**New antiinflammatory sucrose esters in the natural sticky coating of tomatillo
(*Physalis philadelphica*), an important culinary fruit**

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ABSTRACT

Tomatillo is a popular culinary fruit. The sticky material on its surface, consumed as part of the fruit, has never been investigated. Chemical characterization of sticky material on tomatillo fruits yielded five new sucrose esters, as confirmed by spectroscopic methods. The solvent extract of the sticky material from the whole fresh fruit and pure isolates showed antiinflammatory activity as confirmed by in vitro cyclooxygenase enzymes inhibitory assays. Five sucrose esters isolated at 100 $\mu\text{g/mL}$ ((153.8, 138.8, 136.2, 141.6 and 138.8 μM), respectively inhibited cyclooxygenase-1 and -2 enzymes by 50%. The cyclooxygenase enzyme inhibitory activity of extract and isolates at 100 $\mu\text{g/mL}$ was similar to non-steroidal antiinflammatory drugs aspirin, ibuprofen and naproxen, used as positive controls in the assay at 108, 12 and 15 $\mu\text{g/mL}$ (600, 60 and 60 μM), respectively.

Keywords: Tomatillo, sucrose esters, cyclooxygenase enzymes inhibitor, inflammation, functional food

1. Introduction.

The plant *Physalis philadelphica* Lam. (Solanaceae), formerly known as *P. ixocarpa*, is an annual originated in Mexico and grown widely in many countries as one of the vegetable crops. Its fruits, known as tomatillos or husk tomatoes, are green and sour when mature and used in the preparation of many sauces, salsas, enchiladas and as a culinary ingredient. Tomatillo has also been used in traditional medicine to relieve fever, cough, and amygdalitis (Maldonado, Pérez-Castorena, Garcés, & Martínez, 2011). Previous studies on leaves, stems and fruits of *P. philadelphica* afforded a series of withanolides with quinone reductase induction, cytotoxic, antiproliferative and apoptotic activities (Choi, Murillo, Su, Pezzuto, Kinghorn, & Mehta, 2006; Gu et al., 2003; Maldonado et al., 2011; Su et al., 2002; Kennelly et al., 1997). Several ceramides with quinone reductase induction activity were also isolated from leaves and stems of *P. philadelphica* (Su et al., 2002). Tomatillo fruit at harvest is covered by paper-like husk. A sticky material under this husk, not washable by water, covers the entire surface of the fruit. It is therefore automatically integrated in foods prepared from it and interestingly has never been investigated before.

The sticky exudates are found on the aerial surface or trichome of many plants of Solanaceae family and among genus *Lycopersicon*, *Nicotiana*, *Petunia* and *Solanum*. This exudate is believed to protect the plants and fruits from insect and fungus. Glucose and sucrose esters were characterized as the primary compounds present in the trichome or exudate of *Lycopersicon* (King, Calhoun, Singh, & Boucher, 1993), *Nicotiana* (Buta, Lusby, Neal, Waters, & Pittarelli, 1993; Chortyk, Severson, Culter, & Sisson, 1993; Neal, Bute, Pittarelli, Lusby, & Bentz, 1994), *Petunia* (Chortyk, Stanley, & Teng, 1997; Moser, Klaiber, Vogler, & Kraus, 1999) and *Solanum* (King,

Singh, & Calhoun, 1988) species. In this study, five new sucrose esters were isolated from the extract yielded by dipping the whole tomatillo fruit in dichloromethane (CH_2Cl_2) for about a minute. After confirming the chemical identity by NMR, LC-MS and GC-MS methods, the pure isolates were evaluated for antiinflammatory activity using in vitro cyclooxygenase enzymes (COX-1 and -2) inhibitory assays (Kelm, Nair, Strasburg, & DeWitt, 2000; Zhang, Dissanayake, Kevseroğlu, & Nair, 2015).

2. Materials and methods

2.1. General Experimental Procedures

All solvents used for isolation and purification were of ACS reagent grade (Sigma-Aldrich Chemical Company, St. Louis, MO, USA). Merck silica gel (60 mesh size, 35–70 μm , EMD Chemicals, Inc., Gibbstown, NJ, USA) was used for vacuum liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC). 250 and 500 μm silica gel plates (Analtech, Inc., Newark, DE, USA) were used for thin-layer chromatography (TLC). ESIMS spectra were recorded on a Waters Xevo G2-S Q-TOF LC mass spectrometer (Waters Corporation, Milford, MA, USA). GCMS spectra were recorded on an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). NMR spectra were recorded on Agilent DirectDrive2 500 MHz instruments. Aspirin, naproxen and ibuprofen were purchased from Sigma-Aldrich Chemical Company. The nonsteroidal antiinflammatory drug (NSAID) Celebrex[®] (Celecoxib capsules 200 mg, manufactured by Pfizer, Inc., New York, USA) was physician's professional sample. Cyclooxygenase (COX-1 and -2) enzymes were prepared in our laboratory from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford Biomedical Research, Inc., Oxford, MI, USA) and insect cells cloned with human PGHS-2 enzyme, respectively.

Arachidonic acid was purchased from Oxford Biomedical Research, Inc. All enzymes and reagents were stored in the Bioactive Natural Products and Phytochemicals Laboratory at Michigan State University (East Lansing, MI, USA). COX assays were performed in a micro oxygen chamber with oxygen electrode (Instech Laboratories, Plymouth Meeting, PA, USA) attached to an YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH, USA).

2.2. Extraction and isolation

The tomatillo fruits were purchased in December 2014 from a local vegetable market in Lansing, Michigan, USA. After removing the inedible husk, the sticky tomatillo fruits (6.075 kg) were dipped in CH₂Cl₂ kept in a glass beaker (about a minute for each fruit). The sticky material on the surface of the fruit was readily soluble in CH₂Cl₂. Evaporation of solvent under vacuum afforded a gummy extract TC (3.78 g).

An aliquot of the extract TC (2.2 g) was fractionated by silica gel vacuum liquid chromatography (VLC) and eluted under gradient conditions using hexane-acetone (5:1, 1:1, v/v) and then CHCl₃-MeOH (10:1, 4:1, 1:1, v/v) to yield fractions A (251 mg, chlorophyll), B (1027 mg) and C (918 mg). Fr. C was separated by silica gel medium pressure liquid chromatography (MPLC) and eluted with CHCl₃-MeOH (10:1, 5:1, 3:1, 1:1, v/v) to yield fractions C1 (601 mg), C2 (183 mg) and C3 (126 mg). Fr. C3 (30 mg) was purified by preparative TLC using CHCl₃-MeOH (5:1, v/v) as the mobile phase and afforded compound **1** (25 mg). Fr. C2 (32 mg) was analyzed by TLC using CHCl₃-MeOH (8:1, v/v) as the mobile phase and showed two spots. It was then purified by preparative HPLC using isocratic MeOH-H₂O (80:20, v/v) system to afford compounds **2** (15 mg) and **3** (11 mg), respectively (**Figure 1A**). Fractions B and C1 were combined based on

identical TLC profiles to get fraction D and an aliquot (40 mg) of fraction D was purified by repeated preparative HPLC using MeOH-H₂O (85:15, v/v) and then MeOH-H₂O (80:20, v/v) under isocratic system to afford compounds **4** (21 mg) and **5** (10 mg) (Figure 1B).

2.2.1. Compound 1

Colorless gum; ¹H NMR (500 MHz, CDCl₃): δ 5.55 (1H, d, *J* = 3.2 Hz, H-1), 5.40 (1H, dd, *J* = 10.0, 9.3 Hz, H-3), 4.86 (1H, dd, *J* = 10.0, 3.2 Hz, H-2), 4.33 (1H, m, H-3'), 4.31 (1H, m, H-4'), 4.18 (1H, brs, H-5), 3.99 (1H, m, H-6a), 3.89 (1H, m, H-6'a), 3.84 (1H, m, H-5'), 3.79 (1H, brs, H-6'b), 3.74 (1H, m, H-6b), 3.58 (1H, d, *J* = 9.3 Hz, H-1'a), 3.46 (1H, m, H-4), 3.45 (1H, m, H-1'b), 2.24–2.38 (4H, m, -COCH₂-), 1.52–1.60 (4H, m, -COCH₂-CH₂-), 1.22–1.32 (24H, m, -CH₂-), 0.89 (6H, t, *J* = 6.8 Hz, -CH₃); ¹³C NMR (125 MHz, CDCl₃): δ 174.8 (3-CO-), 173.2 (2-CO-), 103.6 (C-2'), 89.0 (C-1), 81.5 (C-5'), 77.7 (C-3'), 74.2 (C-5), 73.0 (C-4'), 72.5 (C-3), 70.4 (C-2), 69.5 (C-4), 64.0 (C-1'), 62.2 (C-6), 60.5 (C-6'), 34.4, 33.9 (-CO-CH₂-), 31.9 (-CH₂-CH₂-CH₃), 29.2–29.7 (-CH₂-), 25.0, 24.7 (-CO-CH₂-CH₂-), 22.7 (-CH₂-CH₃), 14.1 (-CH₃). HRESIMS: *m/z* 673.3779 ([M + Na]⁺ (calcd for C₃₂H₅₈O₁₃Na, 673.3775).

2.2.2. Compound 2

Colorless gum; ¹H NMR (500 MHz, CDCl₃): δ 5.57 (1H, d, *J* = 3.4 Hz, H-1), 5.38 (1H, brt, *J* = 9.8 Hz, H-3), 4.88 (1H, dd, *J* = 10.2, 3.4 Hz, H-2), 4.37 (1H, dd, *J* = 9.3, 8.8 Hz, H-4'), 4.19 (1H, d, *J* = 8.8 Hz, H-3'), 4.14 (1H, t, *J* = 7.3, 6.8 Hz, H-5), 4.10 (1H, d, *J* = 11.7 Hz, H-1'a), 3.99 (1H, m, H-1'b), 3.95 (1H, m, H-6a), 3.91 (1H, m, H-6'a), 3.80 (1H, m, H-5'), 3.76 (1H, m, H-6'b), 3.75 (1H, m, H-6b), 3.48 (1H, brt, *J* = 9.8 Hz, H-4), 2.60 (1H, m, H-2''), 2.26–2.44 (4H, m, -COCH₂-), 1.54–1.62 (4H, m, -COCH₂-CH₂-), 1.22–1.36 (24H, m, -CH₂-), 1.19, 1.18 (each 3H, d, *J* = 6.9 Hz,

113 Me-3", 4"), 0.89 (6H, t, $J = 6.9$ Hz, $-CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.4 (C-1"), 174.8
114 (3-CO-), 173.3 (2-CO-), 102.4 (C-2'), 89.6 (C-1), 81.5 (C-5'), 77.4 (C-3'), 74.6 (C-5), 72.2 (C-3),
115 71.8 (C-4'), 70.1 (C-4), 69.8 (C-2), 62.7 (C-1'), 62.6 (C-6), 59.5 (C-6'), 34.4, 34.0 ($-CO-CH_2-$),
116 33.9 (C-2"), 31.9 ($-CH_2-CH_2-CH_3$), 29.1–29.5 ($-CH_2-$), 24.9, 24.7 ($-CO-CH_2-CH_2-$), 22.7
117 ($-CH_2-CH_3$), 18.9 (C-3", 4"), 14.1 ($-CH_3$). HRESIMS: m/z 743.4203 ($[M + Na]^+$ (calcd for
118 $C_{36}H_{64}O_{14}Na$, 743.4194).

119 2.2.3. Compound 3

120 Colorless gum; 1H NMR (500 MHz, $CDCl_3$): δ 5.58 (1H, d, $J = 3.6$ Hz, H-1), 5.37 (1H, brt, J
121 $= 9.8$ Hz, H-3), 4.89 (1H, dd, $J = 10.3, 3.6$ Hz, H-2), 4.37 (1H, brt, $J = 8.8$ Hz, H-4'), 4.19 (1H, d, J
122 $= 8.8$ Hz, H-3'), 4.14 (1H, m, H-5), 4.11 (1H, d, $J = 11.7$ Hz, H-1'a), 3.99 (1H, m, H-1'b), 3.96 (1H,
123 m, H-6a), 3.90 (1H, m, H-6'a), 3.79 (1H, m, H-5'), 3.75 (1H, m, H-6'b), 3.74 (1H, m, H-6b), 3.49
124 (1H, dd, $J = 9.8, 9.3$ Hz, H-4), 2.42 (1H, m, H-2"), 2.26–2.44 (4H, m, $-COCH_2-$), 1.69 (1H, m,
125 H-4"a), 1.54–1.62 (4H, m, $-COCH_2-CH_2-$), 1.49 (1H, m, H-4"b), 1.22–1.34 (24H, m, $-CH_2-$), 1.16
126 (3H, d, $J = 7.4$ Hz, Me-3"), 0.92 (3H, t, $J = 7.4$ Hz, Me-5"), 0.89 (6H, t, $J = 7.1$ Hz, $-CH_3$); ^{13}C
127 NMR (125 MHz, $CDCl_3$): δ 176.0 (C-1"), 174.8 (3-CO-), 173.3 (2-CO-), 102.4 (C-2'), 89.6 (C-1),
128 81.4 (C-5'), 77.4 (C-3'), 74.6 (C-5), 72.1 (C-3), 71.8 (C-4'), 70.2 (C-4), 69.7 (C-2), 62.7 (C-1'),
129 62.5 (C-6), 59.5 (C-6'), 40.9 (C-2"), 34.4, 34.0 ($-CO-CH_2-$), 31.9 ($-CH_2-CH_2-CH_3$), 29.1–29.8
130 ($-CH_2-$), 26.7 (C-4"), 24.9, 24.7 ($-CO-CH_2-CH_2-$), 22.7 ($-CH_2-CH_3$), 16.5 (C-3"), 14.1 ($-CH_3$), 11.6
131 (C-5"). HRESIMS: m/z 757.4357 ($[M + Na]^+$ (calcd for $C_{37}H_{66}O_{14}Na$, 757.4350).

132 2.2.4. Compound 4

133 Colorless gum; 1H NMR (500 MHz, $CDCl_3$): δ 5.55 (1H, d, $J = 3.9$ Hz, H-1), 5.24 (1H, d, $J =$

8.3 Hz, H-3'), 5.16 (1H, dd, $J = 10.3, 9.3$ Hz, H-3), 4.94 (1H, dd, $J = 10.3, 3.9$ Hz, H-2), 4.62 (1H, brt, $J = 8.8$ Hz, H-4'), 4.06 (1H, m, H-5), 4.02 (1H, d, $J = 11.8$ Hz, H-1'a), 3.96 (1H, m, H-6a), 3.93 (1H, m, H-5'), 3.93 (1H, m, H-6'a), 3.91 (1H, m, H-1'b), 3.75 (1H, m, H-6b), 3.72 (1H, m, H-6'b), 3.55 (1H, dd, $J = 9.8, 9.3$ Hz, H-4), 2.63 (1H, m, H-2'''), 2.60 (1H, m, H-2''), 2.57 (1H, m, H-2''), 2.26–2.42 (2H, m, -COCH₂-), 1.54–1.62 (2H, m, -COCH₂-CH₂-), 1.30, 1.26 (each 3H, d, $J = 6.9$ Hz, Me-3''', 4'''), 1.22–1.34 (12H, m, -CH₂-), 1.20, 1.18 (each 3H, d, $J = 6.8$ Hz, Me-3'', 4''), 1.16, 1.14 (each 3H, d, $J = 6.9$ Hz, Me-3''', 4'''), 0.87 (3H, t, $J = 7.1$ Hz, -CH₃); ¹³C NMR (125 MHz, CDCl₃): δ 178.2 (C-1'''), 177.3 (C-1'''), 176.0 (C-1''), 173.1 (2-CO-), 102.1 (C-2'), 89.6 (C-1), 82.2 (C-5'), 78.4 (C-3'), 74.6 (C-5), 73.0 (C-3), 70.4 (C-4'), 69.8 (C-4), 69.2 (C-2), 64.0 (C-1'), 62.3 (C-6), 59.4 (C-6'), 34.0, 33.9, 33.8 (C-2'', C-2''', C-2''', -CO-CH₂-), 31.8 (-CH₂-CH₂-CH₃), 29.1–29.3 (-CH₂-), 24.6 (-CO-CH₂-CH₂-), 22.6 (-CH₂-CH₃), 19.2, 18.9, 18.7 (C-3'', C-4'', C-3''', C-4''', C-3''', C-4'''), 14.1 (-CH₃). HRESIMS: m/z 729.3678 ([M + Na]⁺ (calcd for C₃₄H₅₈O₁₅Na, 729.3673).

2.2.5. Compound 5

Colorless gum; ¹H NMR (500 MHz, CDCl₃): δ 5.56 (1H, d, $J = 3.9$ Hz, H-1), 5.24 (1H, d, $J = 8.4$ Hz, H-3'), 5.17 (1H, brt, $J = 9.2$ Hz, H-3), 4.93 (1H, dd, $J = 10.3, 3.9$ Hz, H-2), 4.62 (1H, brt, $J = 8.8$ Hz, H-4'), 4.05 (1H, m, H-5), 4.02 (1H, d, $J = 11.8$ Hz, H-1'a), 3.96 (1H, m, H-6a), 3.93 (1H, m, H-5'), 3.93 (1H, m, H-6'a), 3.90 (1H, m, H-1'b), 3.74 (1H, m, H-6b), 3.71 (1H, m, H-6'b), 3.53 (1H, dd, $J = 9.8, 9.3$ Hz, H-4), 2.63 (1H, m, H-2'''), 2.60 (1H, m, H-2''), 2.57 (1H, m, H-2''), 2.26–2.42 (3H, m, -COCH₂-), 1.66 (1H, m, H-4''a), 1.54–1.62 (2H, m, -COCH₂-CH₂-), 1.46 (1H, m, H-4''b), 1.29, 1.26 (each 3H, d, $J = 6.9$ Hz, Me-3''', 4'''), 1.22–1.34 (12H, m, -CH₂-), 1.20, 1.18 (each 3H, d, $J = 6.8$ Hz, Me-3'', 4''), 1.14 (3H, t, $J = 6.9$ Hz, Me-4'''), 0.94 (3H, d, $J = 6.9$ Hz,

Me-3'''), 0.87 (3H, t, $J = 7.1$ Hz, $-CH_3$). HRESIMS: m/z 743.3806 ($[M + Na]^+$ (calcd for $C_{35}H_{60}O_{15}Na$, 743.3830).

2.4. Purification of compounds 2–5 by HPLC

Compounds 2–5 were yielded after repeated purification of silica gel MPLC fractions by HPLC using an XTerra Prep C18 column (19×250 mm i.d.; $10 \mu m$ particle size; Serial # T11551U03, Waters Corporation, Milford, MA) under UV detector at 205 nm. The column was eluted at a flow rate of 3 mL/min under isocratic solvent system (MeOH- H_2O , 80:20 or 85:15, v/v) maintained at 30 °C (Waters column heater module.)

2.4. ESIMS analyses of compounds 1–5.

ESIMS spectra were recorded on a Waters Xevo G2-S Q-TOF LC mass spectrometer by flow injection analysis (FIA) experiments. An aliquot of 10 μL of samples was injected and eluted with CH_3CN-H_2O (50:50, v/v) for 2 min under isocratic condition. The mass range of 50–1500 under electro spray ionization (ESI) positive ion mode was used for the detection of peaks.

2.5. Hydrolysis of compounds 1–5 and GCMS analyses of fatty acid methyl esters.

An aliquot of 1–5 (1 mg each) separately was stirred with KOH in MeOH (3M, 3 h), acidified with HCl and evaporated under vacuum. The resulting fatty acids were then methylated with CH_2N_2 separately to afford fatty acid methyl esters, according to the reported procedure (Ramsewak, Nair, Murugesan, Mattson, & Zasada, 2001). The methyl esters thus obtained from 1–5 were analyzed separately by GC using a capillary column, Agilent J&W VF-5ms GC Column, 30 m x 0.25 mm, 0.25 μm film thickness attached to a 10 m EZ-Guard column with a 7 inch cage.

The conditions for the analyses were 1 μ L sample dissolved in hexane, helium carrier gas at a flow rate of 1.5 mL/min with a split ratio of 10:1 and temperature gradient with an injector port temperature at 240 °C, held for 2 min, raised to 320 °C at a rate of 40 °C and held for another 10 min.

2.6. Cyclooxygenase enzymes (COX-1 and -2) inhibitory assays.

The COX-1 and -2 enzymes inhibitory activities were measured for extract (100 μ g/mL), compounds **1–5** (25, 50 and 100 μ g/mL) and the positive controls (aspirin, ibuprofen, naproxen and Celebrex at 108, 12, 15 and 1 μ g/mL, or 600, 60, 60 and 2.6 μ M, respectively) according to the published procedure (Kelm et al., 2000; Zhang et al., 2015). The assay was carried out by monitoring the initial rate of O₂ uptake using an Instech micro oxygen chamber and electrode attached to an YSI model 5300 Biological Oxygen Monitor at 37 °C. The stock solution of extracts (10 mg/mL), compounds **1–5** (2.5, 5 and 10 mg/mL) and positive controls (aspirin, ibuprofen, naproxen and Celebrex at 10.8, 1.238, 1.514 and 0.1 mg/mL, respectively) were prepared in DMSO. The test samples (6 μ L) were initially added to the chamber full of assay buffer (1 mM Tris-phenol buffer, 600 μ L, pH = 7, and hemoglobin (17 μ g)). COX-1 or -2 enzymes (20 μ L) was then added and incubated for 2 min. The substrate arachidonic acid (10 μ L of solution at 1 mg/mL 1 mM Tris-phenol buffer, pH = 7) was added to initiate the enzyme reaction or formation of prostaglandin endoperoxide. The data were recorded using QuickLog for windows data acquisition. The percent inhibition was calculated with respect to DMSO control and each sample was tested in duplicate.

3. Results and discussion

Although consumed foods prepared from tomatillo contain the sticky material on its surface, there were no attempts to investigate the chemistry and bioactivity of it in the past. The sticky material is present only on the surface of tomatillo and hence collected by rinsing the fruits with CH₂Cl₂ (Nair, Burke, & Mudd, 1990). The resulting extract (TC) was initially evaluated for antioxidant (Arora, Nair, & Strasburg, 1998; Liu & Nair, 2010; Zhang et al., 2015) and antiinflammatory (Kelm et al., 2000; Zhang et al., 2015) activities. However, it showed only COX-1 and -2 enzymes inhibitory activity. TLC analyses of the extract revealed that it contained chlorophyll and compounds that were not visible under 254 and 366 nm. However, they appeared as black spots after 10% H₂SO₄ spray and charring at 120 °C. MPLC, TLC and HPLC purification of the extract afforded five new sucrose esters (**1–5**) (Figure 2). The chemical identity of the pure isolates was determined by spectroscopic methods including 2D- NMR, LC-MS and GC-MS spectral analyses. Among the sucrose esters isolated from tomatillo, tetra-esters of sucrose (**4** and **5**) accounted for 57.4% of the total extract of sticky material. The tri- (**2** and **3**) and di-esters of sucrose (**1**) were 6.8 and 4.8% of the total extract of sticky material, respectively. Sucrose esters have been reported from *Physalis* genus such as *P. viscosa* (Ovenden et al., 2005), *P. nicandroides* var. *attenuate* (Maldonado, Torres, Martínez, & Pérez-Castorena, 2006), *P. sordida* (Pérez-Castorena, Martínez, & Maldonado, 2010), *P. solanaceus* (Pérez-Castorena, Luna, Martínez, & Maldonado, 2012) and *P. peruviana* (Franco, Ocampo, Gómez, De la Puerta, Espartero, & Ospina, 2014), and showed antiinflammatory (Franco et al., 2014; Pérez-Castorena et al., 2010) and antibacterial (Ovenden et al., 2005) activities. However, this is the first report of sucrose esters in the fruits of *P. philadelphica*.

Compound **1**, a colorless gum, possessed the molecular formula of C₃₂H₅₈O₁₃, as determined

by HRESIMS ions at m/z 673.3779 ($[M + Na]^+$, calcd for $C_{32}H_{58}O_{13}Na$, 673.3775). The NMR data of **1** revealed that it contained signals for sucrose and long chain fatty acid ester moieties (Maldonado et al., 2006; Ovenden et al., 2005; Pérez-Castorena et al., 2010). The fatty acids were elucidated as two decanoic acids on the basis of proton and carbon signals and supported by its molecular formula $C_{32}H_{58}O_{13}$. This was further confirmed by GCMS data of fatty acids afforded from it. For example, hydrolysis and GCMS analysis of the resulting methyl ester of fatty acid from **1** gave a single peak at $R_t = 5.20$ min with an m/z of 186, confirming methyl ester of decanoic acid. The two decanoic acid esters in **1** were assigned at C-2 and C-3 on the basis of 1H - 1H COSY and HMBC correlations of H-1/H-2, H-2/H-3, H-2/-CO- and H-3/-CO-, which was supported by the ESIMS fragment at m/z 471 corresponding to didecanoylated glucopyranosyl fragment. Hence, compound **1** was identified as 2,3-di-*O*-decanoyl sucrose (Figure 2).

Compound **2** gave the molecular formula of $C_{36}H_{64}O_{14}$ as determined by HRESIMS ion at m/z 743.4203 ($[M + Na]^+$, calcd for $C_{36}H_{64}O_{14}Na$, 743.4194). The NMR data of **2** also showed characteristic signals of sucrose and fatty acid esters (Maldonado et al., 2006; Ovenden et al., 2005; Pérez-Castorena et al., 2010). Similar to **1**, long chain fatty acid esters were elucidated as two decanoic acid esters assigned at C-2 and C-3 on the basis of 1H - 1H COSY and HMBC correlations and further confirmed by GCMS analysis. In addition, compound **2** showed characteristic proton and carbon signals for isobutyryl group (Maldonado et al., 2006), which was assigned at C-1'' by the HMBC correlations of H₂-1'/C-2', H₂-1'/C-1'', H-2''/C-1'', Me-3''/C-1'' and Me-4''/C-1''. The ESIMS fragments at m/z 471 and 233 corresponded to didecanoylated glucopyranosyl and isobutyrylated fructofuranosyl fragments. This ESIMS data supported the substitution of pyranose by decanoic acids and furanose ring by isobutyric acid, respectively, in compound **2**.

Therefore, compound **2** was identified as 2,3-di-*O*-decanoyl-1'-*O*-isobutyryl sucrose (**Figure 2**).

Compound **3** gave a molecular formula of $C_{37}H_{66}O_{14}$ based on the HRESIMS ion at m/z 757.4357 ($[M + Na]^+$, calcd for $C_{37}H_{66}O_{14}Na$, 757.4350). The NMR spectral data showed signals belonging to sucrose and two decanoic acid ester moieties as in compounds **1** and **2**. In addition, proton and carbon signals belonging to 2-methylbutyryl group (Maldonado et al., 2006) was observed in **3** and assigned at C-1'' based on HMBC correlations of $H_2-1'/-CO-$, $H-2''/-CO-$, $Me-3''/-CO-$, $H_2-4''/-CO-$ and $Me-5''/C-2''$. The methylbutyrylated fructofuranosyl moiety in **3** was further confirmed by the fragment ion at m/z 247. Also, the ESIMS fragment at m/z 471 was consistent with didecanoylated glucopyranosyl unit. Therefore, compound **3** was assigned as 2,3-di-*O*-decanoyl-1'-*O*-(2-methylbutyryl) sucrose (**Figure 2**).

The HRESIMS of compound **4** gave a molecular formula of $C_{34}H_{58}O_{15}$ as evidenced by the molecular ion at m/z 729.3678 ($[M + Na]^+$, calcd for $C_{34}H_{58}O_{15}Na$, 729.3673). Analysis of the NMR spectral data of **4** indicated the presence of one decanoyl and three isobutyryl groups (Maldonado et al., 2006). The decanoyl group was assigned at C-2 and three isobutyryl groups were assigned at C-3, C-1' and C-3' based on the HMBC correlations from H-2, H-3, H_2-1' , H-3', -CH- and -Me groups of isobutyryl chain to each corresponding carbonyl group. The ESIMS fragment ions at m/z 387 and 303, corresponding to 2-decanoyl-3-isobutyryl glucopyranosyl and diisobutyrylated fructofuranosyl units, supported the assigned acyl substitution of pyranose and furanose ring, respectively, in compound **4**. Therefore, compound **4** was identified as 2-*O*-decanoyl-3,1',3'-tri-*O*-isobutyryl sucrose (**Figure 2**).

The molecular ion at m/z 743.3806 ($[M + Na]^+$ in the HRESIMS spectrum of compound **5**

confirmed its molecular formula as $C_{35}H_{60}O_{15}$ (calcd for $C_{35}H_{60}O_{15}Na$, 743.3830) and indicated it contained an additional CH_2 than compound **4**. As in compound **3**, one 2-methylbutyryl group was observed in **5** and assigned at C-3 based on its NMR spectral data. The assigned structure of **5** was also supported by the ESIMS fragment ions at m/z 401 corresponding to a 2-decanoyl-3-methylbutyryl glucopyranosyl unit. Also, the ESIMS fragment at m/z 303 was evident of a diisobutyrylated fructofuranosyl moiety in **5**. Therefore, compound **5** was identified as 2-*O*-decanoyl-3-*O*-(2-methylbutyryl)-1',3'-di-*O*-isobutyryl sucrose (**Figure 2**).

Although not isolated and characterized, trace amounts of other sucrose esters were detected in the HPLC analyses of fractions used for the isolation of compounds **4** and **5**. The ESIMS of fractions also accounted trace quantities of higher molecular weight sucrose esters accounting to mass differences of 14 (CH_2) suggesting the presence of $C_{33}H_{56}O_{15}$, $C_{36}H_{62}O_{15}$, $C_{37}H_{64}O_{15}$ and $C_{38}H_{66}O_{15}$ molecular forms as indicated at m/z 715.3500 ($[M + Na]^+$), 757.3959 ($[M + Na]^+$), 771.4111 ($[M + Na]^+$) and 785.4255 ($[M + Na]^+$), respectively. These were too small in quantities for isolation and NMR characterization.

The antiinflammatory activity of TC and pure isolates **1–5** was determined by measuring the inhibition of cyclooxygenase enzymes COX-1 and -2. These enzymes modulate conversion of arachidonic acid to prostaglandins which in turn regulate inflammatory responses in vivo (Kelm et al., 2000; Zhang et al., 2015). At 100 $\mu g/mL$, the CH_2Cl_2 washing of tomatillo fruit (TC) showed COX-1 and -2 enzymes inhibition by 46 and 51%, respectively (**Figure 3A**). At 25 $\mu g/mL$ (38.5, 34.7, 34.1, 35.4 and 34.7 μM , respectively), sucrose esters **1–5** from TC showed inhibition by around 23 and 31% for COX-1 and COX-2 enzymes, respectively (**Figure 3B**). At 50 $\mu g/mL$

(76.9, 69.4, 68.1, 70.8 and 69.4 μ M, respectively), **1–5** inhibited COX-1 and -2 enzymes by around 43 and 41%, respectively (**Figure 3B**). Accordingly, sucrose esters **1–5** at 100 μ g/mL (153.8, 138.8, 136.2, 141.6 and 138.8 μ M, respectively) inhibited COX-1 and COX-2 enzymes in a similar manner at around 50% (**Figure 3B**). The results showed that these sucrose esters **1–5** at 100 μ g/mL inhibited COX-1 and COX-2 enzymes similar to over the counter non-steroidal antiinflammatory drugs (NSAIDs) aspirin, ibuprofen and naproxen used as positive controls at 108, 12 and 15 μ g/mL (600, 60 and 60 μ M), respectively (**Figure 3A**).

Similar to sucrose esters isolated from other species of *Physalis* genus (Franco et al., 2014; Maldonado et al., 2006; Ovenden et al., 2005; Pérez-Castorena et al., 2010; Pérez-Castorena et al., 2012), the acylation of new sucrose esters in *P. philadelphica* (tomatillo) also occurred at carbons 2, 3, 1' and 3'. The sucrose esters in *P. philadelphica* were acylated with decanoyl (C10), isobutyryl and 2-methylbutanoyl moieties. However, sucrose esters isolated from *P. nicandroides* var. *attenuate* contained decanoyl, isobutyryl, 2-methylbutanoyl and acetyl groups (Maldonado et al. 2006). The sucrose esters in *P. peruviana* contained a different acyl group as 3-methylbutanoyl in addition to the decanoyl and isobutyryl groups (Franco et al., 2014). The major difference in the sucrose esters of *P. sordida* was the presence of lauroyl (C12) moiety in addition to acetyl and isobutyryl groups (Pérez-Castorena et al, 2010). The sucrose esters in *P. solanaceus* also contained lauroyl (C12), isobutyryl and 2-methylbutanoyl groups (Pérez-Castorena et al., 2012), whereas the sucrose esters in *P. viscosa* with lauroyl (C12) and 3-methyl-2-butenoyl groups, unique to this species (Ovenden et al., 2005). It is noteworthy that sucrose esters **1–3** contained two long chain acyl groups (decanoyl), a unique feature of the *P. philadelphica* spp.

Carbohydrate polyesters, especially esters of sucrose and glucose, have been approved as food additives based on safety and studies on its digestion (Akoh & Swanson, 1994; Nakamura, 1999). Sucrose esters with unsaturated fatty acids, short-chain fatty acids or complete acylation are bitter in taste and hence not suitable for use as a food additive (Nakamura, 1999). Earlier, we also reported a series of glucolipids from the oily glands of *Lycopersicon pennellii*, a wild tomato species (Mudd, Burke, Fobes, & Nair, 1988; Nair et al., 1990), where the glucolipid was separated as bitter glucolipid (BG) and non-bitter glucolipid (NBG). BG was characterized as glucolipids with no more than 7 carbons in any of the acylating fatty acids on the glucose molecule. NBG was characterized as glucolipids containing more than 7 carbons in at least one of the acylating fatty acid groups on the glucose molecule (Mudd et al., 1988; Nair et al., 1990). In this study, the sucrose esters **1–5** isolated from tomatillo are di-, tri- and tetra-acylated and contain only saturated fatty acids with majority of it with a chain length of 10. Therefore, these sucrose esters are non-bitter and will not impart bitter taste to foods containing them.

4. Conclusion

This is the first study on the sticky material on the surface of tomatillo fruits and the isolation and identification of new sucrose esters (**1–5**) present in it. These esters accounted for 69% of the total amount of natural sticky material coating the surface of tomatillo fruits. This is also the first report of the sucrose esters from *P. philadelphica*. The natural sticky material on the surface of tomatillo fruits and purified sucrose esters from it showed antiinflammatory activity similar to NSAIDs aspirin, ibuprofen and naproxen. The results presented herein suggest possible functional food benefit of the sticky material naturally present on the surface of tomatillo fruit as

an antiinflammatory food ingredient.

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Figure captions

Figure 1. HPLC profiles: (A) for compounds **2** and **3** in fraction C2 and (B) compounds **4** and **5** in fraction D. Peak D1 was a mixture of four analogues of sucrose esters. Due to paucity of this isolated peak, its composition was determined by LCMS. Fractions C2, C3 and D were obtained from the silica gel column chromatography of the crude extract. Compound **1** was isolated from C3 by preparative TLC.

Figure 2. Structures of sucrose esters **1–5** isolated from the natural sticky coating of tomatillo fruits.

Figure 3. (A), cyclooxygenase enzymes (COX-1 and -2) inhibitory activity of sucrose ester extract at 100 $\mu\text{g/mL}$ and positive controls NSAIDs aspirin, ibuprofen, naproxen and Celebrex[®] (Celecoxib) at 108, 12, 15 and 1 $\mu\text{g/mL}$ (600, 60, 60 and 2.6 μM), respectively and (B) pure isolates **1–5** at 25 $\mu\text{g/mL}$ (38.5, 34.7, 34.1, 35.4 and 34.7 μM , respectively), 50 $\mu\text{g/mL}$ (76.9, 69.4, 68.1, 70.8 and 69.4 μM , respectively) and 100 $\mu\text{g/mL}$ (153.8, 138.8, 136.2, 141.6 and 138.8 μM , respectively), respectively. Vertical bars represent the standard deviation of each data point (n = 2). The varying concentrations of positive controls used were to yield a comparable inhibitory activity values between 50–100% by test extract, compounds and positive controls alike.

Figure 1

Figure 2

Figure 3