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ABSTRACT

Twenty-three phenolic compounds were isolated from a butanol extract of Canadian maple syrup (MS-BuOH) using chromatographic methods. The compounds were identified from their nuclear magnetic resonance and mass spectral data as seven lignans: lyoniresinol (1), secoisolariciresinol (2), dehydroconiferyl alcohol (3), 5’-methoxy-dehydroconiferyl alcohol (4), erythro-guaiacylglycerol-β-O-4’-coniferyl alcohol (5), erythro-guaiacylglycerol-β-O-4’-dihydroconiferyl alcohol (6), and [3-[4-[(6-deoxy-α-L-mannopyranosyl)oxy]-3-methoxyphenyl)methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3H)-furanone (7); two coumarins: scopoletin (8) and fraxetin (9); a stilbene: (E)-3,3’-dimethoxy-4,4’-dihydroxystilbene (10), and thirteen phenolic derivatives: 2-hydroxy-3’,4’-dihydroxyacetophenone (11), 1-(2,3,4-trihydroxy-5-methylphenyl)-ethanone (12), 2,4,5-trihydroxyacetophenone (13), catechaldehyde (14), vanillin (15), syringaldehyde (16), gallic acid (17), trimethyl gallic acid methyl ester (18), syringic acid (19), syringenin (20), (E)-coniferol (21), C-veratroylglycol (22), and catechol (23). The antioxidant activities of MS-BuOH (IC₅₀>1000 µg/mL), pure compounds, vitamin C (IC₅₀=58 µM), and the synthetic commercial antioxidant, butylatedhydroxytoluene (IC₅₀=2651 µM), were evaluated in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay. Among the isolates, the phenolic derivatives and coumarins showed superior antioxidant activity (IC₅₀<100 µM) compared to the lignans and stilbene (IC₅₀>100 µM). Also, this is the first report of sixteen of these twenty-three phenolics i.e. compounds 1, 2, 4-14, 18, 20 and 22, in maple syrup.

Keywords: Acer saccharum; sugar maple; maple syrup; butanol extract; phenolics; antioxidant
INTRODUCTION

Maple syrup is a natural sweetener obtained by concentrating the sap collected from certain maple species including the sugar maple (*Acer saccharum* L.) tree which is native to North America ([1], [2]). Maple syrup is primarily produced in north eastern North America and the vast majority of the world’s supply comes from Canada (85%; primarily Quebec), followed by the United States (15%; primarily New England/New York region) ([2]). Maple syrup is the largest commercially available food product consumed by humans which is derived totally from the sap of deciduous trees.

Maple syrup is produced by thermal evaporation of the colorless watery sap collected from maple trees in late winter to early spring. Because of its high water content, about 40 L of sap is required to produce 1 L of syrup ([1]). During the concentration process of transforming sap to syrup, the characteristic flavor, color, and odor of maple syrup develops. Typically, the color of the syrup becomes darker as the season progresses, and based on Canadian standards, maple syrup is graded as extra light (grade AA), light (grade A), medium/amber (grade B), and dark (grade C) ([2]).

Being a plant-derived natural product, it is not surprising that maple syrup contains phytochemicals (naturally present in the xylem sap), as well as process-derived compounds (formed during thermal evaporation of sap) ([1]-[4]). Apart from sucrose, which is its dominant sugar, maple syrup contains organic acids, amino acids, minerals, and lignin derived flavor compounds ([1]-[4]). Among the phytochemicals which have been previously reported from maple syrup, the phenolic class predominates. For example, vanillin, syringaldehyde, coniferaldehyde, cinnamic acid and benzoic acid derivatives, as well as flavonoids (flavanols and flavonols), have been identified in maple syrup extracts ([3]-[6]).
The presence of a diverse range of phenolic sub-classes in maple syrup is interesting given that this large class of dietary phytochemicals has attracted significant research attention due to their diverse biological functions and potential positive effects on human health (6). Recently, phenolic-enriched extracts of maple syrup were shown to have antioxidant, antimutagenic, and human cancer cell antiproliferative properties (7, 8). Thus, a comprehensive investigation of maple syrup phenolics is necessary in order to evaluate the biological properties and potential human health benefits of this natural sweetener. Previous phytochemical research has been conducted on maple syrup extracts, namely ethyl acetate, chloroform, dichloromethane and diethyl ether extracts (3-6). While these organic solvents are commonly used for the extraction of phytochemicals from complex food matrices, it is possible that higher polarity solvents, such as n-butanol, may contain previously unreported phenolic compounds. However, there have been no prior reported studies of compounds found in butanol extracts of maple syrup (MS-BuOH).

Maple syrup is popularly consumed worldwide and is of significant cultural and economical importance to north eastern North America, particularly in Canada, where it is largely produced. Therefore, increased knowledge of the chemical constituents of Canadian maple syrup would aid in the authentication, characterization, and subsequent detection of intentional adulteration of this premium natural sweetener. Also, characterization of the different chemical sub-classes of bioactive phenolics, and ascertaining their levels, would aid in evaluating the potential human health benefits resulting from consumption of Canadian maple syrup. Towards this end, our objectives were: 1) To isolate and identify the phytochemicals present in a Canadian MS-BuOH, 2) To evaluate the Canadian MS-BuOH, and its purified
constituents, for antioxidant potential in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay.

Here we report the isolation and identification of twenty-three phenolic compounds, 1-23, from MS-BuOH among which sixteen compounds, namely, 1, 2, 4-14, 18, 20 and 22, are being reported from maple syrup for the first time.

MATERIALS AND METHODS

General Experimental Procedures. $^1$H and $^{13}$C Nuclear Magnetic Resonance (NMR) spectra were obtained either on a Bruker 400 MHz or a Varian 500 MHz instrument using deuterated methanol (CD$_3$OD) as solvent. Electrospray ionization mass spectral (ESIMS) data were acquired on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer equipped with a Turbo Ionspray source and were obtained by direct infusion of pure compounds. Analytical high performance liquid chromatography (HPLC) were performed on a Hitachi Elite LaChrom system consisting of a L2130 pump, L-2200 autosampler, and a L-2455 Diode Array Detector all operated by EZChrom Elite software. Semi-preparative scale HPLC were performed on a Beckman-Coulter HPLC system consisting of a Beckman System Gold$^R$ 126 solvent module pump, 168 photodiode array (PDA)-UV/VIS detector, and 508 autosampler all operated by the 32 Karat 8.0 software. All solvents were either ACS or HPLC grade and were obtained from Wilkem Scientific (Pawcatuck, RI). Ascorbic acid (vitamin C), butylatedhydroxytoluene (BHT), and diphenylpicrylhydrazyl (DPPH) reagent were purchased from Sigma-Aldrich (St Louis, MO).

Maple Syrup Butanol Extract (MS-BuOH). Maple syrup (grade C, 20 L) was provided by the Federation of Maple Syrup Producers of Quebec (Canada). The syrup was kept frozen until
extraction when it was subjected to liquid-liquid partitioning with ethyl acetate (10 L x 3) followed by n-butanol (10 L x 3) solvents, to yield ethyl acetate (4.7 g) and n-butanol (108 g) extracts, respectively, after solvent removal in vacuo.

**Analytical HPLC.** All analyses were conducted on a Luna C18 column (250 x 4.6 mm i.d., 5 µM; Phenomenex) with a flow rate at 0.75 mL/min and injection volume of 20 µL. A gradient solvent system consisting of solvent A (0.1% aqueous trifluoroacetic acid) and solvent B (methanol, MeOH) was used as follows: 0-10 min, 10% to 15% B; 10-20 min, 15% B; 20-40 min, 15% to 30% B; 40-55 min, 30% to 35% B; 55-65 min, 35% B; 65-85 min, 35% to 60% B; 85-90 min, 60% to 100% B, 90-93 min, 100% B; 93-94 min, 100% to 10% B; 94-104 min, 10% B.

**Figures 1A and 1B** show the HPLC-UV profiles of the butanol extract and all of the isolated phenolics (combined into one solution/injection), respectively. Unfortunately, due to limited sample quantity, we were not able to include compound 13 in the HPLC-UV injection shown in Figure 1B.

**Isolation of Compounds from the MS-BuOH.** The butanol extract (108 g) of Canadian maple syrup was further extracted with methanol (100 mL x 3) to afford methanol soluble (57 g; dark-brown powder) and methanol insoluble (51 g; off-white powder) fractions. Analytical HPLC-UV analyses of the methanol soluble extract revealed a number of peaks characteristic of phenolic compounds at 220, 280 and 360 nm (see above for details of methodology; see Figure 1A for chromatogram). Therefore, this fraction was selected for further purification by repeated chromatography on a Sephadex LH-20 column (4.5 x 64 cm), eluting with a gradient system of MeOH: H₂O (3:7 v/v to 7:3 v/v to 100:0 v/v), and then with acetone: H₂O (7:3 v/v). Based on analytical HPLC-UV profiles, twelve combined fractions, Fr. 1-12, were obtained. Fr. 4 (1.5 g) was subjected to column chromatography on a Sephadex LH-20 column (4.5 x 64 cm) using a
gradient solvent system of MeOH: H$_2$O (3:7 v/v to 7:3 v/v) to afford twelve sub-fractions, Fr. 4.1-4.12. These were individually subjected to a series of semi-prep HPLC-UV separation using a Waters Sunfire Prep C$_18$ column (250 x 10 mm i.d., 5 µm; flow 2 mL/min) and eluting with a MeOH:H$_2$O gradient system to yield compounds 1 (4.6 mg), 3 (3.8 mg), 5 (4.0 mg), 6 (41.6 mg), 7 (6.6 mg), 11 (3.5 mg), 15 (0.3 mg), 16 (0.8 mg), 18 (0.2 mg), 20 (1.3 mg), 22 (1.5 mg) and 23 (3.0 mg). Similarly, Fr. 5 (0.47 g) was purified by semi-prep HPLC-UV using a Waters XBridge Prep C$_18$ column (250 x 19 mm i.d., 5 µm; flow 3.5 mL/min) and a gradient solvent system of MeOH:H$_2$O to afford four subfractions Fr. 5.1-5.4. These subfractions were separately subjected to a combination of semi-prep HPLC-UV and/or Sephadex LH-20 column chromatography with gradient solvents systems of MeOH:H$_2$O to afford compounds 2 (1.9 mg), 4 (1.9 mg), 8 (2.0 mg), 9 (2.3 mg), 14 (2.5 mg), 17 (2.4 mg), 19 (1.8 mg) and 21 (1.3 mg). Similarly, Fr. 6 (0.2 g) afforded compounds 12 (1.4 mg) and 13 (1.3 mg) and Fr. 11 yielded compound 10 (4.8 mg).

Identification of Compounds. All of the isolated compounds (Figure 2) were identified by examination of their $^1$H and/or $^{13}$C NMR and mass spectral data, and by comparison of these to published literature reports, when available (Table 1). The NMR data for compounds 12 and 13 have not been previously published and are provided here for the first time.

$(+)$-Lyoniresinol (1). Yellowish amorphous powder; (+) ESIMS, m/z 443.1719 [M+Na]$^+$, calcd. for molecular formula C$_{22}$H$_{28}$O$_8$; $^1$H-NMR (CD$_3$OD, 400 MHz) δ: 1.64 (1H, m, H-8), 1.95 (1H, m, H-8'), 2.59 (1H, m, H-7a), 2.72 (1H, m, H-7b), 3.36 (3H, s, 3-OCH$_3$), 3.51 (2H, m, H-9a, 9a'), 3.61 (2H, m, H-9b, 9b'), 3.75 (6H, s, 3', 5'-OCH$_3$), 3.87 (3H, s, 5-OCH$_3$), 4.31 (1H, d, J=5.6 Hz, H-7'), 6.39 (2H, s, H-2', 6'), 6.60 (1H, s, H-6); $^{13}$C-NMR (CD$_3$OD, 100 MHz) δ: 149.09 (C-3', 5'), 148.77 (C-5), 147.80 (C-3), 139.44 (C-4, 1'), 130.27 (C-1), 135.00 (C-4'), 126.36 (C-2), 107.84 (C-6), 106.91 (C-2', 6'), 66.87 (C-9), 64.21 (C-9'), 60.26 (3-OCH$_3$), 56.85 (3', 5'-OCH$_3$),
56.69 (5-OCH$_3$), 49.01 (C-8'), 42.43 (C-7'), 40.98 (C-8), 33.71 (C-7). $^1$H and $^{13}$C NMR data were consistent with literature (9).

Secoisolariciresinol (2). Yellowish amorphous powder; (+) ESIMS m/z 385.1447 [M+Na]$^+$, calcd. for molecular formula C$_{20}$H$_{26}$O$_6$; $^1$H-NMR (CD$_3$OD, 500 MHz) $\delta$: 1.89 (2H, m, H-8, 8'), 2.55 (2H, m, H-7a, 7a'), 2.66 (2H, m, H-7b, 7b'), 3.58 (4H, m, H-9, 9'), 3.74 (6H, s, 3, 3'-OCH$_3$), 6.55 (2H, d, J=8.0 Hz, H-6, 6'), 6.58 (2H, s, H-2, 2'), 6.66 (2H, s, H-5, 5'); $^{13}$C-NMR (CD$_3$OD, 125 MHz) $\delta$: 147.38 (C-3, 3'), 144.05 (C-4, 4'), 132.45 (C-1, 1'), 121.28 (C-6, 6'), 114.34 (C-5, 5'), 111.93 (C-2, 2'), 60.69 (C-9, 9'), 54.74 (3, 3'-OCH$_3$), 42.69 (C-8, 8'), 34.61 (C-7, 7'). $^1$H and $^{13}$C NMR data were consistent with literature (10).

Dehydroconiferyl alcohol (3). Yellowish amorphous powder; (+) ESIMS m/z 383.1208 [M+Na]$^+$, calcd. for molecular formula C$_{20}$H$_{24}$O$_6$; $^1$H-NMR (CD$_3$OD, 400 MHz) $\delta$: 1.81 (2H, m, H-8'), 2.64 (2H, m, H-7'), 3.48 (1H, m, H-8), 3.58 (2H, m, H-9'), 3.70 (1H, m, H-9a), 3.80 (1H, m, H-9b), 3.82 (3H, s, 3-OCH$_3$), 3.86 (3H, s, 3'-OCH$_3$), 5.50 (1H, d, J=6.0 Hz, H-7), 6.74 (2H, s, H-4', 6'), 6.76 (1H, d, J=8.0 Hz, H-5), 6.82 (1H, d, J=8.0 Hz, H-6), 6.96 (1H, s, H-2); $^{13}$C-NMR (CD$_3$OD, 100 MHz) $\delta$: 149.20 (C-3), 147.61 (C-4, 2'), 145.34 (C-3'), 137.03 (C-5'), 134.92 (C-1), 129.79 (C-1'), 119.81 (C-6), 118.01 (C-6'), 115.97 (C-5), 114.10 (C-4'), 110.56 (C-2), 89.11 (C-7), 65.09 (C-9), 62.35 (C-9'), 56.81 (3-OCH$_3$), 56.41 (3'-OCH$_3$), 35.99 (C-8), 33.05 (C-7'). $^1$H and $^{13}$C NMR data were consistent with literature (11).

5-methoxydehydroconiferyl alcohol (4). Yellowish amorphous powder; (+) ESIMS m/z 413.1464 [M+Na]$^+$, calcd. for molecular formula C$_{21}$H$_{26}$O$_7$; $^1$H-NMR (CD$_3$OD, 500 MHz) $\delta$: 1.80 (2H, m, H-8'), 2.60 (2H, m, H-7'), 3.47 (1H, m, H-8), 3.58 (2H, m, H-9'), 3.76 (1H, m, H-9a), 3.80 (6H, s, 3, 5-OCH$_3$), 3.84 (1H, m, H-9b), 3.86 (3H, s, 3'-OCH$_3$), 5.49 (1H, d, J=5.5 Hz, H-7), 6.64 (2H, s, H-2, 6), 6.72 (2H, s, H-4', 6'); $^{13}$C-NMR (CD$_3$OD, 125 MHz) $\delta$: 147.91 (C-3, 5), 146.10 (C-
′), 143.80 (C-3′), 135.56 (C-4, 5′), 132.64 (C-1), 128.40 (C-1′), 116.49 (C-6′), 112.71 (C-4′), 110.79 (C-3′), 87.68 (C-7), 63.75 (C-9), 60.80 (C-9′), 55.36 (3, 5-OCH₃), 55.32 (3′-OCH₃), 54.17 (C-8), 34.39 (C-8′), 31.48 (C-7′).

H and ¹³C NMR data were consistent with literature (12).

Erythro-guaiacylglycerol-β-O-4′-coniferyl alcohol (5). Yellowish amorphous powder; (+) ESIMS m/z 399.1156 [M+Na]⁺, calcd. for molecular formula C₂₀H₂₄O₇; ¹H-NMR (CD₃OD, 400 MHz) δ: 3.81 (6H, s, 3, 2′-OCH₃), 3.87 (2H, m, H-9), 4.20 (2H, d, J=5.6 Hz, H-9′), 4.37 (1H, m, H-8), 6.24 (1H, dd, J=6.0, 16.0 Hz, H-8′), 6.52 (1H, d, J=16.0 Hz, H-7′), 6.73 (1H, d, J=8.0 Hz, H-5), 6.84 (1H, d, J=8.0 Hz, H-6), 6.88 (2H, brs, H-5′, 6′), 7.01 (1H, s, H-3′), 7.03 (1H, s, H-2); ¹³C-NMR (CD₃OD, 100 MHz) δ: 151.80 (C-2′), 149.00 (C-1′), 148.61 (C-3), 147.22 (C-4), 134.18 (C-1), 133.11 (C-4′), 130.81 (C-7′), 128.57 (C-8′), 121.13 (C-6), 120.77 (C-5′), 118.95 (C-6′), 115.74 (C-5), 111.92 (C-2), 110.79 (C-3′), 86.31 (C-8), 74.19 (C-7), 63.90 (C-9′), 62.32 (C-9), 56.58 (3, 2′-OCH₃). ¹H and ¹³C NMR data were consistent with literature (13).

Erythro-guaiacylglycerol-beta-O-4′-dihydroconiferyl alcohol (6). Yellowish amorphous powder; (+) ESIMS m/z 401.1602 [M+Na]⁺, calcd. for molecular formula C₂₀H₂₆O₇; ¹H-NMR (CD₃OD, 400 MHz) δ: 1.81 (2H, m, H-8′), 2.62 (2H, m, H-7′), 3.47 (1H, m, H-9a′), 3.58 (2H, m, H-9), 3.72 (1H, m, H-9b′), 3.82 (3H, s, 5-OCH₃), 3.85 (3H, s, 2′-OCH₃), 4.21 (1H, m, H-8), 4.90 (1H, m, H-7), 6.71 (1H, d, J=8.0 Hz, H-5′), 6.77 (1H, d, J=8.0 Hz, H-3), 6.86 (1H, s, H-3′), 6.88 (1H, d, J=8.0 Hz, H-2), 7.03 (1H, s, H-6); ¹³C-NMR (CD₃OD, 100 MHz) δ: 151.80 (C-2′), 148.95 (C-5), 147.69 (C-4), 147.29 (C-1′), 138.35 (C-4′), 133.88 (C-1), 122.18 (C-5′), 120.93 (C-2), 119.74 (C-6′), 116.01 (C-3), 114.02 (C-3′), 87.88 (C-8), 87.88 (C-8).
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74.29 (C-7), 62.36 (C-9), 62.01 (C-9’), 56.65 (2’-OCH₃), 56.48 (5-OCH₃), 35.71 (C-8’), 32.86 \(^1\)H and \(^{13}\)C NMR data were consistent with literature (14).

\[3\]-\(4\)-\{(6-deoxy-\(\alpha\)-L-mannopyranosyl)oxy\}-3-methoxyphenyl)methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3H)-furanone (7). Yellowish amorphous powder; (+) ESIMS \(m/z\) 573.1913 \([M+Na]^+\), calcd. for molecular formula \(C_{27}H_{34}O_{12}\).

\(^1\)H NMR (400Hz, CD\(_3\)OD): \(\delta\) 1.25 (3H, \(d, J=6.4Hz\), H-6’”), 2.46 (1H, \(m\), H-8’”), 3.06 (1H, \(d, 6\ J=13.2Hz\), H-7b), 3.35 (1H, \(d, J=13.2Hz\), H-7a), 3.5-3.90 (3H, \(m\), H-3’”, 4’”, 5’”), 3.55 (1H, \(m\), 9’b), 3.63 (3H, \(s\), 4’-OCH₃), 3.79 (3H, \(s\), 3’-OCH₃), 3.80 (3H, \(s\), 3-OCH₃), 3.95 (1H, \(m\), 9’a), 4.07 (1H, \(s\), H-2”), 5.10 (1H, \(d, J=9.2Hz\), H-7’), 5.31 (1H, \(s\), H-1”), 6.37 (1H, \(s\), H-2’), 6.62 (1H, \(d, J=8.0Hz\), H-6’), 6.85 (1H, \(d, J=8.0Hz\), H-6), 6.87 (1H, \(d, J=8.4Hz\), H-5’), 6.97 (1H, \(s\), H-2), 7.05 (1H, \(d, J=8.4Hz\), H-5); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): \(\delta\) 179.64 (C-9), 152.11 (C-3), 151.04 (C-13’), 150.74 (C-4’), 146.15 (C-4), 132.66 (C-1), 132.45 (C-1’), 124.54 (C-6), 120.92 (C-6’), 120.11 (C-5), 116.36 (C-2), 112.60 (C-5’), 110.39 (C-2’), 101.82 (C-1”), 82.89 (C-7’), 79.47 (C-8), 73.94 (C-4”), 72.33 (C-3”), 72.25 (C-2”), 71.02 (C-5”), 58.69 (C-9’), 56.75, 56.50 (C3,3’4’-OCH₃), 51.79 (C-8’), 42.75 (C-7), 18.18 (C-6”). \(^1\)H and \(^{13}\)C NMR data were consistent with literature (15).

Scopoletin (8). Yellowish amorphous powder; (+) ESIMS \(m/z\) 193.0787 \([M+H]^+\), calcd. for molecular formula \(C_{10}H_{8}O_{4}\). \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 3.81 (3H, \(s\), 6-OCH₃), 6.10 (1H, \(d, J=9.4Hz\), H-3), 6.67 (1H, \(s\), H-8), 7.01 (1H, \(s\), H-5), 7.75 (1H, \(d, J=9.4Hz\), H-4). \(^1\)H NMR data were consistent with literature (15).

Fraxetin (9). Yellowish amorphous powder; (+) ESIMS \(m/z\) 209.0639 \([M+H]^+\), calcd. for molecular formula \(C_{10}H_{8}O_{5}\). \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 3.82 (3H, \(s\), 6-OCH₃), 6.22 (1H, \(d, J=9.4Hz\), H-3), 6.67 (1H, \(s\), H-8), 7.01 (1H, \(s\), H-5), 7.75 (1H, \(d, J=9.4Hz\), H-4). \(^1\)H NMR data were consistent with literature (15).
$J=9.4, \ H-3), \ 6.73 \ (1H, \ s, \ H-5), \ 7.85 \ (1H, \ d, \ J=9.4, \ H-4)$. $^1H$ NMR data were consistent with literature (16).

(E)-3,3′-dimethoxy-4,4′-dihydroxystilbene (10). Yellowish amorphous powder; (+) ESIMS m/z 294.9650 [M+Na]$^+$, calcd. for molecular formula C$_{16}$H$_{16}$O$_4$; $^1H$ NMR (400 MHz, CD$_3$OD): $\delta$ 3.83 (6H, s, 3, 3′-OCH$_3$), 6.76 (2H, d, $J=8.0$, H-5, 5′), 6.92 (2H, s, H-7, 7′), 6.95 (2H, d, $J=8.0$, H-6, 6′), 7.12 (2H, s, H-2, 2′); $^{13}$C-NMR (CD$_3$OD, 100 MHz) $\delta$: 148.72 (C-3, 3′), 147.35 (C-4, 4′), 131.70 (C-1, 1′), 127.40 (C-7, 7′), 116.45 (C-5, 5′), 110.40 (C-2, 2′), 56.53 (3, 3′-OCH$_3$). $^1H$ and $^{13}$C NMR data were consistent with the literature (17).

2-hydroxy-3′,4′-dihydroxyacetophenone (11). Brown amorphous powder; (+) ESIMS m/z 191.0227 [M+Na]$^+$, calcd. for molecular formula C$_8$H$_8$O$_4$; $^1H$ NMR (500 MHz, CD$_3$OD): $\delta$ 4.68 (2H, s, H-8), 6.72 (1H, d, $J=8.0$, H-6), 7.27 (1H, d, $J=8.0$, H-7), 7.29 (1H, s, H-3). $^1H$ NMR data were consistent with the literature (18).

1-(2,3,4-trihydroxy-5-methylphenyl)-ethanone (12). Brown amorphous powder; (-) ESIMS m/z 181.0691 [M-H]$^-$, calcd. for molecular formula C$_9$H$_{10}$O$_4$; $^1H$ NMR (500 MHz, CD$_3$OD): $\delta$ 2.15 (3H, s, CH$_3$), 2.51 (3H, s, CH$_3$CO), 7.08 (1H, s, H-7).

2,4,5-trihydroxyacetophenone (13). Brown amorphous powder; (-) ESIMS m/z 167.0601 [M-H]$^-$; calcd. for molecular formula C$_8$H$_8$O$_4$; $^1H$ NMR (500 MHz, CD$_3$OD): $\delta$ 2.48 (3H, s, CH$_3$), 6.28 (1H, s, H-5), 7.16 (1H, s, H-7).

Catechaldehyde (14). Brown amorphous powder; (-) ESIMS m/z 137.0341 [M-H]$^-$, calcd. for molecular formula C$_7$H$_6$O$_3$; $^1H$ NMR (400 MHz, CD$_3$OD): $\delta$ 6.92 (1H, d, $J=8.0$, H-5), 7.31 (2H, brs, H-2, 6), 9.70 (1H, s, CHO). $^1H$ NMR data were consistent with literature (19).

Vanillin (15). White amorphous powder; (-) ESIMS m/z 151.0667 [M-H]$^-$, calcd. for molecular formula C$_8$H$_8$O$_2$; $^1H$ NMR (500 MHz, CD$_3$OD): $\delta$ 6.94 (1H, d, $J=8.0$, H-5), 7.43 (1H, d, $J=8.0$, H-7), 7.85 (1H, s, H-7).
H-6), 7.44 (1H, s, H-2), 9.75 (1H, s, CHO). \(^1\)H NMR data were consistent with the literature (20).

**Syringaldehyde** (16). White amorphous powder; (-) ESIMS \(m/z\) 181.0768 [M-H]\(^-\), calcd. for molecular formula C\(_9\)H\(_{10}\)O\(_4\): \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 3.86 (6H, s, 3, 5-OCH\(_3\)), 7.24 (2H, s, H-2, 6), 9.76 (1H, s, CHO). \(^1\)H NMR data were consistent with literature (20).

**Gallic acid** (17). Brown amorphous powder; (-) ESIMS \(m/z\) 169.1226 [M-H]\(^-\), calcd. for molecular formula C\(_7\)H\(_6\)O\(_5\): \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 7.02 (2H, s, H-2, 6). \(^1\)H NMR data were consistent with the literature (21).

**Trimethylgallic acid methyl ester** (18). Brown amorphous powder; (+) ESIMS \(m/z\) 249.0735 [M+Na]\(^+\), calcd. for molecular formula C\(_{11}\)H\(_{14}\)O\(_5\): \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 3.35 (3H, s, 10-COOCH\(_3\)), 3.92 (9H, s, 3, 4, 5-OCH\(_3\)), 7.34 (2H, s, H-2, 6). \(^1\)H NMR data were consistent with the literature (22).

**Syringic acid** (19). White amorphous powder; (-) ESIMS \(m/z\) 197.0256 [M-H]\(^-\), calcd. for molecular formula C\(_9\)H\(_{10}\)O\(_5\): \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 3.90 (6H, s, 3, 5-OCH\(_3\)), 7.34 (2H, s, H-2, 6). \(^1\)H NMR data were consistent with literature (20).

**Syringenin** (20). Brown amorphous powder; (+) ESIMS \(m/z\) 233.0630 [M+Na]\(^+\), calcd. for molecular formula C\(_{11}\)H\(_{14}\)O\(_4\): \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 3.75 (6H, s, 3, 5-OCH\(_3\)), 4.10 (2H, \(d, J=5.5\text{Hz}, \text{H-9}\)), 6.12 (1H, \(d, J=16.0, \text{H-8}\)), 6.39 (1H, \(d, J=16.0, \text{H-7}\)), 6.60 (2H, s, H-2, 6). \(^1\)H NMR data were consistent with literature (20).

**(E)-coniferol** (21). Brown amorphous powder; (-) ESIMS \(m/z\) 179.0833 [M-H]\(^-\), calcd. for molecular formula C\(_{10}\)H\(_{12}\)O\(_3\): \(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 3.88 (3H, s, 3-OCH\(_3\)), 4.20 (2H, \(d, J=5.0\text{Hz}, \text{H-9}\)), 6.20 (1H, \(d, J=16.0, \text{H-8}\)), 6.51 (1H, \(d, J=16.0, \text{H-7}\)), 6.74 (1H, \(d, J=8.0, \text{H-5}\)), 6.86 (1H, \(d, J=8.0, \text{H-6}\)), 7.01 (1H, s, H-2). \(^1\)H NMR data were consistent with literature (23).
C-veratroylglycol (22). Brown amorphous powder; (+) ESIMS m/z 235.0582 [M+Na]^+, calcd. for molecular formula C\textsubscript{10}H\textsubscript{12}O\textsubscript{5}; \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD): \(\delta\) 3.78 (1H, \(m\), H-9a), 3.90 (1H, \(m\), H-9b), 3.93 (3H, \(s\), OCH\textsubscript{3}), 5.13 (1H, \(dd\), \(J=3.5, 5.5\)Hz, H-8), 6.89 (1H, \(d\), \(J=8.0\), H-5), 7.60 (1H, \(d\), \(J=8.0\), H-6), 7.61 (1H, \(s\), H-2); \textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD): \(\delta\) 199.52 (C-7), 153.11 (C-4), 150.04 (C-3), 128.14 (C-1), 125.19 (C-6), 116.03 (C-5), 112.51 (C-2), 75.59 (C-8), 66.39 (C-9). \textsuperscript{1}H and \textsuperscript{13}C NMR data were consistent with literature (24).

Catechol (23). Brown amorphous powder; (-) ESIMS m/z 109.0448 [M-H], calcd. for molecular formula C\textsubscript{6}H\textsubscript{6}O\textsubscript{2}; \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD): \(\delta\) 6.66 (2H, \(m\), H-2, 5), 6.76 (2H, \(m\), H-3, 4); \textsuperscript{13}C NMR (100Hz, CD\textsubscript{3}OD): \(\delta\) 144.67 (C-1, 6), 121.04 (C-2, 6), 116.52 (C-3, 4). \textsuperscript{1}H and \textsuperscript{13}C NMR data were consistent with the literature (25).

**Antioxidant Assay.** The antioxidant potential of MS-BuOH, the sugar fraction of maple syrup, and the pure compounds were determined on the basis of the ability to scavenge the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical as previously reported (26). The DPPH radical scavenging activity of ascorbic acid (vitamin C) and the synthetic commercial antioxidant, butylated hydroxytoluene (BHT), were also assayed as positive controls (see Table 2). The assay was conducted in a 96-well format using serial dilutions of 100 \(\mu\)L aliquots of test compounds (ranging from 2500-26 \(\mu\)g/mL), ascorbic acid (1000-10.4 \(\mu\)g/mL), and BHT (250,000-250 \(\mu\)g/mL). Then DPPH (150 \(\mu\)L) was added to each well to give a final DPPH concentration of 137 \(\mu\)M. Absorbance was determined after 30 min at 515 nm, and the scavenging capacity (SC) was calculated as SC% = [(A0-A1/A0)] x 100 where A0 was the absorbance of the reagent blank, and A1 was the absorbance with test samples. The control contained all reagents except the compounds and all tests were performed in triplicate. IC\textsubscript{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.
RESULTS AND DISCUSSION

Isolation and Identification of Compounds in Canadian Maple Syrup Butanol Extract (MS-BuOH). The primary objective of this study was to isolate and identify the phytochemicals present in Canadian maple syrup butanol extract. Because the constituents of ethyl acetate, chloroform, dichloromethane, and diethyl ether extracts of maple syrup have already been reported (3-6), we focused our isolation and structural elucidation efforts on the butanol extract. We speculated that the butanol extract may contain phenolic compounds not previously identified from the aforementioned organic extracts of maple syrup.

Figure 1A shows the HPLC-UV profile of MS-BuOH which revealed several peaks at 280 and 360 nm which are characteristic of phenolic compounds. The extract was subjected to a series of chromatographic isolation procedures to yield twenty-three (1-23) phenolics. Figure 1B shows the HPLC-UV profile of the purified isolates all combined into a single injection. All of the compounds were identified based on their $^1$H and/or $^{13}$C NMR and mass spectral data and by correspondence to published literature data where available (Table 1). Figure 2 shows the structures of the compounds and they are grouped into their individual phenolic sub-classes for ease of discussion as follows.

Lignans. Seven lignans were isolated from MS-BuOH and identified as lyoniresinol (1), secoisolariciresinol (2), dehydroconiferyl alcohol (also known as dihydrodehydrodiconiferyl alcohol) (3), 5′-methoxydehydroconiferyl alcohol (4), erythro-guaiacylglycerol-β-O-4′-coniferyl alcohol (5), erythro-guaiacylglycerol-β-O-4′-dihydroconiferyl alcohol (6), and [3-[4-[[6-deoxy-α-L-mannopyranosyl]oxy]-3-methoxyphenyl]methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3H)-furanone (7).
With the exception of dehydroconiferyl alcohol (3), which has been previously reported as a lignin-derived flavor compound in maple syrup (1, 2), this is the first reported occurrence of all of the other lignans in maple syrup. Notably, compound 7 was recently described as a constituent of the hardwood collected from the sugar maple tree, A. saccharum (15), and thus its occurrence in maple syrup is not surprising. Also, apart from dehydroconiferyl alcohol (3), previously found in maple syrup (1, 2), and lyoniresinol (1), previously reported from leaves of A. truncatum (27), this may be regarded as the first reported occurrence of these lignans in the Acer genus.

Lignan-rich foods, such as flaxseed which contains secoisolariciresinol (2), have attracted significant research attention for their biological effects (28, 29). Thus the presence of these compounds in maple syrup is interesting from a human health perspective. However, determination of the levels of these lignans (as well as the other bioactive phenolic sub-classes described below) in different grades of maple syrup consumed by humans, and whether these compounds achieve physiologically relevant levels after maple syrup consumption, would be required to evaluate their impact on human health.

**Coumarins.** Two coumarins, not previously reported from maple syrup, were isolated from MS-BuOH and identified as scopoletin (8) and fraxetin (9). Notably, scopoletin (8), has recently been identified from the wood of A. saccharum (15), and has also been reported from the bark of A. nikoense (30). From a biosynthetic perspective, it is interesting that coumarinolignans have been previously reported from the heartwood of A. nikoense (31), which would account for the occurrence of these two individual phenolic sub-classes, namely coumarins and lignans, in maple syrup.
**Stilbene.** A stilbene was isolated from MS-BuOH and identified as (E)-3,3′-dimethoxy-4,4′-dihydroxystilbene (10). While stilbene glycosides have been previously reported from the leaves of *A. mono* (32), this is the first reported occurrence of a stilbenoid in maple syrup. Foods containing stilbenes have attracted immense public attention for their potential human health benefits due in large part to emerging research on resveratrol, a stilbene present in red wine, grapes, and berries (33).

**Phenolic derivatives.** Thirteen phenolic derivatives were found in MS-BuOH including 2-hydroxy-3′,4′-dihydroxyacetophenone (11), 1-(2,3,4-trihydroxy-5-methylphenyl)-ethanone (12), 2,4,5-trihydroxyacetophenone (13), catechaldehyde (14), vanillin (15), syringaldehyde (16), gallic acid (17), trimethyl gallic acid methyl ester (18), syringic acid (19) syringenin (20), (E)-coniferol (21), C-veratroylglycol (22), and catechol (23). While several of these compounds have been previously found in maple syrup (3, 4), this is the first report of catechaldehyde (14), trimethyl gallic acid methyl ester (18), syringenin (20) and C-veratroylglycol (22) in maple syrup.

**Other unidentified compounds.** It is noteworthy that similar to the observations of Abou Zaid et al (2008), a number of peaks/compounds in maple syrup still remain unidentified (see Figure 1A). Despite starting our initial extraction protocol with 20 L of maple syrup, several compounds were unobtainable either due to rapid degradation/decomposition on our columns and/or low yields.

In addition, we cannot rule out the presence of compounds previously reported in the other organic extracts of maple syrup (3-6), such as ethyl acetate (MS-EtOAc), being present in the MS-BuOH. Towards this end, we conducted HPLC-UV comparisons of the retention times of authentic phenolic standards of several of these previously reported compounds with the
unidentified peaks in Figure 1A, along with comparisons of HPLC-UV chromatograms of MS-BuOH and MS-EtOAc (data not shown). However, due to considerable overlapping and co-elution of compounds in these HPLC-UV profiles, our results were inconclusive. Our future work will include the isolation and identification of compounds in MS-EtOAc in order to have a comprehensive phytochemical/phenolic characterization of maple syrup.

Finally, we speculate that apart from the ‘natural products’ identified here, that there are ‘un-natural, artifacts or process-derived’ compounds present in maple syrup, possibly formed under the conditions of intensive heating involved in transforming sap to syrup. These compounds could potentially be formed in situ as: 1) decomposition/degradation products from the natural compounds, and 2) due to chemical reactions between native and process-derived compounds. Further research to identify these compounds is warranted since their contribution towards the potential health benefits and biological activity of maple syrup may be significant.

**Antioxidant activity.** Phenolic compounds identified from maple syrup and maple syrup extracts have been reported to show antioxidant activity (4, 7, 8). Therefore, MS-BuOH, the sugar fraction of maple syrup, and the pure isolates along with positive controls, vitamin C and the synthetic commercial antioxidant, BHT, were evaluated for antioxidant potential in the DPPH assay (Table 2). Vitamin C (ascorbic acid) and BHT showed IC$_{50}$ values of 58 µM (ca. 17 µg/mL) and 2651 µM (ca. 583 µg/mL), respectively. While the antioxidant activity of the MS-BuOH (IC$_{50}$>1000 µg/mL), the sugar fraction (IC$_{50}$>1000 µg/mL), the stilbene (10), and vanillin (15), all exceeded that of BHT, compounds 11, 12 and 14 all showed superior antioxidant activity compared to vitamin C. Among the diverse phenolic sub-classes of compounds identified in MS-BuOH, the general trend in antioxidant activity was phenolic derivatives, coumarins > stilbene, lignans.
In summary, twenty-three phenolics (1-23) with varying antioxidant activities were isolated and identified from MS-BuOH. Among the isolates, sixteen compounds (1, 2, 4-14, 18, 20 and 22) are being reported from maple syrup for the first time. However, to get a comprehensive phenolic profile and characterization of maple syrup, further isolation work on other extracts (e.g. MS-EtOAc) would be necessary. The results of the current study suggest that the ‘cocktail’ of bioactive phenolics present in Canadian maple syrup may impart potential health benefits to this natural sweetener. However further research would be required to confirm this.

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**ACKNOWLEDGEMENTS**

This project was supported by the Conseil pour le développement de l'agriculture du Québec (CDAQ), with funding provided by Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) program. The Federation of Quebec Maple Syrup Producers participated in the collection and donation of maple syrup samples from Quebec, Canada. Mass spectral data were acquired from an instrument located in the RI-INBRE core facility located at the University of Rhode Island (Kingston, RI, USA) obtained from Grant # P20RR016457 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH).
Table 1. Total Compounds isolated from a Butanol Extract of Canadian Maple Syrup (MS-BuOH) showing those reported for the first time from Maple Syrup.

<table>
<thead>
<tr>
<th>Identification</th>
<th>References of NMR data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  lyoniresinol</td>
<td>(9)</td>
</tr>
<tr>
<td>2  secoisolariciresinol</td>
<td>(10)</td>
</tr>
<tr>
<td>3  dehydroconiferyl alcohol</td>
<td>(11)</td>
</tr>
<tr>
<td>4  5'-methoxydehydroconiferyl alcohol*</td>
<td>(12)</td>
</tr>
<tr>
<td>5  guaiacylglycerol-β-O-4′-coniferyl alcohol*</td>
<td>(13)</td>
</tr>
<tr>
<td>6  guaiacylglycerol-β-O-4′-dihydroconiferyl alcohol*</td>
<td>(14)</td>
</tr>
<tr>
<td>7  [3-[4-[(6-deoxy-α-L-mannopyranosyl)oxy]-3-methoxyphenyl][methyl]-5-(3,4-dimethoxyphenyl)dihydro-3-hydroxy-4-(hydroxymethyl)-2(3H)-furanone*</td>
<td>(15)</td>
</tr>
<tr>
<td>8  scopoletin*</td>
<td>(15)</td>
</tr>
<tr>
<td>9  fraxetin*</td>
<td>(16)</td>
</tr>
<tr>
<td>10 (E)-3,3′-dimethoxy-4,4′-dihydroxystilbene*</td>
<td>(17)</td>
</tr>
<tr>
<td>11 2-hydroxy-3′,4′-dihydroxyacetophenone*</td>
<td>(18)</td>
</tr>
<tr>
<td>12 1-(2,3,4-trihydroxy-5-methylphenyl)-ethanonea,*</td>
<td>-</td>
</tr>
<tr>
<td>13 2,4,5-trihydroxyacetophenonea,*</td>
<td>-</td>
</tr>
<tr>
<td>14 catechaldehyde*</td>
<td>(19)</td>
</tr>
<tr>
<td>15 vanillin</td>
<td>(20)</td>
</tr>
<tr>
<td>16 syringaldehyde</td>
<td>(20)</td>
</tr>
<tr>
<td>17 gallic acid</td>
<td>(21)</td>
</tr>
<tr>
<td>18 trimethyl gallic acid methyl ester*</td>
<td>(22)</td>
</tr>
<tr>
<td>19 syringic acid</td>
<td>(20)</td>
</tr>
<tr>
<td>20 syringenin*</td>
<td>(20)</td>
</tr>
<tr>
<td>21 (E)-coniferol</td>
<td>(23)</td>
</tr>
<tr>
<td>22 C-veratroylglycol*</td>
<td>(24)</td>
</tr>
<tr>
<td>23 catechol</td>
<td>(25)</td>
</tr>
</tbody>
</table>

aNMR data provided for the first time herein
*First report from maple syrup
Table 2. Antioxidant Activities of Pure Compounds* Isolated from a Butanol Extract of Canadian Maple Syrup (MS-BuOH) showing 50% Inhibitory Concentrations (IC$_{50}$) in the Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Assay. Values are means ± Standard Deviations. BHT= the synthetic commercial antioxidant, Butylated Hydroxytoluene.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101.5 ± 5.9</td>
<td>12</td>
<td>31.3 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>147.9 ± 3.6</td>
<td>14</td>
<td>35.5 ± 3.7</td>
</tr>
<tr>
<td>3</td>
<td>1040.9 ± 103</td>
<td>15$^b$</td>
<td>&gt;2600</td>
</tr>
<tr>
<td>4</td>
<td>136.7 ± 3.9</td>
<td>16$^a$</td>
<td>357.1</td>
</tr>
<tr>
<td>5</td>
<td>943.5 ± 21.9</td>
<td>17</td>
<td>20.3 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>1335.9 ± 47.6</td>
<td>19</td>
<td>191.85±20.99</td>
</tr>
<tr>
<td>7</td>
<td>679.3 ± 45.6</td>
<td>21$^a$</td>
<td>115</td>
</tr>
<tr>
<td>8</td>
<td>68.2 ± 31.2</td>
<td>22</td>
<td>641 ±1 0.6</td>
</tr>
<tr>
<td>9</td>
<td>46.5 ± 3.6</td>
<td>23</td>
<td>89.5 ± 2.7</td>
</tr>
<tr>
<td>10$^b$</td>
<td>&gt;2600</td>
<td>vitamin C</td>
<td>58.6 ± 10.7</td>
</tr>
<tr>
<td>11</td>
<td>51.8 ± 8.1</td>
<td>BHT</td>
<td>2651.5 ± 285.9</td>
</tr>
</tbody>
</table>

$^a$Only tested once because of the quantity
$^b$Stated as >2600 µM when IC$_{50}$ values of sample exceeds that of BHT; MS-BuOH and sugar fraction of maple syrup had IC$_{50}$ values >1000 µg/mL.

*All compounds were evaluated except 13, 18, and 20 (because of limited sample quantity)
Figure 1. HPLC-UV chromatogram of: 1A. Butanol extract of Canadian maple syrup (MS-BuOH); 1B. Twenty-three phenolic compounds isolated and identified in MS-BuOH.

Figure 2. Structures of phenolic compounds (1-23) isolated and identified from a butanol extract of Canadian maple syrup (MS-BuOH).
Figure 2