nm. IR Spectra: *Mattson Galaxy* FT-IR spectrometer; KBr pellet; ν in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian VRX-500* spectrometer; ^1H-NMR spectra at 500 MHz,

^{13}C-NMR spectra at 125 MHz, in (D_6)DMSO, unless otherwise indicated; chemical shifts δ in ppm with respect to the residual solvent signal of (D_6)DMSO or CD_3OD; J in Hz; HMBC was optimized for $J = 8.0$ Hz. GC-MS: *HP 6890* system equipped with an electron-capture detector operating at 250°, *HP-5MS* (30 m × 250 μ, × 0.25 μm) column and a *7673* model injector operating at 250° in splitless mode. FAB-MS: *JEOL HX-110* double-focusing mass spectrometer (positive ionization mode); *m*/*z*.

Preparation of Standards. Both aspirin and ibuprofen (racemate) were purchased from *Sigma* (St. Louis, MO). Aspirin (5.4 mg) was dissolved in DMSO (500 μl), and 10 μl of this stock soln. was added to 590 μl of assay buffer to give 180 ppm. Similarly, ibuprofen (6.19 mg) was dissolved in DMSO (500 μl), and the resulting soln. was diluted 100-fold in DMSO to yield a stock soln. An aliquot of 10 μl of the stock soln. was added to 590 μl assay buffer to give 2.06 ppm. The nonsteroidal anti-inflammatory drug (NSAID), rofecoxib was prepared from a physician's sample of *Vioxx* (supplied by Dr. *S. Gupta*, Sparrow Hospital Pain Center, MI); each *Vioxx* tablet contained 50 mg of rofecoxib. One Vioxx tablet (400.8 mg) was ground to a fine powder with a mortar and pestle, 1.6 mg of powder was dissolved in DMSO (2 ml), and an aliquot of 10 μl was added to the assay chamber (600 μl). The final concentration of rofecoxib in the assay was 1.67 ppm.

Plant Material. Dried and milled powder of *M. oleifera* fruits was purchased from *Phytomyco Research Co.*, Greensville, NC on November 12, 2001. The powder was kept at −20° before extraction.

Isolation of Compounds **1**–**8**. The dried and milled fruits of *M. oleifera* (700 g) were extracted sequentially with hexane (3 × 2 l), EtOAc (3 × 2 l), and MeOH (3 × 2 l) by percolating the dry powder with solvents (8 h for each extraction). The extracts were combined for each solvent and evaporated under vacuum. The resulting yields of extracts were 3.1, 0.4, and 20.8%, resp., for hexane, EtOAc, and MeOH.

The hexane and EtOAc extracts were not further investigated. The MeOH (3 g) extract was fractionated by MPLC on silica gel under gradient conditions (MeOH/$CHCl_3$ 1:1 to 100% MeOH) at a flow rate of 4 ml/min. Fractions 1–5, 120 ml each, were collected with MeOH/$CHCl_3$ 1:1; fractions 6–9, 90 ml each, were collected with MeOH/$CHCl_3$ 3:1; fractions 10–12, 120 ml each, were eluted with MeOH/$CHCl_3$ 3:1. The column was then eluted with 100% MeOH and fractions 13–15, 120 ml each, 16–20, 60 ml each, 21–28, 90 ml each, and 29–30, 150 ml each were collected. The fractions were analyzed by TLC with $CHCl_3$/AcOH/MeOH/H_2O 64:32:12:8 as the mobile phase and pooled to yield fractions *A* (162 mg), *B* (872 mg), *C* (610 mg), *D* (467 mg), *E* (444 mg), *F* (49.5 mg), and *G* (57.9 mg). These fractions induced insulin secretion or inhibited lipid peroxidation and COX enzymes and were then purified by prep. HPLC with UV and RI detection.

HPLC Analysis of fraction *G* showed the presence of three major components. Purification of fraction *G* (58 mg) by HPLC with MeCN/H_2O 2:98 at 4 ml/min, yielded a major fraction at 38 min. The solvent front contained a peak and was collected. Repeated HPLC purification of the fraction at 38 min, eluting with MeCN/H_2O 25:75 at 2 ml/min; yielded pure **1** (3.5 mg, $R_t = 36.1$ min) as light-yellow needle-like crystals. The component in the solvent front was purified by prep. TLC on silica gel with $CHCl_3$/AcOH/MeOH/H_2O 64:32:12:8. The band at $R_f = 0.10$ was collected and eluted with MeOH (37 mg). Final purification of this band, carried out by prep. HPLC with MeCN/H_2O 25:75, yielded **6** ($R_t = 19.7$ min, 11 mg). Similarly, prep. HPLC of fraction *D* (465 mg) with MeCN:H_2O 25:75 at 2.5 ml/min yielded fractions at 26, 35, and 49.2 min. The fraction at 49.2 min was further purified by repeated HPLC to yield **2** (22 mg) as an amorphous powder. The fraction at 35 min was purified by HPLC MeCN/H_2O 2:98 at 3.5 ml/min under isocratic conditions. A fraction collected at 35 min, further purified at 2 ml/min with the same mobile phase, yielded **3** (14.1 mg). For fraction *C* (608 mg), MeCN/H_2O 5:95 at 3 ml/min was used and yielded two fractions at 50.4 and 62 min. Further purification of the fraction at 62 min by repeated HPLC yielded **4** (9 mg, $R_t = 62$ min). Purification of the fraction at 50.4 min by repeated TLC ($CHCl_3$/AcOH/MeOH/H_2O 64:32:12:8 yielded **5** (120 mg). Also, HPLC purification of fraction *B* with MeCN/H_2O 5:95 at 4 ml/min gave **7** (8 mg) and **8** (2 mg), at 80 and 85 min, resp. The yields of **1**–**8** were 0.02, 0.13, 0.15, 0.05, 1.2, 0.07, 0.008, 0.002%, resp., with respect to the dry weight of the fruit powder.

Data of **2**. Colorless, amorphous powder. ^{1}H-NMR (CD_3OD)[1]): 1.22 (*d*, *J* = 6.0, Me); 3.45 (*dd*, *J* = 9.5, 9.5, H−C(4′)); 3.64 (*dd*, *J* = 3.5, H−C(2′)); 3.83 (*dd*, *J* = 9.5, 3.5, H−C(3′)); 3.99 (*dq*, *J* = 9.0, 6.0, H−C(5′)); 3.58 (*s*, CH_2(7)); 3.66 (*s*, MeO); 5.39 (*d*, *J* = 2.0, H−C(1′)); 7.0 (*d*, *J* = 9.5, H−C(2), H−C(6)); 8.5 (*d*, *J* = 9.5, H−C(3), H−C(5)). ^{13}C-NMR[1]): 17.98 (C(6′)); 40.89 (C(7)); 52.39 (C(9)); 70.63 (C(5′)); 73.92 (C(4′)); 72.11 (C(3′)); 72.34 (C(2′)); 100.05 (C(1′)); 117.67 (C(2), C(6)); 129.36 (C(4)); 131.39 (C(3), C(5)); 157.01 (C(1)); 174.19 (C(8)). HRFABMS: 313.1288 ($[M+H]^+$, $C_{15}H_{20}O_7$; calc. 313.1309). FABMS: 147.12, 166.12, 239.19.

Data of **3**. Colorless, amorphous powder. ^{1}H-NMR[1]): 1.11 (*d*, *J* = 6.12, Me-6′); 2.96 (*m*); 3.03 (*m*, H−C(5′)); 3.1 (*dd*, *J* = 9.5, 9.5, H−C(4′)); 3.25 (*dd*, *J* = 9.4, 9.3, H−C(3″)); 3.40 (*dd*, *J* = 10.5, 6.5, H−C(2′)); 3.47 (*m*, H−C(5′)); 3.62 (*ddd*, *J* = 9.4, 3.4, 3.4, H−C(5″)); 3.70 (*dd*, *J* = 11.5, 3.5, H_a−C(6″)); 3.80

[1]) Arbitrary numbering.

(*m*, H–C(2″)); 3.90 (*dd*, *J* = 11.5, 3.5, H_b–C(6″)); 4.03 (*d*, *J* = 16, H–C(7)); 4.32 (*d*, *J* = 2.0, H–C(1′)); 4.33 (*t*, *J* = 10, H–C(8)); 4.63 (br. *s*, HO–C(3″)); 4.79 (br. *s*, HO–C(6″), HO–C(2″)); 4.99 (br. *s*, HO–C(3′), HO–C(2′)); 5.10 (br. *s*, HO–C(4″)); 5.4 (br. *s*, HO–C(4′)); 5.32 (*d*, *J* = 6.5, H–C(1′)); 6.98 (*d*, *J* = 8.5, H–C(2), H–C(6)); 7.21 (*d*, *J* = 8.5, H–C(3), H–C(5)). ^{13}C-NMR[1]): 15.59 (C(6′)); 37.74 (C(7)); 61.66 (C(6″)); 70.17 (C(5′)); 70.46 (C(5″)); 70.94 (C(2″)); 71.16 (C(2′)); 72.52 (C(3′)); 73.0 (C(3″)); 78.73 (C(4′)); 81.93 (C(4″)); 99.22 (C(1′)); 110.8 (C(1″)); 117.24 (C(2), C(6)); 129.83 (C(3), C(5)); 130.50 (C(4)); 155.71 (C(9)); 155.75 (C(1)). HRFABMS: 492.1505 ($[M+H]^+$, $C_{20}H_{29}O_{11}NS$; calc. 492.1461), FABMS: 369.2 ($[M+H]^+ -$ 7 OH), 93 (C_6H_4O).

Data of **5**. Colorless, amorphous powder. ^{1}H-NMR[1]): 1.08 (*d*, *J* = 6.0, Me); 3.25 (*dd*, *J* = 9.0, 9.0, H–C(4′)); 3.45 (*dd*, *J* = 9.0, 6.0, H–C(5′)); 3.61 (*dd*, *J* = 5.0, 2.0 H–C(2)); 3.80 (br. *s*, H–C(3′)); 5.30 (*d*, *J* = 2.0, H–C(1′)); 6.94 (*d*, *J* = 7.0, H–C(2), H–C(6)); 7.21 (*m*, H–C(3), H–C(4), H–C(5)). ^{13}C-NMR[1]): 17.84 (C(6′)); 69.36 (C(5′)); 70.17 (C(3′)); 70.42 (C(2′)); 71.80 (C(4′)); 98.5 (C(1′)); 116.18 (C(2), C(4), C(6)); 128.46 (C(3), C(5)); 154.84 (C(1)). HRFABMS: 241.1074 ($[M+H]^+$, $C_{12}H_{16}O_5$; calc. 240.0998). FABMS: 77.0 (C_6H_5), 163.10 ($[M - C_6H_5]$).

Data of **6**. Colorless, amorphous powder. ^{1}H-NMR[1]): 1.08 (*d*, *J* = 6.25, Me); 3.25 (*dd*, *J* = 9.4, 9.4, H–C(4′)); 3.44 (*m*, H–C(5′)); 3.62 (*dd*, *J* = 9.4, 2.5, H–C(3′)); 3.81 (br. *s*, H–C(2′), H–C(2″)); 3.94 (*s*, CH_2(7)); 5.35 (*d*, *J* = 1.7, H–C(1′)); 7.04 (*d*, *J* = 8.7, H–C(2), H–C(6)); 7.26 (*d*, *J* = 8.7, H–C(3), H–C(5)); 5.01 (*d*, *J* = 3.30, HO–C(2′)); 4.98 (br. *s*, HO–C(2″)); 4.91 (br. *s*, H–C(3′), HO–C(3″)); 4.8 (br. *s*, HO–C(4″)); 3.16 (*m*, H–C(4″)); 3.43 (*m*, H–C(5″)); 3.61 (br. *s*, CH_2(6″)); 3.66 (*dd*, *J* = 9.4, 3.0, H–C(3″)); 4.31 (*d*, *J* = 9.0, H–C(1″)). ^{13}C-NMR[1]): 18.57 (C(6′)); 22.27 (C(7)); 61.82 (C(6″)); 70.19 (C(5′)); 70.31 (C(5″)); 70.84 (C(3′)); 71.1 (C(3″)); 72.45 (C(2″)); 72.46 (C(2′)); 78.71 (C(4″)); 82.0 (C(4′)); 90.73 (C(1″)); 99.09 (C(1′)); 117.58 (C(2), C(6)); 120.05 (C(4)); 125.08 (C(8)); 130.0 (C(3), C(5)); 156.16 (C(1)). HRFABMS: 442.1630 ($[M+H]^+$, $C_{20}H_{27}O_{10}N$; calc. 442.1635). FABMS: 93, 215, 307.

Insulin-Secretion Assay. INS-1 Cells used in the assay were rat pancreatic β-cells derived from the parental RINm5f lines [20]. Cells were grown at 37° under 5% CO_2 in a humidified incubator with RPMI 1640 medium containing 11.1 mM glucose supplemented with 10% fetal bovine serum (FBS), 1 mM pyruvate, 10 mM HEPES, 50 mM 2-mercaptoethanol, and 100 U penicillin/ml and were passaged every week by detaching with trypsin EDTA. Cells (0.75×10^6) were plated in 24 well plates, incubated for 36 h, and the growth medium was then replaced with RPMI-1640 containing 4 mM glucose plus supplements and incubated for another 24 h. Cells were then incubated for 60 min at 37° in *Krebs–Ringer* bicarbonate (KRB) buffer (118.5 mM NaCl, 2.54 mM $CaCl_2$, 1.19 mM KH_2PO_4, 4.74 mM KCl, 25 mM $NaHCO_3$, 1.19 mM $MgCl_2$, 10 mM HEPES, pH 7.4, 0.1% bovine serum albumin) containing 4.0 mM glucose. Cells were then incubated for 20 min at 37° in KRB buffer containing 4 mM glucose, 100 mM isobutylmethylzanthine (IBMX), and vehicle or extract or 100 ppm compounds **1**–**8**. Concentrations of insulin released into the media were determined by insulin enzyme-linked immunosorbent assay by a modification of the procedure of *Kekow et al.* [21]. Insulin released into the medium was normalized to cellular protein concentrations determined by *Lowry* assay.

Cyclooxygenase Enzyme Inhibitory Assay. COX-1 Activity was recorded with an enzyme preparation from ram seminal vesicles and COX-2 activity with a preparation of human prostaglandin H synthase isozyme 2 (hPGHS-2) cloned in insect cells. COX Assays were performed by monitoring the initial rate of O_2 uptake in an *Instech* micro chamber with an O_2 electrode attached to a *YSI* model *5300* biological O_2 monitor as reported earlier [22][23]. Each assay mixture contained 12 ml 0.1M Tris/1 mM phenol buffer (pH 7) and 340 mg hemoglobin. The test samples and the enzyme (20 µl) were allowed to incubate for 10 min before the addition of 10 µl arachidonic acid soln. (0.25 mg/0.5 ml Tris buffer). Data was recorded with Quicklog for Windows data acquisition and control software (*Strawberry Tree Inc.*, Sunnyvale, CA). All extracts and fractions were tested at 250 ppm, whereas the pure compounds were tested at 83 ppm. For comparison purposes, the activities of commercial NSAIDs rofecoxib (*Vioxx*) (1.67 ppm), aspirin (180 ppm), and ibuprofen (2.06 ppm) were also carried out.

Antioxidant Activity. This assay was conducted by recording the fluorescence at 384 nm of model liposome system. The lipid, 1-stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine and the fluorescent probe 3-[4-(6-phenylhexa-1,3,5-trienyl)phenyl]propanoic acid were mixed in DMF and evaporated to dryness. Large unilamellar vesicles (LUVs) were prepared by resuspending the lipid-probe mixture in MBSE buffer (=0.15M NaCl, 0.1 mM EDTA, and 0.01M MOPS passed over *Chelex* resin) followed by ten freeze-thaw cycles in a dry ice/EtOH bath and extrusion through a membrane (pore size 100 nm) [22][23]. The assay volume was brought to 2 ml by adding 100 µl HEPES buffer, 200 µl 1M NaCl, 1.64 ml N_2-sparged water, 20 µl of test sample in DMSO or DMSO (solvent control), and the 20 µl aliquot of liposome suspension. Peroxidation, initiated by the addition of 20 µl $FeCl_2 \cdot 4\ H_2O$ (0.5 mM), was monitored by observing the fluorescence at 0, 1, 3, and every 3 min. thereafter

up to 21 min. The decrease in relative fluorescence intensity with time indicated the rate of peroxidation. All compounds were tested at 50 ppm. The inhibition of lipid peroxidation was also recorded for commercial antioxidants butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and *tert*-butyl hydroquinone (TBHQ) at 1.66, 2.2, and 1.8 ppm, respectively.

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