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Antioxidant activity and polyphenol composition of sugarcane molasses extract

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ABSTRACT

The antioxidant activity of sugarcane molasses ethanol extract (ME) and its fraction (ME-RBF) was evaluated using ABTS, ORAC 6.0 and CAA assays and ME-RBF demonstrated 26-fold, 12-fold and 2-fold higher values, respectively than ME. Likewise, total polyphenol and flavonoid concentration in ME-RBF are more than 10-fold higher than ME, that suggested antioxidant activity is correlated with polyphenol composition. Quantitative analysis of 13 polyphenols (chlorogenic acid, caffeic acid, sinapic acid, syringic acid, vanillin, homoorientin, orientin, vitexin, swertisin, diosmin, apigenin, tricin and diosmetin) was carried out by LCMS. MS/MS analysis allowed the tentative identification of seven apigenin-*C*-glycosides, three methoxyluteolin-*C*-glycosides and three tricin-*O*-glycosides some of which have not been reported in sugarcane before to the best of our knowledge. The results demonstrated that sugarcane molasses can be used as potential source of polyphenols that can be beneficial to health.

1. Introduction

Sugarcane (*Saccharum officinarum*) molasses is an undervalued process stream product of the sugar mill industry. It has GRAS (generally recognised as safe) classification and has found use in the food industry as an alternative sweetener (Edwards, Rossi, Corpe, Butterworth, & Ellis, 2016; Phillips, Carlsen, & Blomhoff, 2009). In the last decade, there has been a growing interest in the health benefits of sugarcane juice and molasses. A metabolic study in rats found that molasses lowered the peak and global responses of glucose, and improved insulin, amylin and gastric inhibitory polypeptide after oral ingestion (St-Pierre et al., 2014). In a human study, it was found to reduce plasma glucose and insulin responses to carbohydrate (Wright, Ellis, & Ilag, 2014). Recent preclinical studies on a polyphenol rich sugarcane extract showed therapeutic potential to regulate carbohydrate metabolism and protect against metabolic disorders such as type-2 diabetes by upregulation of insulin production in dysfunctional pancreatic cells and modulate glucose and fructose transport across epithelial membranes in Caco-2 cells (Ji, Yang, Flavel, Shields, & Kitchen, 2019). Sugarcane molasses was reported to act as anti-mutagen in a bacterial model and had shown inhibitory effect on nitric oxide production in lipopolysaccharide stimulated macrophages that suggested anti-inflammatory activity (Wang et al., 2011).

The protective effect of sugarcane molasses against DNA oxidative damage was demonstrated *in vitro* against an induced oxidative stress in human HepG2 cells that had comparable effect with positive control α-tocopherol, which is a potent antioxidant acting as peroxyl radical scavenger (Valli et al., 2012). The inhibitory effect of sugarcane molasses against DNA oxidative damage was also observed by decreas-

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ing deoxyribose degradation and DNA scission, which directly correlated with the antioxidant activity of the molasses fractions tested (Guimarães et al., 2007). Several groups have reported the antioxidant activity of sugarcane molasses (Asikin et al., 2013; Guan et al., 2014; Wang et al., 2011; Yu, Xu, & Yu, 2017).

Polyphenols have been demonstrated to exhibit antioxidant activity. However, they have activities beyond the ability to scavenge free radicals. They play a role in regulating carbohydrate metabolism, enzyme inhibition and in prevention of diseases such as cardiovascular diseases and neurodegenerative diseases (Ji et al., 2019; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005). Polyphenols can be quantified by spectrophotometric analysis with Folin-Ciocalteu's phenol reagent (Kim, Jeong, & Lee, 2003) that offers a rapid assessment of the total polyphenol composition of an extract. However, this assay also has a disadvantage of responding to other reducing compounds present in the sample being tested such as reducing sugars, certain amino acids, dehydroascorbic acid that can result to an overestimation of measured values (Capanoglu, Kamiloglu, Ozkan, & Apak, 2017; Sánchez-Rangel, Benavides, Heredia, Cisneros-Zevallos, & Jacobo-Velázquez, 2013).

To have a better understanding of the types of polyphenols present in an extract and to determine the identity of these compounds, a widely used method is reversed-phase high performance liquid chromatography (RP-HPLC) with diode array detector since polyphenols have distinct chromophores (Leme et al., 2014). However, polyphenols can be glycosylated, which usually makes the differentiation of glycosides with the same aglycone problematic. Liquid chromatography coupled to mass spectrometry (LCMS) is an alternative method of analysis that provides information on molecular mass and structural features, whereby detailed information obtained from fragment ions and fragmentation patterns can be used to identify the compounds (Ferreres, Gil-Izquierdo, Andrade, Valentão, & Tomás-Barberán, 2007).

Sugarcane molasses is a rich source of polyphenols, but the complex nature of molasses poses a challenge in its characterisation. Because molasses undergoes several processing steps in sugar cane milling, its composition is highly variable. However, determining the chemical composition will provide guidance to further explore the reported biological activities associated with sugarcane molasses. The aim of this study is to assess the antioxidant activity of molasses against several ROS using different assays such as ABTS, ORAC 6.0 and CAA, and to determine its polyphenol composition using routine colorimetric methods and mass spectral analysis.

2. Materials and methods

2.1. Sample and sample preparation

Sugarcane molasses ethanol extract was provided by The Product Makers (Keysborough, Victoria, Australia) that was prepared using a proprietary process. Briefly, sugarcane molasses sample was diluted to 50 °Bx with water. Food grade ethanol was added to a concentration of 74–76% and the mixture was allowed to stand to settle the gelatinous precipitate. The supernatant was recovered, filtered and then concentrated *in vacuo* at 40–45 °C as the molasses ethanol extract (ME). A subsample of ME (approximately 20 mL) was taken and immediately sent for LCMS analysis and the bulk ME was stored in the fridge at 5 °C until further analysis. The subsample of ME was immediately prepared for analysis upon receipt at the Centre for AgriBioscience and the rest of the sample was stored in the fridge at 5 °C.

ME (1 mL) was transferred into pre-weighed vials, freeze-dried (Christ Alpha 1–4 LO plus, John Morris Scientific Pty Ltd., Australia) for 3 days and then the dried extracts were weighed (Sartorius, Sartorius AG Germany) to obtain sample weight on dry basis (% moisture content = 23.9 ± 0.5). The dried ME was reconstituted in 1 mL of 80:20 methanol-Milli-Q water (v/v), vortexed, sonicated for 5 min and analysed by LCMS.

2.2. Fractionation of ME

2.2.1. Small-scale fractionation

ME was partially purified to remove the sugars and obtain more concentrated phenolic fraction by passing through a C18 solid phase extraction (SPE) cartridge. ME (1 mL) was diluted with Milli-Q water (6 mL) and eluted through a Waters 3 cc SPE Vac C18 cartridge that was initially activated with methanol (MeOH) and then conditioned with Milli-Q water. The polar components were eluted with Milli-Q water (6 mL) which was discarded. The remaining metabolites on the SPE cartridge were then eluted with MeOH (2 × 3 mL) into a pre-weighed vial and the solvent was evaporated to dryness under nitrogen gas. The ME SPE-MeOH fraction was further dried overnight in the freeze dryer and then weighed to obtain the dry weight of fraction. The extract was reconstituted in 200 μL 80:20 MeOH-H_2O (v/v) and analysed by LCMS.

2.2.2. Large-scale fractionation

ME was fractionated using Amberlite FPX66 resin (Dow Chemical Company, USA). Prior to use the resin was successively washed with deionised water, food grade ethanol (95%) and finally with deionised water. The washed resin was filtered under vacuum through a Buchner Funnel with glass microfiber filter paper, 90 mm diameter (Westlab Pty Ltd, VIC, Australia). ME was diluted to 20 °Bx with deionised water and mixed at room temperature until homogeneous. The washed resin was added to the diluted ME at a ratio of 500 g wet resin:1 L of diluted ME and stirred gently for 10 min, then filtered under vacuum through a Buchner Funnel with filter paper. The resin with the bound polyphenols was washed twice with deionised water by resuspension in 1 L of deionised water and then filtered. The filtrate and water washings were discarded. The resin (with the bound polyphenols) was suspended in 1 L of 70% ethanol, stirred for 10 min and filtered under vacuum through a Buchner Funnel with filter paper. This step was repeated three times and the filtrates were combined and concentrated *in vacuo* at 40–45 °C, which is the ME resin-bound fraction (ME-RBF). ME-RBF was analysed for antioxidant activity, total polyphenols, total flavonoids and by LCMS.

2.3. Total polyphenol analysis

The total polyphenol content was determined using the Folin-Ciocalteu colorimetric method with gallic acid as reference standard as described by Kim et al. (2003) with modifications. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Gallic acid standard solutions were prepared at a concentration range of 40 to 200 mg/mL in distilled water. Weighed samples were initially diluted with distilled water to 50 °Bx, which were further diluted 1:200 (v/v) with distilled water. A 0.20 mL aliquot of each of the diluted samples and standard solutions were made up to 2 mL with distilled water in separate tubes and mixed on a vortex mixer. Folin-Ciocalteu reagent (0.20 mL) was added into each tube, mixed thoroughly for 5 min, then 2 mL of 7% sodium carbonate was added. Distilled water (0.8 mL) was added into each tube, mixed, and the mixture was allowed to stand at room temperature for 90 min for complete colour development. The absorbance was measured at 750 nm on a UV–Vis spectrophotometer and the polyphenol content was extrapolated from the gallic acid calibration curve. Total polyphenol was reported as gallic acid equivalent (GAE)/g of sample. Analysis was carried out in triplicate.

2.4. Total flavonoid analysis

The total flavonoid content was determined using the aluminium chloride colorimetric method with catechin as reference standard as described by Zhishen, Mengcheng, and Jianming (1999) with modifications. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Catechin standard solutions were prepared at a concentration range of 50 to 250 mg/mL in distilled water and samples were diluted 1:50 (v/v) with distilled water. A 0.5 mL aliquot of each of the diluted samples and standard solutions were transferred into separate tubes. Into each tube, 2 mL distilled water and 0.15 mL of 5% sodium nitrite solution were added, mixed thoroughly and allowed to stand for 5 min. A 10% aluminium chloride solution (0.15 mL) was added and mixed thoroughly and after 1 min, 1 mL of 1 M NaOH was added. The solutions were diluted by adding 1.2 mL of distilled water then mixed and absorbance was measured at 510 nm on a UV–Vis spectrophotometer. The total flavonoid was reported as catechin equivalent (CE)/g of sample. Analysis was carried out in triplicate.

2.5. Antioxidant activity assays

2.5.1. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) antioxidant assay

The antioxidant capacity of the samples was determined according to the method described by Re et al. (1999) with some modifications. This method determines capacity of the molecules in the test substance to reduce the radical cation of ABTS. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). A mixed solution (1:1, v/v) of 14 mM ABTS and 4.9 mM potassium persulfate in distilled water was prepared 16 h before analysis and stored in a cool, dark place to develop the ABTS radical. A stock solution of 100 μg/mL gallic acid in distilled water was prepared and a concentration range of 5, 10, 15, 20 and 25 μg/mL was used for the calibration curve. Samples were prepared by diluting with distilled water (1:500, v/v).

Three mL aliquots of the prepared ABTS radical solution were dispensed into clean test tubes and the tubes were incubated in a water bath at 26 °C for 5 min. One hundred fifty μL of each of the standards, samples and blank (distilled water) were added into separate test tubes, mixed thoroughly using a vortex mixer and incubated on a water bath at 26 °C for 45 min. The reaction was measured at 734 nm on a UV–Vis spectrophotometer. Antioxidant activity of the samples was calculated from the gallic acid (GA) standard calibration curve using a linear regression equation from the plot of GA concentration and corresponding corrected absorbance reading (absorbance reading minus the blank reading). Values were expressed as mg gallic acid equivalent (GAE)/g of sample. Analysis was carried out in triplicate.

2.5.2. Oxygen radical absorbance capacity (ORAC) assay

The *in vitro* assay ORAC 6.0 was contracted to Brunswick Laboratories, 200 Turnpike Road Southborough, MA 01772, USA (www.brunswicklabs.com). The ORAC assay measured the antioxidant capacity of the test samples against the reactive oxygen species (ROS), such as peroxyl radical, hydroxyl radical, peroxynitrite radical, superoxide anion, singlet oxygen radical and hypochlorite and the total antioxidant values for these six ROS is referred to as ORAC 6.0. The ORAC values were measured using fluorescein (3′,6′-dihydroxyspiro[2-*benzofuran*-3,9′-*xanthene*]-1-one) as the fluorescent probe (Ou, Hampsch-Woodill, & Prior, 2001; Zhang et al., 2009). The sample was prepared at 5.8 mg/mL in 75 mM phosphate buffer solution (PBS; GE Healthcare Life Sciences, PA, USA) for the assay. The fluorescence was measured at excitation wavelength of 485 ± 20 nm and emission filter of 510 ± 30 nm on a Synergy™ HT multi-detection microplate reader (BioTek Instruments Inc, VT, USA). The test was carried out in duplicate and values were reported as μmoles Trolox equivalent (TE)/g of sample.

2.5.3. Cellular antioxidant activity (CAA) assay

The CAA assay was contracted to Brunswick Laboratories, 200 Turnpike Road Southborough, MA 01772, USA (www.brunswicklabs.com). This assay measures the capacity of a test substance to protect a fluorescent probe (fluorescein) from damage by ROS (peroxyl radical) in intracellular environment using human HepG2 cells (McDowell, Thompson, Stark, Ou, & Gould, 2011; Wolfe & Liu, 2007). Human HepG2 cells (Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, China) were grown in Dulbecco's modified Eagle's medium (DMEM; GE Healthcare Life Sciences, PA, USA) supplemented with 0.1 mg/mL penicillin–streptomycin (Sigma Aldrich, MO, USA) and were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Cell cytotoxicity of ME and ME-RBF to HepG2 cells was tested using a Promega CellTiter-Glo® luminescent assay kit (Promega US, Madison, WI, USA). The concentration of ME and ME-RBF tested were 2.9 to 23.2 mg/mL, which were diluted in DMEM. The assay was carried out in duplicate.

For the CAA assay, ME and ME-RBF were diluted in DMEM and the concentrations used for the assay were 0.18 to 5.8 mg/mL, which are not cytotoxic to the cells. HepG2 cells were seeded at 6×10^4/well on a 96-well flat-bottom plate in 100 μL of DMEM and incubated for 24 h, after which DMEM was removed and the wells washed with PBS. Wells were incubated for 1 h with 100 μL of the test sample plus 25 μM 2′,7′-dichlorofluorescin diacetate (DCFH-DA; Sigma Aldrich, MO, USA) dissolved in DMEM. Fluorescence was measured at excitation wavelength of 485 nm and emission filter of 538 nm on a Synergy™ HT multi-detection microplate reader (BioTek Instruments Inc, VT, USA). The assay was carried out in duplicate and values were reported as μmoles quercetin equivalent (QE)/g of sample.

2.6. Analytical standards used for quantitative analysis

Reference standards of chlorogenic acid (**S01**), caffeic acid (**S02**), syringic acid (**S03**), vanillin (**S04**), sinapic acid (**S07**), vitexin (**S08**), diosmin (**S10**) and diosmetin (**S13**) were purchased from Sigma Aldrich (St. Louis, MO, USA); homoorientin (**S05**), orientin (S06), apigenin (**S11**) and tricin (**S12**) were from Chromadex (Irvine, CA, USA); and swertisin (S09) was from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Stock solution of each standard was prepared in methanol (HPLC grade, Thermo Scientific, USA) and subsequently, mixtures of the standards were prepared in 80:20 methanol-Milli-Q water (v/v) with concentrations ranges of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, and 50 μg/mL for quantitative analysis. Syringic acid was prepared at higher concentrations of 3, 6, 12, 30, 60, 120, 300 and 600 μg/mL and diosmin at 20, 30, 50, 100, 200 and 500 μg/mL. The LOQ in μg/mL for each analyte: **S01** ≤ 1.0; **S02** ≤ 0.05; **S03** ≤ 3; **S04** ≤ 0.05; **S05** ≤ 0.02; **S06** ≤ 0.1; **S07** ≤ 0.1; **S08** ≤ 0.02; **S09** ≤ 0.02; **S10** ≤ 1; **S11** ≤ 0.001; **S12** ≤ 0.01; **S13** ≤ 0.01.

2.7. Liquid chromatography-mass spectroscopy (LCMS)

Analysis was carried out on an Agilent 1290 Infinity UHPLC system (Agilent Technologies, USA) with solvent degasser, binary pump, temperature-controlled sampler/auto injector (maintained at 15 °C) and temperature regulated column compartment maintained at 30 °C, coupled with LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). The UHPLC system was controlled by ChemStation B.04.03-SP2 and the MS was controlled by Xcalibur software version 3.0.63. Separation was carried out on a Thermo Hypersil Gold C_{18} column, 1.9 μ, 150 mm × 2.1 mm (Thermo Fisher Scientific Inc.,

MA, USA). Mobile phases used were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) (Optima® LCMS grade, both purchased from Thermo Fisher Scientific Inc., MA, USA) using the following gradient program: 2 to 20% B, 0–15 min; 20 to 100% B, 15–25 min; 100% B, 25–30 min; 100 to 2% B, 30–30.2 min; 2% B, 30.2–35 min, with 0.3 mL/min flow rate. Injection volume was 3 μL.

A divert valve at the front of the MS was in use with the first 6 min of the run (contained sugars and highly polar metabolites) diverted to waste. MS analysis was carried out using an electrospray ionisation (ESI) interface in negative ion mode. For quantitative analysis, resolution was set at 30,000 in full scan mode and mass range measured from *m/z* 80 to 2000. Nitrogen was used as the sheath and auxillary gas at a flow rate of 60 and 20 L/min, respectively. Spray voltage was 3.2 kV, and the capillary and source heater temperatures were 300 and 250 °C; respectively. MS data were processed using LCquan 2.7.0.20 and Xcalibur Qual Browser v2.3.26 (Thermo Fisher Scientific Inc., MA, USA). Peak identity was determined by comparison of retention time with reference standards and extracted ion technique. The concentration of each analyte was determined by integration of peaks and interpolation from standard calibration curves using Excel worksheet. Results were expressed as average concentration ± standard deviation (SD).

MS/MS analysis of ME-RBF was carried out using the same LCMS system and chromatography parameters as above. MS^2-MS^3 activation is by collision-induced dissociation (CID) with normalised collision energy of 35 and resolution set at 7500 in full scan mode with mass range measured from *m/z* 80 to 2000. Nitrogen was used as the sheath and auxillary gas at a flow rate of 37 and 13 L/min, respectively. Spray voltage was 3.3 kV, and the capillary and source heater temperatures were 300 and 350 °C; respectively. MS data were processed using Xcalibur Qual Browser v3.0.63 (Thermo Fisher Scientific Inc., MA, USA).

2.8. Statistical analysis

Reported values were calculated using Microsoft Office Excel software and standard deviations of duplicate or triplicate analyses were calculated using the Statistical function of Microsoft Office Excel.

3. Results

3.1. Quantitative analysis of polyphenols

The total polyphenol (PP) and total flavonoid content of ME were assessed using colorimetric methods and the values obtained were 17.4 mg GAE/g and 5.2 mg CE/g, respectively (Table 1). A study of natural sweeteners by St-Pierre et al. (2014) reported a total polyphenol content of 92 μg GAE/mL for an ethyl acetate extract of molasses that included phenolic acids, flavonoids, lignans, and phlorizin. ME is a molasses ethanol extract that has an average density of 1.35 g/mL and thus has an equivalent total PP of 23,490 μg GAE/mL. This demonstrates that ethanol as a solvent has better extracting ability for polyphenols than ethyl acetate.

Preliminary LCMS profiling of ME showed a complex chromatogram with dominant peaks eluting in the first few minutes of the chromatogram indicating the presence of polar metabolites, which are most likely sugars (data not shown) and were thus excluded from the analysis. A sample clean up using a C18 SPE cartridge was carried out to remove most of the sugars and/or highly polar metabolites and at the same time concentrate the polyphenols in the extract. Preliminary MS analysis had identified 13 polyphenols in ME by comparison of retention time and mass spectrum with reference standards and quantitative analysis of 13 polyphenols was carried out by LCMS for ME and its SPE MeOH fraction (Fig. 1a and b, respectively) using 13 external reference standards. The results are summarised in Table 2 and the chemical structures of the compounds quantified in ME and fraction are shown in Fig. 2.

Table 1
Total polyphenols, total flavonoid and antioxidant activity of sugarcane molasses ethanol extract (ME) and its fraction ME-RBF.

Analysis	ME	ME-RBF
Total polyphenols, mg GAE/g	17.4 ± 1.4	205.0 ± 7.0
Total flavonoids, mg CE/g	5.2 ± 0.4	55.0 ± 1.7
Antioxidant assays		
ABTS, mg GAE/g sample	2.3 ± 0.1	60 ± 10
ORAC 6.0 [a], μmole TE/g sample	1599	19,437
ORAC against peroxyl radicals [b]	260 ± 18	2336 ± 233 [c]
ORAC against hydroxyl radicals [b]	808 ± 121	13,785 ± 988 [c]
ORAC against peroxynitrite [b]	49 ± 1	255 ± 13 [c]
ORAC against superoxide anion [b]	110 ± 11	450 ± 12 [c]
ORAC against singlet oxygen [b]	278 ± 33	2011 ± 60 [c]
ORAC against hypochlorite [b]	94 ± 9	600 ± 46
Cellular antioxidant activity (CAA), μmole QE/g sample	91.5 ± 1.8	229 ± 28 [c]

[a] ORAC 6.0 represents the sum of ORAC values against peroxyl radicals, hydroxyl radicals, peroxynitrite, superoxide anion, singlet oxygen and hypochlorite anion.
[b] ORAC values reported as μmole TE/g sample.
[c] Published data (Ji et al., 2019).

Diosmin was observed to have the highest concentration (19.45 μg/g) in ME followed by syringic acid (10.90 μg/g) and chlorogenic acid (6.53 μg/g). The concentration of the other 10 polyphenols analysed were of varying proportions all at less than 1 μg/g. Sample clean up by C18 SPE resulted in an increase in the concentration of all the 13 polyphenols in the SPE MEOH fraction (Table 2). Syringic acid had the highest concentration (182.15 μg/g) followed by chlorogenic acid (37.76 μg/g) and diosmin (37.57 μg/g). All the other 10 polyphenols exhibited at least a 10-fold increase in concentration in the SPE MEOH fraction compared to its extract ME, with the concentration of apigenin consistently the lowest for both ME and its SPE MeOH fraction. The polyphenols were concentrated in the SPE MeOH fraction with a total polyphenol concentration of 307.03 μg/g, which is 8-fold higher than that of its source ME (38.74 μg/g).

A large-scale fractionation of ME was carried out using Amberlite FPX66 resin that yielded the resin bound fraction (ME-RBF). The total PP and total flavonoid contents of ME-RBF was analysed by colorimetric methods and ME-RBF gave a total PP of 205 mg GAE/g and total flavonoids of 55 mg CE/g (Table 1), which were more than 10-fold higher than that of ME. The total PP and total flavonoid of a polyphenol-rich sugarcane extract together with its physical properties were recently reported by Ji et al. (2019).

3.2. Antioxidant activity of sugarcane molasses extracts

ME and ME-RBF gave antioxidant values of 2.3 and 60 mg GAE/g, respectively, with the latter showing more than 25-fold higher activity than the former (Table 1). Further antioxidant assays were carried out on ME and ME-RBF using ORAC assays for different ROS such as peroxyl, hydroxyl, peroxynitrile, superoxide anion, singlet oxygen and hypochlorite (Table 1). The ORAC 6.0 value of ME-RBF is 19,437 μmole TE/g, which is more than 12-fold higher than the ORAC 6.0 value of ME (1599 μmole TE/g). The ORAC 5.0 data for polyphenol-rich sugarcane extract (peroxyl radical, hydroxyl radical, peroxynitrite radical, superoxide anion and singlet oxygen radical) were recently reported by Ji et al. (2019). The ORAC values for individual ROS showed an increase in antioxidant activity in ME-RBF compared to ME (Table 1) that suggested the concentration of the antioxidant metabolites in ME-RBF. Among the ROS, both ME and ME-RBF showed the highest ORAC value against hydroxyl radicals. To further assess the antioxidant potential of ME and ME-RBF in a cellular system using human HepG2 cells, CAA was determined. ME-RBF exhibited CAA value of 229

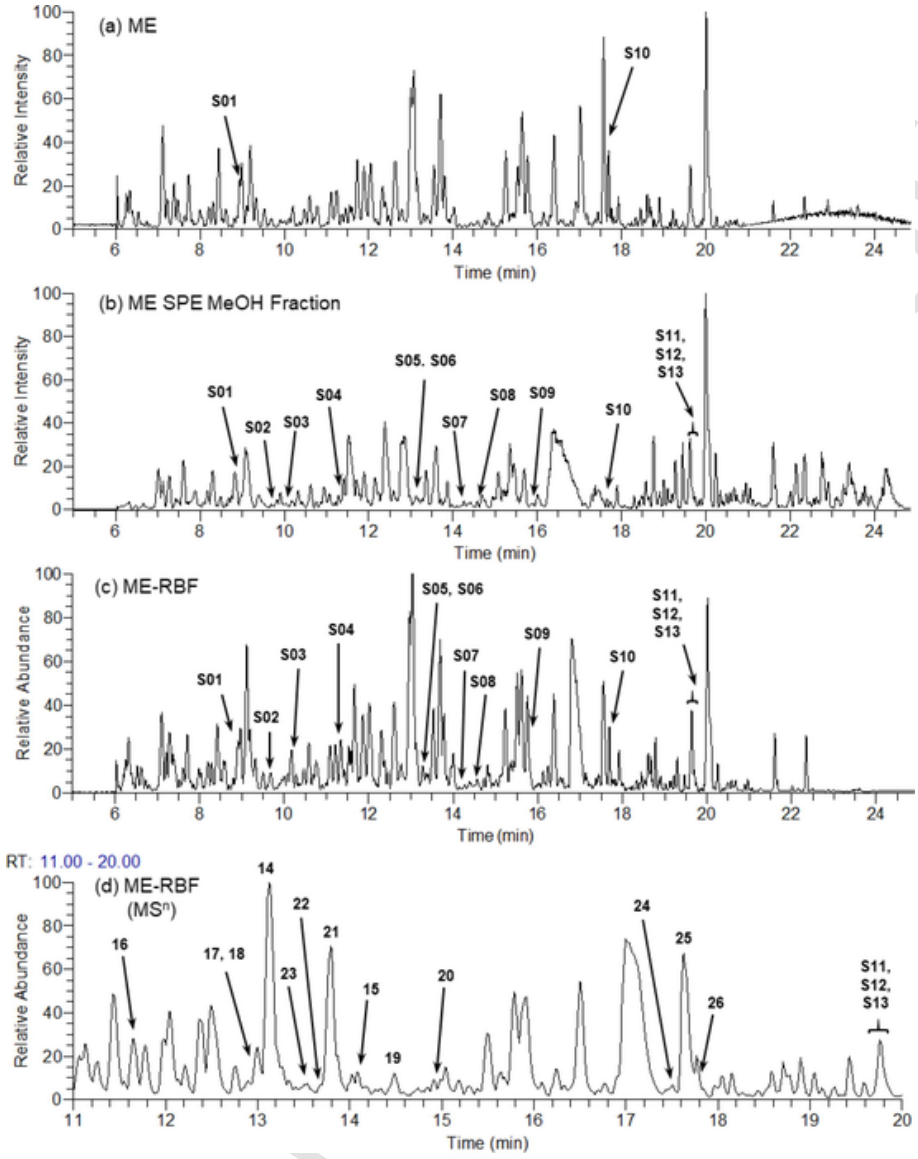


Fig. 1. LCMS chromatogram (full scan, negative mode, from 6 min retention time) of: (a) sugarcane molasses ethanol extract (ME); (b) ME SPE MeOH fraction; (c) ME-RBF; and (d) expanded 10–20 min region of ME-RBF (MS/MS). Labelled peaks **S01** to **S13** were identified by comparison with reference standards: **S01** – chlorogenic acid; **S02** – caffeic acid; **S03** – syringic acid; **S04** – vanillin; **S05** – homoorientin; **S06** – orientin; **S07** – sinapic acid; **S08** – vitexin; **S09** – swertisin; **S10** – diosmin; **S11** – apigenin; **S12** – tricin; **S13** – diosmetin. Labelled peaks **14** to **26** were tentatively identified by MS/MS analysis: **14** – isoschaftoside; **15** – schaftoside; **16** – apigenin-6,8-*C*-diglucoside; **17** – apigenin-6″-*O*-glucosyl-8-*C*-glucoside; **18** – schaftoside-2″-*O*-glucoside; **19** – apigenin 6-*C*-rhamnosyl-8-*C*-glucoside; **20** – apigenin-6,8-*C*-diarabinoside; **21** – methoxyluteolin-8-*C*-glucoside; **22** – methoxyluteolin-6″-*O*-rutinosyl-8-*C*-glucoside; **23** – methoxyluteolin-6″-*O*-glucosyl-8-*C*-glucoside; **24** – tricin 7-*O*-neohesperidoside; **25** – tricin 7-*O*-rhamnosylglucuronide; 2**6** – tricin-7-*O*-glycoside.

μmole QE/g (Ji et al., 2019), which is almost 3-fold higher than that of ME (91.5 μmole QE/g).

3.3. Analysis of ME-RBF

The LCMS chromatogram (full scan, negative mode) of ME-RBF is shown in Fig. 1c. Using extracted ion technique and comparison of retention time with reference standards, the 13 polyphenols quantified in ME were all found to be present in ME-RBF. Analysis showed diosmin concentration is higher in ME-RBF (227 μg/g) compared to ME (Table 2). Syringic acid (108 μg/g) and chlorogenic acid (74 μg/g) was higher in ME-RBF compared to ME and the concentration of the other 10 polyphenols in ME-RBF were of varying proportions.

MS/MS analysis was carried out to identify some of the compounds in ME-RBF and Fig. 1d shows the expanded region of the chromatogram (11–20 min) of ME-RBF. The dominant peak at 13.1 min retention time the mass spectrum gave $(M-H)^-$ of *m/z* 563.1395 (Table 3), which corresponded to a chemical formula of $C_{26}H_{27}O_{14}$. The molecular radical ion $(M\bullet)^-$ for this peak was observed with *m/z* 564.1461 that corresponded to a molecular formula of $C_{26}H_{28}O_{14}$. Analysis of the MS^2 fragmentation of this peak showed an ion fragment *m/z* 545.1325 that corresponded to a loss of water $(M-H-H_2O)^-$. The other fragment ions observed were *m/z* 503, 473, 443, 383, 353 that corresponded to $(M-H-60)^-$, $(M-H-90)^-$, $(M-H-120)^-$, $(M-H-180)^-$, and $(M-H-210)^-$, respectively (Table 3). The observed loss of masses in the fragment ions are characteristic of *C*-glycosides and these $^{k,l}X$ fragments (fragment nomenclature based on Domon & Costello, 1988) are due to the intraglycosidic cleavages of a hexose or pentose (Guo et al., 2013; Picariello et al., 2017). Furthermore, the occurrence of the $(M-H-60)^-$, $(M-H-90)^-$ and $(M-H-120)^-$ fragment ions further supports the compound to be an asymmetrical di-*C*-glycosyl flavonoid (Becchi & Fraisse, 1989). With a hexose and a pentose as the sugar moieties, this further suggested that the aglycone would have a molecular mass of 270 that could correspond to apigenin. *C*-glycosides

Table 2
Polyphenol composition of sugarcane molasses ethanol extract (ME) and fractions by LCMS analysis.

Analyte	RT, min	Molecular Formula	Exact Mass, $(M-H)^-$	Concentration, μg/g dry weight		
				ME	ME SPE MeOH Fraction	ME-RBF*
Chlorogenic acid (S01)	8.9	$C_{16}H_{18}O_9$	353.0878	6.53 ± 0.04	37.76 ± 2.15	74.29
Caffeic acid (S02)	9.7	$C_9H_8O_4$	179.0350	0.29 ± 0.01	9.10 ± 0.24	7.54
Syringic acid (S03)	10.1	$C_9H_{10}O_5$	197.0455	10.90 ± 0.24	182.15 ± 18.26	107.57
Vanillin (S04)	11.4	$C_8H_8O_3$	151.0401	0.15 ± 0.05	11.77 ± 0.84	2.13
Homoorientin (S05)	13.2	$C_{21}H_{20}O_{11}$	447.0933	0.04 ± 0.00	0.71 ± 0.02	0.58
Orientin (S06)	13.3	$C_{21}H_{20}O_{11}$	447.0933	0.25 ± 0.01	8.54 ± 0.02	4.50
Sinapic acid (S07)	14.3	$C_{11}H_{12}O_5$	223.0612	0.18 ± 0.02	4.98 ± 0.08	1.73
Vitexin (S08)	14.6	$C_{21}H_{20}O_{10}$	431.0984	0.08 ± 0.001	1.85 ± 0.02	1.62
Swertisin (S09)	15.8	$C_{22}H_{22}O_{10}$	445.1140	0.69 ± 0.01	9.50 ± 0.02	5.25
Diosmin (S10)	17.6	$C_{28}H_{32}O_{15}$	607.1668	19.45 ± 1.05	37.57 ± 0.91	227.00
Apigenin (S11)	19.6	$C_{15}H_{10}O_5$	269.0455	0.001 ± 0.000	0.01 ± 0.001	0.01
Tricin (S12)	19.7	$C_{17}H_{14}O_7$	329.0667	0.029 ± 0.001	0.55 ± 0.02	0.40
Diosmetin (S13)	19.7	$C_{16}H_{12}O_6$	299.0561	0.15 ± 0.01	2.56 ± 0.10	0.16
Total Polyphenols				38.74	307.03	432.78

* No duplicate.

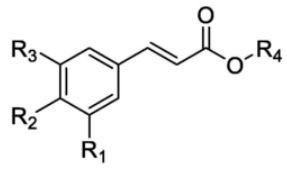
have the sugar substituent(s) usually linked to carbon of the flavonoid aglycone generally at the C-6 and/or C-8 positions; the *O*-glycosides have the sugar substituent(s) linked to the hydroxyl group of the flavonoid aglycone (Guo et al., 2013). Thus, this compound (**14**) at 13.1 min is most probably an apigenin with pentosyl and hexosyl moieties either in C-6 and C-8 positions. Coutinho et al. (2016) reported the presence of several glucose, arabinose, rhamnose and glucuronic acid *C*-glycosides of apigenin, luteolin and diosmetin from sugar cane leaf extracts, and this suggested that the base peak ion fragment *m/z* 473 [(M−H−90)$^-$] could correspond to a cleavage of arabinose ($^{0,2}X_0$), which is a pentose, and the ion fragment *m/z* 443 [(M−H−120)$^-$] to a cleavage of glucose ($^{0,2}X_0$), which is a hexose.

Becchi and Fraisse (1989) studied the mass fragmentation of di-*C*-glycosides by FAB CAD mass-analysed ion kinetic energy and observed that the sugar at the C-6 position of the aglycone undergoes more fragmentation and thus gives the most intense ion fragment. They also reported that for 6-*C* and 8-*C* glycoside isomers, the $^{0,2}X$ cleavage is favoured. Study had shown that the most intense fragment ion in 6-*C*-glycosides is the $^{0,2}X_0$ due to the ease of elimination of water between the hydroxyl groups at the C-2″ position of the C-6 sugar moiety and at C-5 or C-7 positions of the aglycone (Guo et al., 2013). The base peak of compound **14** at *m/z* 473 is equivalent to (M−H−90)$^-$, which has to correspond to $^{0,2}X_0$ of a pentosyl moiety at the C-6 position of apigenin. The second most intense fragment ion is *m/z* 443 which is equivalent to (M−H−120)$^-$ and has to be $^{0,2}X_0$ of a hexosyl at the C-8 position of apigenin. Becchi and Fraisse (1989) used several di-*C*-glycosides as examples including schaftoside (apigenin-6-*C*-glucosyl-8-*C*-arabinoside) and isoschaftoside (apigenin-6-*C*-arabinosyl-8-*C*- glucoside) and the observed base peaks for these two compounds are (M−H−120)$^-$ and (M−H−90)$^-$, respectively. Ferreres, Silva, Andrade, Seabra, and Ferreira (2003) studied *C*-glycosyl flavones from seeds of quince by ion trap ESI/MS/MS and they were able to identify several compounds through their fragmentation patterns that included schaftoside and isochaftoside. The group observed the base peak of schaftoside is (M−H−120)$^-$ and isoschaftoside is (M−H−90)$^-$. By comparison of the mass spectral data of **14** with those reported in the literature, it was deduced that **14** is apigenin-6-*C*-arabinosyl-8-*C*-glucoside or isoschaftoside ($C_{26}H_{28}O_{14}$; exact mass = 564.1474).

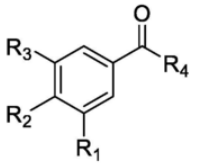
Using extracted ion technique, another peak with $(M-H)^-$ of *m/z* 563.1398 was observed at 14.1 min. Analysis of MS/MS data (Table 3) showed similar fragmentation pattern with **14** except the base peak was observed at *m/z* 443 [(M−H−120)$^-$] that could correspond to ($^{0,2}X$) fragment of a hexose and suggested a glucose linked at the C-6 position of the aglycone. Thus, this peak at 14.1 min was deduced to be apigenin-6-*C*- glucosyl-8-*C*-arabinoside or schaftoside (**15**). The elution order of **14** and **15** on a C18 analytical LC column is consistent with what have been reported in the literature (Ferreres et al., 2003; Picariello et al., 2017).

Several apigenin glycosides were tentatively identified based on the observed fragmentation patterns and fragment ion masses (Table 3). Two peaks with $(M-H)^-$ of *m/z* 593.15 at 11.7 and 12.9 min were tentatively identified as apigenin with two hexosyl substituents most probably glucose since it is the most common hexose reported in molasses. The peak at 11.7 min (**16**) showed a base peak at *m/z* 473, which could correspond to (M−H−120)$^-$ that is typical of $^{0,2}X$ fragmentation of a *C*-linked hexose and another fragment ion *m/z* 503 could correspond to (M−H−90)$^-$ or $^{0,3}X$ fragmentation. Fragment ions *m/z* 383 and 353 are equivalent to (A + 113)$^-$ and (A + 83)$^-$ that corresponds to the aglycone and the residue of sugar that remained linked to it and thus suggesting the presence of two hexose units with *C*-linkage to the aglycone and thus confirming apigenin as the aglycone. This peak was tentatively identified as apigenin-6,8-*C*-diglucoside (**16**). The peak at 12.9 min (**17**) showed a base peak at *m/z* 311 that is equivalent to (M−H−162−120)$^-$, which suggested an *O*-linked hexose to another hexose that is *C*-linked to the aglycone. The interglycosidic linkages commonly observed is either (1 → 2) or (1 → 6). The presence of the fragment ion *m/z* 341 in **17** that is equivalent to (M−H−162−90)$^-$ suggested that the *O*-linked hexose is not in the C-2″ position of the *C*-linked hexose but most probably at the C-6″ position. The aglycone was deduced to be apigenin and the *C*-linkage is most probably at the C-8 position of the aglycone since there is no observed loss of water molecule in the ion fragments that is typical of C-6 glycosides of apigenin (Coutinho et al., 2016; Ferreres et al., 2007). Compound **17** was tentatively identified as apigenin-6″-*O*-glucosyl-8-*C*-glucoside or vitexin-2″-*O*-glucoside.

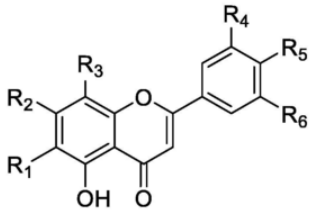
An observed mass at 12.9 min has $(M-H)^-$ of *m/z* 725.1909 that showed a base peak at *m/z* 563, which suggested a schaftoside or isoschaftoside substructure based on other observed fragment ions in this peak. The base peak *m/z* 563 indicated a loss of a hexose moiety [(M−H−162)$^-$] that is most probably glucose, which has to be *O*-linked to another glucose due to the presence of fragment ions *m/z* 443 and 413 that is equivalent to (M−H−162−120)$^-$ and (M−H−162−150)$^-$. These fragment ions further suggest that the interglycosidic linkage



	R_1	R_2	R_3	R_4
Chlorogenic acid (**S01**)	OH	OH	H	quinyl
Caffeic acid (**S02**)	OH	OH	H	H
Sinapic acid (**S07**)	OCH_3	OH	OCH_3	H



	R_1	R_2	R_3	R_4
Syringic acid (**S03**)	OCH_3	OH	OCH_3	OH
Vanillin (**S04**)	OCH_3	OH	H	H



	R_1	R_2	R_3	R_4	R_5	R_6
Homoorientin (**S05**)	C-Glc	OH	H	OH	OH	H
Orientin (**S06**)	H	OH	C-Glc	OH	OH	H
Vitexin (**S08**)	H	OH	C-Glc	H	OH	H
Swertisin (**S09**)	C-Glc	OCH_3	H	H	OH	H
Diosmin (**S10**)	H	O-Rutin	H	OH	OCH_3	H
Apigenin (**S11**)	H	OH	H	H	OH	H
Tricin (**S12**)	H	OH	H	OCH_3	OH	OCH_3
Diosmetin (**S13**)	H	OH	H	OH	OCH_3	H
Isoschaftoside (**14**)	C-Ara	OH	C-Glc	H	OH	H
Schaftoside (**15**)	C-Glc	OH	C-Ara	H	OH	H
Scoparin (**21**)	H	OH	C-Glc	OCH_3	OH	H

Fig. 2. Chemical structures of some compounds identified in molasses ethanol extract and fractions.

is glucose (1 → 2) glucose linkage and thus, this compound was tentatively identified as apigenin-2″-*O*- glucosyl-6-*C*-glucosyl-8-*C*-arabinoside or schaftoside-2″-*O*- glucoside (**18**).

A peak at 14.5 min with $(M-H)^-$ of *m/z* 577.1558 gave a base peak at *m/z* 473, which corresponds to $(M-H-104)^-$ that could be equivalent to $^{0,2}X$ fragmentation of a rhamnose. The fragment ion at *m/z* 413 corresponds to $^{0,2}X$ fragmentation of a glucose that is most probably at the C-8 position of the aglycone due to the absence of $(M-H-118)^-$ fragment ion (Ferreres et al., 2003). This compound was tentatively identified as apigenin 6-*C*-rhamnosyl-8-*C*-glucoside or vitexin-6-*C*-rhamnoside (**19**). A peak at 14.9 min showed $(M-H)^-$ of *m/z* 533.1296 and the fragmentation pattern indicated a di-*C*-glycoside with the base peak at *m/z* 443 indicating a $^{0,2}X$ fragmentation of a pentose most probably arabinose. The compound was deduced as apigenin-6,8-*C*-diarabinoside (**20**).

A dominant peak at 13.8 min gave (M–H)- of *m/z* 461.1078 (Table 3). The base peak at *m/z* 341 corresponds to (M–H–120)- that is equivalent to $^{0,2}X$ fragmentation of a hexose, which is most probably glucose that is *C*-linked to an aglycone. The *C*-glycosylation could either be at the C-6 or C-8 position of the aglycone but the absence of a further loss of water molecule suggests glycosylation at the C-8 position of the aglycone (Ferreres et al., 2003). The presence of a hexose (glucose) moiety in this compound indicated an aglycone with molecular mass of 300 that could be a methoxyflavone such as chrysoeriol (3′-methoxyluteolin) or diosmetin (4′-methoxyluteolin), which only differs in the position of the methoxy substituent in the B ring of luteolin. Although this compound can be deduced as a *C*-glucoside, the identification of the aglycone cannot be confirmed. Thus, this compound was tentatively identified as methoxyluteolin-8-*C*-glucoside (**21**), which can either be chrysoeriol-8-*C*-glucoside (scoparin) or diosmetin-8-*C*-glucoside.

By relationship, two other peaks at 13.5 and 13.7 min were observed with *m/z* 461 fragment ion (Table 3) that could correspond to the methoxyluteolin-8-*C*-glucosyl moiety (**21**). The compound at 13.7 min showed $(M-H)^-$ of *m/z* 769.2184 with *m/z* 461 as base peak that is equivalent to $(M-H-308)^-$, which could correspond to a loss of a rutinosyl moiety. The fragment ion *m/z* 341 corresponds to $(M-H-308-120)^-$ that suggests the rutinosyl moiety is *O*-linked to a hexose (probably glucose), which is *C*-linked to the aglycone. The interglycosidic linkage of rutinose and glucose is most likely at the C-6″ position ((1 → 6) linkage) with the glucose *C*-linked to the aglycone methoxyluteolin. This compound was tentatively identified as methoxyluteolin-6″-*O*-rutinosyl-8-*C*-glucoside (**22**). The compound at 13.5 min showed $(M-H)^-$ of *m/z* 623.1615 with *m/z* 341 as base peak (Table 3) that is equivalent to $(M-H-162-120)^-$, which suggests two hexoses that are *O*-linked to each other with one of the hexoses *C*-linked to the aglycone (methoxyluteolin). This further suggests that the interglycosidic linkage is (1 → 6) and this compound was tentatively identified as methoxyluteolin-6″-*O*-glucosyl-8-*C*-glucoside (**23**).

Three tricin glycosides were tentatively identified with $(M-H)^-$ of *m/z* 637.1777, 651.1548 and 491.1183 at 17.5, 17.6 and 17.8 min, respectively. The peaks at 17.5 and 17.6 min both showed a base peak of *m/z* 329 that could correspond to a tricin aglycone. Tricin and its glycosides naturally occur in sugarcane and the most common are the 7-*O*-glycosides (Zhou & Ibrahim, 2010). The compound at 17.5 min showed a loss of 308 that suggests a rhamnosyl and a glucosyl moieties $[(M-H-146-162)^-]$ and was tentatively identified as tricin-7-*O*-neohesperidoside (**24**). The loss of 322 amu in the compound at 17.6 min suggested a rhamnose and glucuronic acid $[(M-H-146-176)^-]$ and this compound was tentatively identified as tricin-7-*O*-rhamnosylglucuronide (**25**). The peak at 17.8 min also gave a fragment ion at *m/z* 329 equivalent to a loss of a glucosyl unit and the base peak at *m/z* 476 corresponds to a loss of a methyl group. This compound was tentatively identified as tricin-7-*O*-glucoside (**26**). Compounds **24**, **25** and **26** have been previously reported from sugarcane leaves (Colombo, Yariwake, & McCullagh, 2008; Coutinho et al., 2016).

4. Discussion

Sugar cane molasses extract (ME) exhibited antioxidant activity and its fractionation resulted to an increased antioxidant activity in ME-RBF. Sugarcane molasses is an alternative sweetener to refined sugar and compared to other sweeteners such as honey, agave nectar, corn syrup, brown rice malt syrup, barley malt syrup, wheat malt syrup, and maple syrup, molasses exhibited the highest FRAP value (Phillips et al., 2009). When compared with sugar beet molasses, sugar cane molasses exhibited higher total antioxidant capacity (TAC) by DPPH assay and greater effectiveness in protecting HepG2 cells from induced oxidative stress (Valli et al., 2012).

A higher total polyphenol and total flavonoid content was observed in ME-RBF compared to ME that suggested a correlation of the polyphenol composition to antioxidant activity. In a study of phytochemical content of two sugarcane cultivars, Feng and co-workers (Feng, Luo, Zhang, Zhong, & Lu, 2014) reported a positive correlation between both total phenolic content and total flavonoid con-

Table 3
Compounds tentatively identified in ME-RBF with the corresponding observed $(M-H)^-$ and fragment ions.

Peak ID	RT (min)	Tentative identification	Observed $(M-H)^-$	*m/z* (% RI)	Fragment description
14	13.1	Apigenin-6-*C*-arabinosyl-8-*C*-glucoside (Isoschaftoside)	563.1395	564 (61)	$(M\cdot)^-$
				545 (21)	$(M-H-H_2O)^-$
				503 (39)	$(M-H-60)^-$
				485 (8)	$(M-H-60-H_2O)^-$
				473 (100)	$(M-H-90)^-$
				455 (7)	$(M-H-90-H_2O)^-$
				443 (91)	$(M-H-120)^-$
				425 (9)	$(M-H-120-H_2O)^-$
				413 (6)	$(M-H-150)^-$
				383 (53)	$(M-H-180)^-$
				365 (6)	$(M-H-180-H_2O)^-$
				353 (85)	$(M-H-120-90)^-$
15	14.1	Apigenin-6-*C*-glucosyl-8-*C*-arabinoside (Schaftoside)	563.1398	545 (16)	$(M-H-H_2O)^-$
				503 (15)	$(M-H-60)^-$
				485 (8)	$(M-H-60-H_2O)^-$
				473 (93)	$(M-H-90)^-$
				455 (19)	$(M-H-90-H_2O)^-$
				443 (100)	$(M-H-120)^-$
				383 (27)	$(M-H-180)^-$
				353 (54)	$(M-H-120-90)^-$
16	11.7	Apigenin-6,8-*C*-diglucoside	593.1503	594 (29)	$(M\cdot)^-$
				575 (11)	$(M-H-H_2O)^-$
				503 (49)	$(M-H-90)^-$
				473 (100)	$(M-H-120)^-$
				413 (7)	$(M-H-180)^-$
				383 (50)	$(A+113)^-$
				353 (79)	$(A+83)^-$
17	12.9	Apigenin-6″-*O*-glucosyl-8-*C*-glucoside (Vitexin-6″-*O*-glucoside)	593.1505	594 (53)	$(M\cdot)^-$
				503 (6)	$(M-H-90)^-$
				473 (42)	$(M-H-120)^-$
				431 (55)	$(M-H-162)^-$
				353 (7)	A + 83
				341 (28)	$(M-H-162-90)^-$
				311 (100)	$(M-H-162-120)^-$
				297 (15)	$(M-H-296)^-$
				283 (23)	$(M-H-310)^-$

Table 3 (*Continued*)

Peak ID	RT (min)	Tentative identification	Observed (M–H)$^-$	*m/z* (% RI)	Fragment description
18	12.9	Schaftoside-2″-*O*-glucoside	725.1909	707 (4)	(M–H–H_2O)$^-$
				665 (7)	(M–H–60)$^-$
				635 (8)	(M–H–90)$^-$
				605 (15)	(M–H–120)$^-$
				563 (100)	(M–H–162)$^-$
				545 (6)	(M–H–180)$^-$
				443 (11)	(M–H–162–120)$^-$
				413 (66)	(M–H–162–150)$^-$
				383 (8)	A + 113
				353 (12)	A + 83
				293 (47)	(M–H–432)$^-$
19	14.5	Apigenin 6-*C*-rhamnosyl-8-*C*-glucoside (Vitexin-6-*C*-rhamnoside)	577.1558	559 (23)	(M–H–H_2O)$^-$
				503 (60)	(M–H–74)$^-$
				487 (10)	(M–H–90)$^-$
				473 (100)	(M–H–104)$^-$
				457 (82)	(M–H–120)$^-$
				439 (9)	(M–H–138)$^-$
				413 (6)	(M–H–104–60)$^-$
				383 (59)	(M–H–104–90)$^-$
				353 (65)	(M–H–104–120)$^-$
20	14.9	Apigenin-6,8-*C*-diarabinoside	533.1296	534 (43)	(M·)$^-$
				515(27)	(M–H–H_2O)$^-$
				473 (61)	(M–H–60)$^-$
				455 (8)	(M–H–60–18)$^-$
				443 (100)	(M–H–90)$^-$
				425 (11)	(M–H–90–H_2O)$^-$
				413 (12)	(M–H–120)$^-$
				383 (26)	(M–H–150)$^-$
				353 (24)	(M–H–180)$^-$
21	13.8	Methoxyluteolin-8-*C*-glucoside	461.1078	462 (10)	(M·)$^-$
				371 (14)	(M–H–90)$^-$
				341 (100)	(M–H–120)$^-$
				313 (74)	(M–H–148).
				298 (25)	(M–H–163)$^-$
22	13.7	Methoxyluteolin-6″-*O*-rutinosyl-8-*C*-glucoside	769.2184	461 (100)	(M–H–308)$^-$
				341 (26)	(M–H–308–120)$^-$
23	13.5	Methoxyluteolin-6″-*O*-glucosyl-8-*C*-glucoside	623.1615	624 (22)	(M·)$^-$
				609 (5)	(M–H–14)$^-$

Table 3 (*Continued*)

Peak ID	RT (min)	Tentative identification	Observed (M−H)⁻	*m/z* (% RI)	Fragment description
				608 (31)	(M−H−15)⁻
				533 (6)	(M−H−90)⁻
				503 (40)	(M−H−120)⁻
				461(51)	(M−H−162)⁻
				445 (5)	(M−H−178)⁻
				443 (9)	(M−H−180)⁻
				383 (8)	(M−H−240)⁻
				371 (21)	(M−H−252)⁻
				341 (100)	(M−H−162−120)⁻
				327 (20)	(M−H−296)⁻
				313 (8)	(M−H−310)⁻
				312 (10)	(M−H−311)⁻
				298 (20)	(M−H−325)⁻
24	17.5	Tricin 7-*O*-neohesperidoside	637.1777	329 (100)	(M−H−308)⁻
				314 (14)	(M−H−323)⁻
25	17.6	Tricin 7-*O*-rhamnosyl-glucuronide	651.1548	329 (100)	(M−H−322)⁻
				314 (16)	(M−H−337)⁻
				299 (11)	(M−H−352)⁻
26	17.8	Tricin-7-*O*-glycoside	491.1183	476 (100)	(M−H−CH_3)⁻
				461 (7)	(M−H−30)⁻
				343 (8)	(M−H−148)⁻
				329 (29)	(M−H−162)⁻
				314 (6)	(M−H−150)⁻

tent with DPPH and FRAP activities. A similar trend was reported by Kadam and co-workers for sugarcane juice (Kadam et al., 2008).

Among the 13 analytes (**S01** to **S13**) that were quantitatively determined by LCMS in sugarcane molasses samples, diosmin gave the highest concentration followed by syringic acid and chlorogenic acid. These compounds exhibit antioxidant activities (Cikman et al., 2015; Naveed et al., 2018) and in addition, diosmin was reported to possess cardio-protective effects in a small animal study (Senthamizhselvan, Manivannan, Silambarasan, & Raja, 2014). Tricin and apigenin concentration were relatively low.

MS/MS spectral analysis of ME-RBF tentatively identified seven apigenin-*C*-glycosides (**14**–**20**), three methoxyluteolin-*C*-glycosides (**21**–**23**) and three tricin-*O*-glycosides (**24**–**26**). The isomers isoschaftoside (**14**) and schaftoside (**15**), together with **16**, **17**, **19** and **20** have been previously identified in sugarcane extracts through MS/MS studies (Asikin et al., 2013; Colombo et al., 2008; Coutinho et al., 2016). The apigenin-6,8,-*C*-diglycosides isoschaftoside (**14**) and schaftoside (**15**) have been described as one of the main polyphenols in seeds of quince (*Cydonia oblonga*) (Ferreres et al., 2003) and reported to function as phytoalexins (Picariello et al., 2017). Schaftoside-2″-*O*-glucoside (**18**) has not been reported in sugarcane before to the best of our knowledge.

Three *C*-glycosides of methoxyluteolin (**21**, **22** and **23**) were tentatively identified in this study whereby the methoxyluteolin can either be chrysoeriol or diosmetin. Chrysoeriol has been established as an intermediate in tricin biosynthesis in rice (Lam, Liu, & Lo, 2015), which belongs to the same family as sugarcane, the grass family. Although chrysoeriol has not been reported in sugarcane extract before (to the best of our knowledge), its presence in sugarcane is possible since tricin is a known methoxyflavone in sugarcane. Diosmetin is present in the sugarcane molasses extract used in this study (Table 2) and its glycoside diosmetin 8-*C*-glucoside has been reported in sugarcane leaves and juice (Colombo, Lanças, & Yariwake, 2006). Its diglucoside derivative, diosmetin-6″-*O*-glucosyl-8-*C*-glucoside, however, has not been reported in sugarcane extract before to the best of our knowledge.

The three tricin-*O*-glycosides tentatively identified in this study (**24**, **25** and **26**) have been reported in sugarcane before (Colombo et al., 2008; Coutinho et al., 2016). The biological activities of tricin has been reported and the review of Zhou and Ibrahim (2010) highlights the potential of tricin and its derivatives as nutraceutical.

Polyphenols are ubiquitous in plants and this group of compounds have been a growing subject of interest due to their biological activities such as antioxidant activity. However, the availability of these polyphenols is influenced by their stability during food processing. Polyphenols have been reported in sugarcane juice (Kadam et al., 2008) and leaf extracts (Colombo et al., 2006; Coutinho et al., 2016). The presence of these compounds in molasses indicates the stability of the compounds despite the processing steps that molasses had undergone in the manufacture of sugar, which makes molasses a sustainable source of polyphenols.

5. Conclusion

Sugarcane molasses extract (ME) and its fraction (ME-RBF) have demonstrated antioxidant activity and the increased antioxidant activity in ME-RBF can be correlated to the increased total polyphenol and total flavonoid content of ME-RBF. In this study, quantitative analysis of 13 analytes was carried out by comparison with reference standards using LCMS. In addition, seven apigenin-*C*-glycosides, three methoxyluteolin-*C*-glycosides and three tricin-*O*-glycosides have been tentatively identified by MS/MS analysis and comparison with data reported in the literature. Complete identity of these compounds can be confirmed by comparison with a reference standard or by inference from MS/MS data of reference standards of similar compounds. The majority of the compounds described and tentatively identified in this study have been reported from sugarcane extracts before but to the best of our knowledge, schaftoside-2″-*O*- glucoside (**18**) has not been identified in sugarcane extract before. The identification of methoxyluteolin can either be the isomers chrysoeriol or diosmetin. Chrysoeriol has not been identified in sugarcane before but it was recently reported that chrysoeriol is an intermediate in tricin biosynthesis, which makes the presence of chrysoeriol and its glycosides in molasses possible. In contrast, diosmetin and its glycosides have been reported in sugarcane.

Polyphenols have been demonstrated to exhibit antioxidant activity, but the complex composition of sugarcane molasses poses a challenge in underpinning the components that can be attributed to its antioxidant property, which is true for most extracts of natural origin. The studies to date demonstrate the potential of sugarcane molasses as a source of compounds that can be beneficial to health. The deconvolution of this complex matrix will provide a basis in further understanding how to improve its processing to add value to its use as a nutraceutical and/or food supplement.

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CRediT authorship contribution statement

Myrna A. Deseo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Aaron Elkins:** Methodology, Validation, Formal analysis. **Simone Rochfort:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. **Barry Kitchen:** Conceptualization, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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