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ORIGINAL RESEARCH

Sugarcane waste products as source of phytotoxic compounds for agriculture

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Abstract

Purpose This article aims to evaluate the phytotoxic potential of metabolites present in the waste from sugarcane processing industry, such as vinasse, filter cake and bagasse, in order to reuse them as raw materials for the production of natural herbicides.

Method Vinasse, filter cake and bagasse were submitted to different treatments, which originated 15 different samples. They were chemically identified by negative-ion mode electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI(-)FT-ICR MS), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography–mass spectrometry (LC-MS). Furthermore, they were submitted to phytotoxic assays, and to total phenolic content determination. Correlation between chemical and biological methods was performed through chemometric analysis and multiple linear regression.

Results From vinasse, dichloromethane (VDiCl) and ethyl acetate (VAcOEt) samples were the most phytotoxic fractions at the concentrations of 500 mg L^{-1} and 250 mg L^{-1}. VDiCl inhibited *L. sativa* root growth by 72.6% and 59.7%, respectively, while VAcOEt inhibited by 62.13% and 30.67%, respectively. The IC_{50} values established for VDiCl e VAcOEt were 168.4 mg L^{-1} e 262.3 mg L^{-1}, respectively. The set of analyzes provided evidence that the synergistic action between fatty acids and phenolic compounds was of paramount importance for greater phytotoxicity of fractions.

Conclusion The results indicate that the waste from the sugarcane processing industry, especially vinasse, can be reused as raw material for the production of natural herbicides, minimizing the environmental risks of incorrect disposal.

Keywords *Saccharum officinarum* L., Industrial residues, Fatty acids, Phenolic compounds, Natural herbicides, Chemometrics

Electronic supplementary material

The online version of this article (Doi:10.30486/IJROWA.2020.1885536.1007) contains supplementary material, which is available to authorized users.

Introduction

Sugarcane (*Saccharum officinarum* L.) is an important crop in several countries. It is a raw material for production of sugar and alcohol (Del Río et al. 2015). Brazil is the largest agricultural producer of sugarcane, and its industrial park is composed of 411 sugar and ethanol producing plants (CONAB 2018). For these reasons, an enormous amount of waste material is generated, highlighting the need for research dedicated to finding new applications for these agroindustrial waste, thus simultaneously addressing both economic

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and ecological issues (PNRS 2011). In particular, agroindustrial wastes from sugarcane like vinasse, filter cake and bagasse have important applications (Cheavegatti-Gianotto et al. 2011). Formerly, it is seen as environmental contaminants. Today, vinasse has economic value in the cultivation of sugarcane itself when diluted in irrigation water, constituting a modern process called fertigation owing to its content in nitrogen, sulfur, calcium, and magnesium, which are essential micronutrients to plants (Carrilho et al. 2016; Clementson and Gopaul 2020). Filter cake is rich in organic matter and minerals like nitrogen, phosphorus and calcium, and, as such, is used in bovine cattle feeding (Yadav and Solomon 2006; George et al. 2010; Kumar et al. 2010). Another application of filter cake involves the extraction of wax with organic solvents to replace different types of natural and petroleum-derived waxes (Basanta et al. 2007). Sugarcane bagasse, in turn, is the largest waste of Brazilian agroindustry (Rabelo et al. 2011). Its main applications include boiler fuel, cellulose production, confined livestock feed (George et al. 2010; Teixeira et al. 2014) and biochar production (Nwajiaku et al. 2018). Of note, *Saccharum officinarum* presents phenolic compounds and fatty acids in its leaves and stems (Sampietro et al. 2006; Colombo et al. 2006; Singh et al. 2015; Attard et al. 2015; Gomes et al. 2016). In a previous work, we described how a mixture of fatty acids and phenolic compounds can influence the phytotoxicity of its leaves on weeds, namely *Calopogonium mucunoides*, a vigorous, hairy annual or short-lived perennial trailing legume (Gomes et al. 2016). In fact, before a new crop is planted, leaves are left on the ground to prevent the growth of weeds (Sampietro and Vattuone 2006). This influence of metabolites of a given plant on the growth of another plant is known as allelopathy (Macías et al. 2010). Some classes of compounds, among them fatty acids and phenolic compounds, are considered to be allelopathic (Gomes et al. 2016; Li et al. 2010; Alamsjah et al. 2008; Macías et al. 2008; Wu et al. 2006) and appear to act synergistically in their phytotoxic effect (Gomes et al. 2016). In this paper, we turn our attention to the wastes of the sugar and alcohol industry in an attempt to make them raw material for the production of natural herbicides, taking into account their content in fatty acids and phenolic compounds. We emphasize that this is the first work to compare the phytotoxic potential of metabolites found in wastes (vinasse, filter cake and bagasse), produced by the sugar and alcohol industry, it is also the pioneer in reporting the presence of oxylipins in *Saccharum officinarum* L.

Materials and Method

Material

Sugarcane bagasse was obtained from the local sugarcane juice trade (Vitória, Espírito Santo, Brazil). Vinasse and filter cake were supplied by Usina Paineiras, which is located in the municipality of Itapemirim, Espírito Santo, Brazil, at the beginning of the harvest in May, 2016. All industrial wastes were immediately frozen. Solvents used were of HPLC grade (methanol, dichloromethane, ethyl acetate, acetone and hexane), and water was deionized. For determination of total phenolic contents, Folin–Ciocalteu SPECTRUM® (10% w/v in H_2O) solution was used.

Extract preparation

From Vinasse

The waste sample was thawed and centrifuged to give a dark decanted solid (5 g) and a yellowish liquid solution (1.2 L). The latter was subjected to successive liquid/liquid partitions with organic solvents (hexane, dichloromethane, ethyl acetate) to give 4 fractions: hexane - VHex (0.036 g, 0.30%), dichloromethane - VDiCl (0.0348 g, 0.29%), ethyl acetate - VAcOEt (0.1284 g, 1.07%) and aqueous - VAq (11.8 g, 98.1%). Two extracts of the decanted solid (2 g each) were shaken for 24 h with 20 mL of acetone and ethanol, respectively. After that time, each extract was concentrated on a rotary evaporator to produce 0.114 g (ADV, 5.70%) and 0.86 g (EDV, 4.3%), respectively. Another sample obtained was the so-called total vinasse (VB), which was concentrated in a rotary evaporator without previe ous centrifugation. Approximately 100 mL of vinasse yields 1 g of dry extract. Therefore, 7 extracts were produced from vinasse.

From Filter Cake

Three extracts of filter cake (10 g each), a solid waste, were shaken for 24 h with 100 mL of dichloromethane, ethanol and water, respectively. After that time, the first two extracts were concentrated on a rotary evaporator to produce 0.567 g (TFDiCl, 5.67%) and 0.538 g (TFE-

tOH, 5.38%), and the last one was lyophilized to yield 0,248 g (TFaq, 2.48%).

Three new extracts of filter cake (10 g each) were refluxed with methanol/KOH (0.5 mol L^{-1}) at 60^{0}C for 30 min. One was filtered and concentrated to yield TF-MeOH (0.125 g, 1.25%), and the other two were acidified with HCl (1 mol L^{-1}) and the solutions concentrated to eliminate the methanol. The aqueous solutions were extracted with hexane (TFsapoH, 0.157 g, 1.57%) or dichloromethane (TFsapoD, 0.117 g, 1.17%). Therefore, 6 extracts were produced from the filter cake.

From Bagasse

Bagasse (10 g) was refluxed either in MeOH (100 mL) or in 0.5 mol L^{-1} MeOH/KOH (100 mL) at 60 °C for 10 min. From the first extraction, after filtration and rotary evaporator concentration, BMeOH (0.17 g) was obtained in 17% yield. From the second extraction, the above procedure was repeated, but the dried extract was suspended in acidified water (1 mol L^{-1} HCl) and extracted with dichloromethane. BsapoD was obtained after concentration of the extract (0.015 g, 15%).

Identification of fatty acids and/or phenolic compounds

ESI(-)FT-ICR MS and ESI-(-)MS/MS

The analyzes were performed according to Oliveira et al. 2016. The acquired mass spectra were analysed in the Data Analysis program (Bruker Daltonics, Bremen, Germany). The elemental composition was determined from mass/charge ratio (m/z) of each signal. The molecular formula proposals for the signals were obtained from the chemical data described in the literature for isolated and/or identified *Saccharum officinarum* molecules.

GC-MS

Except for VB, Vaq and TFaq, all above extracts (2 mg) were methylated with ethereal diazomethane (3.0 mL) overnight at -8°C. After evaporation of the solvent, the methylated extracts were solubilized in CH_2Cl_2 for GC-MS analyses. GC-MS analyzes were performed according to Spindola et al. 2016. The NIST 11 library was used to identify the compounds. A mixture of *n*-alkanes (Sigma Aldrich - C-11 to C-34) was injected in the same chromatographic conditions noted above for Kovats index calculation of the extracts peaks.

LC-MS

An Agilent 1200 series liquid chromatograph coupled to a triple quadrupole mass spectrometer ABSciex API 3200™ MS/MS system was used to identify two oxylipins in the VDiCl extract, which were separated on a Kinetex® 5µm EVO C18 150 x 4,6 mm column from Phenomenex. The mobile phase, consisting of (A) formic acid aqueous solution (0.1%, v v^{-1}) and (B) formic acid methanolic solution (0.1%, v v^{-1}), was pumped through the column at a flow rate of 400 µL min^{-1} in a gradient of concentration as follows: 0-0.25 min (45% A), 0.25-1.0 min (45-40% A), 1.0-8.0 min (40-27% A), 8.0-8.01 min (27-0% A), 8.01-15.50 min (0% A), 15.50-15.51 min (0-45% A), and 15.51-20.51 min (45% A). The extract (1 mg) was solubilized in 1 mL of methanol (HPLC grade) and diluted 4 times before injection (10 µL). Ionization was performed by ESI(-) mode (−4500 V). Collision-induced dissociation (CID) was used to generate MS^2 fragments from the $[M-H]^-$ ions with *m/z* 327 and 329. At Q1, the ions were selected and then analyzed at Q3 as follows: from *m/z* 327 (309, 291, 255 and 239) and from *m/z* 329 (293, 281, 271, 239, 229, 211, 202, 183, 201, and 171).

Determination of the total phenolic concentration of the extracts

The determination of the phenolic concentration of the extracts was according to Guss et al. 2017. Chlorogenic acid (Sigma-Aldrich) at concentrations of 6.25, 12.5, 25.0, 37.5, 50.0, 75.0 and 100 mg L^{-1} was used to construct the analytical curve. From the equation of the line obtained, the total phenolic content in mg CAE g^{-1} of the extract was calculated. Chlorogenic acid has been determined as the standard most suitable for obtaining the analytical curve since the phenolic compounds present in *S. officinarum* are derived from chlorogenic acid (Duarte-Almeida et al. 2006; Caderby et al. 2013).

Phytotoxic activity

The analyses of seed germination inhibition test, inhibition of root and hypocotyl growth were performed according to Gomes et al. 2016. The results of the inhibition of root tests were evaluated by analysis of variance

(ANOVA) according to Tukey's test at a significance level of 5%, and Mean Inhibitory Concentration (IC_{50}) was calculated by nonlinear regression using GraphPad Prism software.

Multiple Linear Regression

Multiple Linear Regression (MLR) was performed in the Excel program for Office 365 running on Windows 10. *Lactuca sativa* root growth inhibition values for the 15 extracts were correlated with their total phenolic contents, along with the intensity of oxylipin ion signals at *m/z* 329 and *m/z* 327 in mass spectrometry and the intensity of the identified phenolic compounds. The coefficient of determination values (R^2) were used to infer correlations.

Chemometrics Analysis

Data chemometrics treatment was performed by principal component analysis (PCA) with the 15 mass spectra from the extracts obtained from the filter cake, bagasse and vinasse. The phytotoxicity assay and total phenolic content measurement aimed to find extracts clusters by chemical similarity.

PCA was applied to retain most information of the mass spectra in a few components and enable the visualization of natural clusters. The use of PCA results in the reduction of dimensionality which facilitated the grouping, or not, of extracts by similarity, that is, between species without called "score" space (T). Close extracts in the space of the main components tend to have similar characteristics (Brereton 2003; Bro and Smilde 2014; Ferreira 2015). In this paper, two PCA models were constructed using MATLAB software, version 7.12. The first model was obtained from a data matrix containing 15 extracts and mass data (78140 variables). In the second model, the matrix was composed of 15 extracts and 8 selected variables (*m/z* = 163, 167, 177, 193, 197, 223, 327, and 329). The data for both models were pre-processed with normal standard variate (SNV) (Fearn et al. 2009) and centered on the mean. The results of the constructed models were correlated with the data of phytotoxicity and total phenolic content.

Results and discussion

From the wastes, sugarcane bagasse provided the lowest extractive yields, 0.17% and 0.15% for the methanolic extract (BMeOH) and for the dichloromethane soluble saponified fraction (BSapoD), respectively. The filtered vinasse from the fermentation of the sugarcane juice mainly provided a hydrophilic fraction, Vaq (98.1%). The hexane Vhex (0.30%), dichloromethane (0.29%) and ethyl acetate VAcOEt (1.07%) fractions yielded very little organic matter mass, while the vinasse sediment yielded a larger mass with ethanol EDV (4.3%) and acetone ADV extractions (5.70%). These values showed, as expected, the presence of major hydrophilic substances (sugar and phenolics) (Singh et al. 2015; Duarte-Almeida et al. 2006) from the original sugarcane juice. The filter cake, on the other hand, provided extracts with higher yields of lipophilic substances, as can be deduced from the mass values of the six extracts obtained from TFaq (2.48%), TFEtOH (5.38%), TFDiCl (5.67%) and TFMeOH (1.25%). As the filter cake is formed from suspended material in the sugarcane juice and therefore insoluble in it, the greater presence of lipophilic compounds, such as fatty acids, can be explained (George et al. 2010; Kumar et al. 2010; Baikow 1982).

Except for VB, Vaq and TFaq, all extracts were methylated with diazomethane and analysed by GC-MS. Fourteen compounds were identified based on their similarity (SI) and Kovats indexes (KI) (Table 1S, Fig 1S-12S: In supplementary file). The fifteen nonmethylated extracts were also analysed by ESI(-)FT-ICR MS (Table 2S, Fig 13S-15S: In supplementary file).

Both techniques were complementary in the identification of 17 compounds. Palmitic acid (**1**) was detected in all 12 methylated extracts, as its methyl ester, and also in the 15 nonderivatized extracts. The other fatty acids found, either as methyl esters by GC-MS, or as deprotonated molecules, i.e., $[M-H]^-$ ions, were myristic (**2**), stearic (**3**), palmitoleic (**4**), oleic (**5**), and linoleic (**6**) acids. With respect to the methylated derivatives of the phenolic compounds, the original molecules were identified by ESI(-)FT-ICR MS in the nonmethylated extracts. Thus, 3,4-dimethoxybenzoic acid methyl ester was obtained from the methylation of veratric acid (**7**), having the $[M-H]^-$ ion, *m/z* 181.05071, and corresponding to the molecular formula (M) $C_9H_{10}O_4$ with mass error equal at -0.44 mg L^{-1}. From the same identification strategy, other compounds were identified, including methylation of vanillic acid (**8**; $[M-H]^-$ ion; *m/z* 167.03508; M = $C_8H_8O_4$; and error = 0.59 mg L^{-1}), which afforded 3,4 dimethoxybenzoic

acid methyl ester; methylation of *p*-coumaric acid (**9**; [M-H]$^-$ ion; *m/z* 163.04005, M = $C_9H_8O_3$; and error = 0.08 mg L^{-1}), which gave the *p*-methoxycinnamic acid methyl ester; methylation of eudesmic acid (**10**; [M-H]$^-$ ion; *m/z* 211.06132; M = $C_{10}H_{12}O_5$; and -0.59 mg L^{-1}) and syringic acid (**11**, [M-H]$^-$ ion; *m/z* 197.04564; M = $C_9H_{10}O_5$; and -0.47 mg L^{-1}), which afforded 3,4,5-trimethoxybenzoic acid methyl ester; and methylation of caffeic acid (**12**; [M-H]$^-$; *m/z* 179.03496; M = $C_9H_8O_4$; and error = -0.14 mg L^{-1}) and ferulic acid (**13**; [M-H]$^-$; *m/z* 193.0578; M = $C_{10}H_{10}O_4$; and error = -0.77 mg L^{-1}), which gave 3,4-dimethoxycinnamic acid methyl ester. Finally, *p*-hydroxybenzoic acid (**14**) was originated from *p*-methoxybenzoic acid methyl ester. Although [M-H]$^-$ ion at *m/z* 137 was not present in the (-)-ESI mass spectrum (only ions above *m/z* 155 are detected by the technique), this acid has been shown to occur in *Saccarum officinarum* (Singh et al. 2015). Another phenolic substance present in the more polar extracts was sinapic acid (**15**). However, such compound was not present in the methylated extracts, but was identified by ESI(-)FT-ICR MS. Fig 1 shows the major fatty and phenolic acids identified in the agroindustrial waste products.

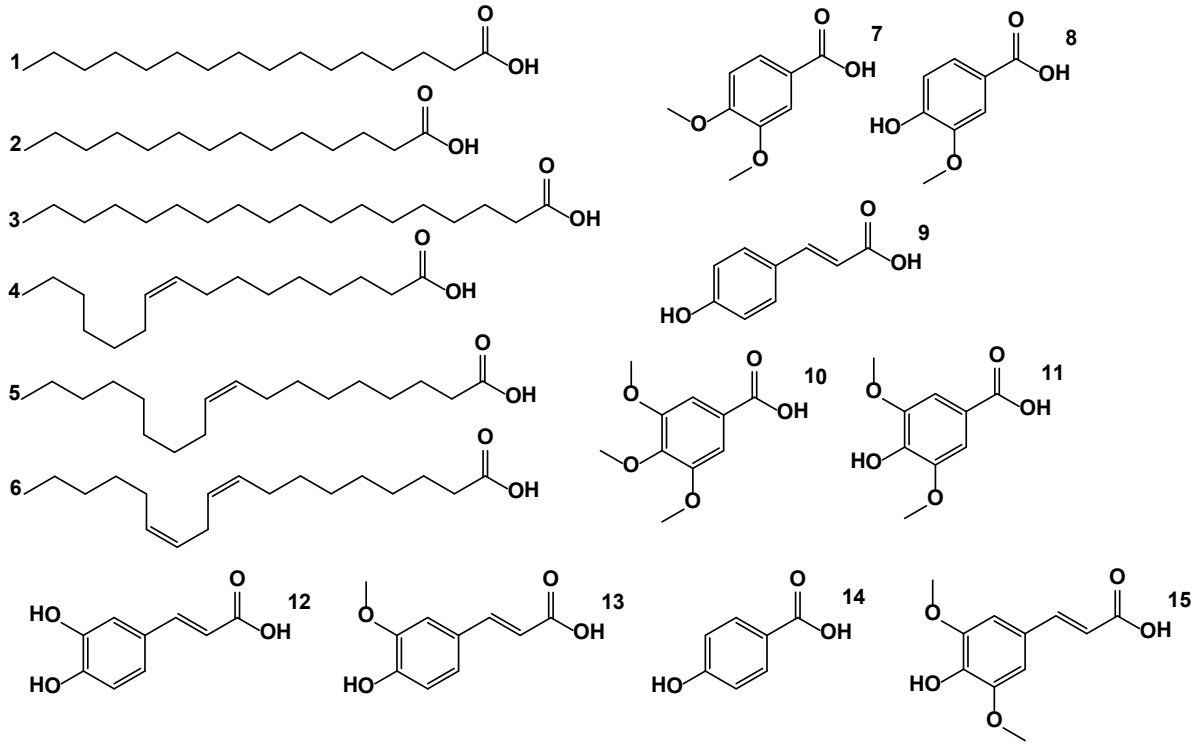

Fig. 1 Main fatty acids and phenolic acids identified in sugar cane agroindustrial products based on ESI(-)FT-ICR MS

Since diazomethane is a methylating agent of acids and phenols, compounds with alcohol function are not derivatized for gas chromatography. Therefore, ESI(-)FT-ICR MS and LC-MS/MS were used for the structural identification of polyhydroxy fatty acids, the so-called oxylipins. In the extracts from vinasse (VHex, VDiCl and VAcOEt), two intense ions were visualized in the (-) ESI-FT-ICR mass spectra, [M-H]$^-$ 327 and 329. Both techniques were complementary in their identification. Exact mass of both ions with low mass defects led to the identification of the molecular formulas $C_{18}H_{32}O_5$ and $C_{18}H_{34}O_5$, respectively. CID experiments of the ions led to the identification of 9,12,13-trihydroxy-10,15-octadecadienoic acid (**16**) for [M-H]$^-$ ion at *m/z* 327 and the mixture of acids, 9,12,13-trihydroxy-10-octadecenoic acid (**17**) and 9,10,13 trihidroxi-11-octadecenoic acid, for [M-H]$^-$ ion at *m/z* 329 (18) (Fig 16S: In supplementary file).

LC-MS/MS was operated in Multiple Reaction Monitoring (MRM) for the VDiCl extracts and confirmed the proposed structures shown in Fig 2.

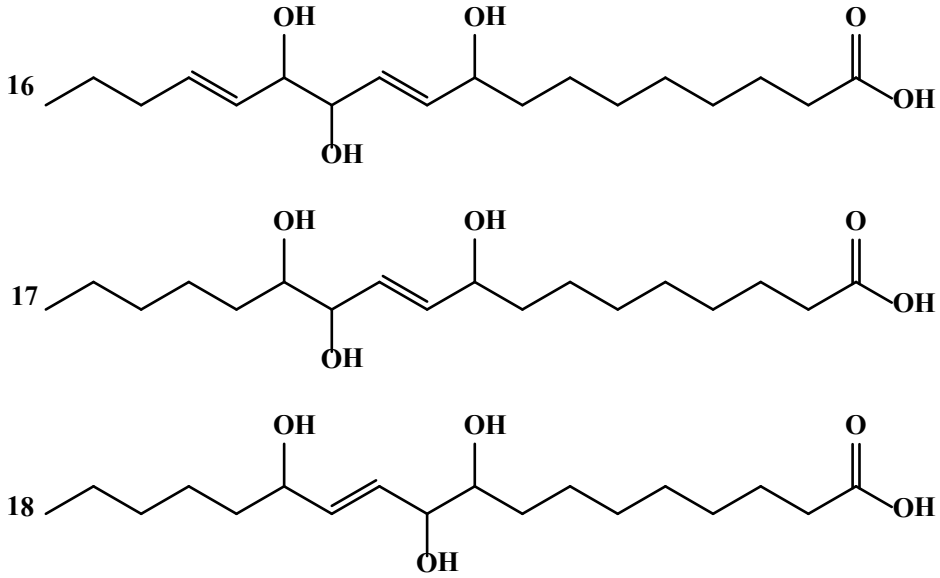

Fig. 2 Oxylipins as major fatty acid derivatives identified in vinasse from ESI(-)FT-ICR MS and LC-MS data

Two more intense peaks at 14.8 and 15.1 min were obtained in the total ion chromatogram monitored for the precursor ion at *m/z* 329 and only one peak at 14.6 min for *m/z* 327. Fragmentation of *m/z* 329 provided, as daughter ions, fragments at *m/z* 229, 211, 171, 293 and 183. All were related to the chromatographic signal at 14.8 min. On the other hand, ions *m/z* 201 and 293, similarly originated from the precursor ion 329, were related to the chromatographic signal at 15,1 min. In relation to the precursor ion of *m/z* 327, the obtained daughter ions *m/z* 291 and 239 were related to a chromatographic signal at 14.6 min. Daughter ions at 229, 211 and 171 were described as major fragments from compound **17** (pinnelic acid) by Bin and Peterson (2016) and Moraes (2017). Moreover, Bin and Peterson (2016) described an isomer for pinnelic acid, which is tianshic acid, the double bond of which is located at C-11 and the hydroxyl groups at C-9, C-10 and C-13 (Bin and Peterson 2016) (Fig 17S-25S: In supplementary file). From this compound, 293 and 201 were major daughter peaks (Bin and Peterson 2016). As shown in Fig 3, the diagnostic peak for **17** was 171, and for **18,** it was 201, both originated from the cleavage of the C-C bond next to a double bond and a CHOH bond. Loss of 2 H_2O leading to peak 293 was common to both molecules.

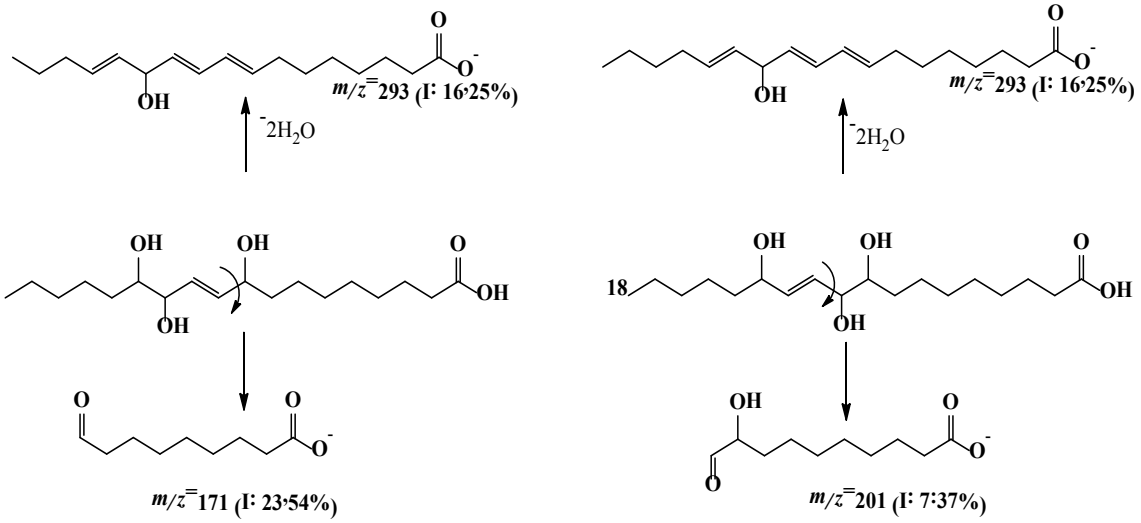

Fig. 3 Proposed fragmentation mechanismm for pinnelic (17) and tianshic (18) acids. (I=signal strength in %)

Pinnelic and tianshic acids are herein described for the first time in *Saccharum officinarum*, although already known in *Urtica dioica* (Gansser and Spiteller 1995), *Triticum vulgare* (Bin and Peterson 2016) and in *Pseudomonas* broth incubated with linoleic acid (Martin-Arjol et al. 2010). Compound **16** (9,12,13-trihydroxy-10,15-octadecadienoic acid) was described along with **17** in *Vicia faba* (Fabaceae) contaminated with *Bean Rust Pathogen Uromyces fabae* (Walters et al. 2006). The fragments *m/z* 309, 291, 239, 265 and 255 (Fig 4) are indicative of the proposed structure.

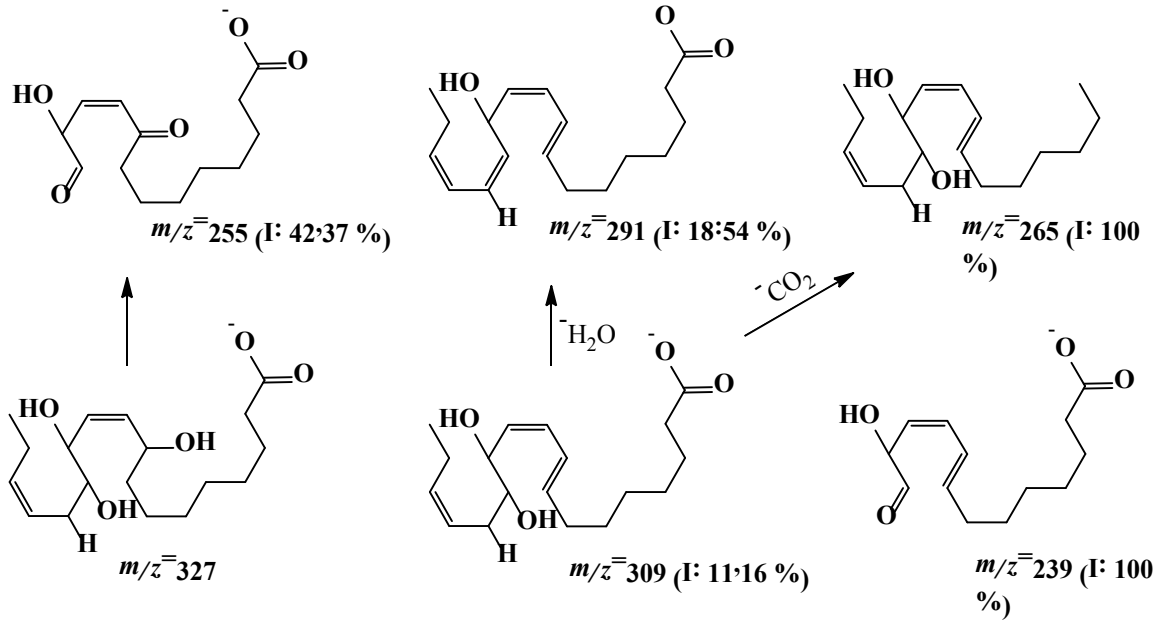

Fig. 4 Proposed fragmentation mechanism for oxylipin 16 from ESI(-)MS / MS data. (I=signal strength in %)

As phenolic compounds are important phytotoxic substances, the determination of total phenolic content for the 15 extracts was performed. Table 1 shows the results obtained and compared with an analytical curve of chlorogenic acid equivalent (CAE).

Table 1 Content of total phenolic substances in agroindustrial wastes: vinasse, filter cake and bagasse expressed as mg CAE g $^{-1}$ equivalent

Extracts	Total phenolic substances (mg g^{-1})
VB	42.2 ± 2.1
VHex	40.3 ± 2.4
VDiCl	123.5 ± 8.4
VAcOEt	244.8 ± 8.5
VAq	32.9 ± 1.9
EDV	87.37 ± 0.64
ADV	84.0 ± 5.8
TFAq	25.3 ± 4.8
TFDICl	23.5 ± 1.9
TFEtOH	31.0 ± 1.2
TFMeOH	43.8 ± 3.9
TFSapoH	16.15 ± 0.68
TFSapoD	198.1 ± 31.1
BMeOH	106.4 ± 8.5
BSapoD	389.9 ± 27.7

Although sugarcane bagasse provides low extractive yields in organic compounds, its extracts have the highest content of phenolic compounds. This can be seen by comparing each extracted waste , for example, with methanol, or with whole vinasse, BMeOH (106.45 ± 8.54 mg CAE g^{-1}), TFMeOH (43.84 ± 3.88 mg CAE g^{-1}) and VB (42.15 ± 2.05 mg CAE g^{-1}). Saponification of bagasse and filter cake with KOH/MeOH further increased the content of total phenolics as in BSapoD (389.88 ± 27.68 mg CAE g^{-1}) and in TFSapoD (198.11 ± 31.06 mg CAE g^{-1}). In addition, extraction with dichloromethane after saponification demonstrates the presence of phenolic compounds of low molar mass, such as phenolic acids. Zheng et al. (2017), found that the total content of phenolics in bagasse varied according to the composition of the extracting solvent. The highest content was found when 30% hydroalcoholic extract was obtained, reaching 170.68 ± 3.25 mg CAE g^{-1}. Vinasse was also a good source of phenolic substances, as can be seen in the VAcOEt (244.78 ± 8.49 mg CAE g^{-1}) and VDiCl (123.51 ± 8.35 mg CAE g^{-1}) fractions. These values are important because vinasse is one of the most abundant waste of the sugar-alcohol industry. According to Duarte-Almeida et al. (2006), the average total phenolic content in sugarcane juice is 160 µg CAE L^{-1}. The increase of phenolic concentration after a fermentation process with *Saccharomyces cerevisae* was described by Gomes et al. (2016) who observed higher levels of phenolic acids and flavonoids in the fermented methanolic fractions of sugarcane juice compared to those of unfermented methanol fractions.

All 15 extracts produced from vinasse, filter cake and bagasse were tested in phytotoxicity assays for inhibition of seed germination (Table 3S: In supplementary file), root growth (radicles) (Fig 5 - 6) and hypocotyl growth (Fig 26S – 27S: In supplementary file) in *Lactuca sativa* (lettuce), respectively tabulated data are presented (Table 4S: In supplementary file).

The germination was evaluated by root protrusion at 24 hours after sowing. At concentrations of 250 and 500 mg L^{-1}, low inhibition values were observed. BSapoD at 500 mg L^{-1} showed the highest inhibition of seed germination, only 18.9% (Table 3S: In supplementary file). Lettuce roots were more sensitive to extracts than hypocotyls. The most pronounced inhibitory effects were from VDiCl and VAcOEt vinasse extracts. At concentrations of 500 mg L^{-1} and 250 mg L^{-1}, VDiCl inhibited *L. sativa* root growth by 72.6% and 59.7%, respectively. While at the same concentrations, VAcOEt inhibited *L. sativa* root growth by 62.13% and 30.67%, respectively. Because of the higher inhibitory values, IC_{50} was determined for both fractions. For VDiCl and VAcOEt, 168.4 mg L^{-1} and 262.3 mg L^{-1} were the values found, respectively (Luz 2018), aqueous methanol extract of *Chrysopogon aciculatus* (Retz.), Poaceae, inhibited lettuce roots growth and an IC_{50} value of 4700 mg L^{-1} was established (Islam et al. 2019) and methanol extract of *Echinochloa colona* (L.) Link. (Poaceae) inhibited rice

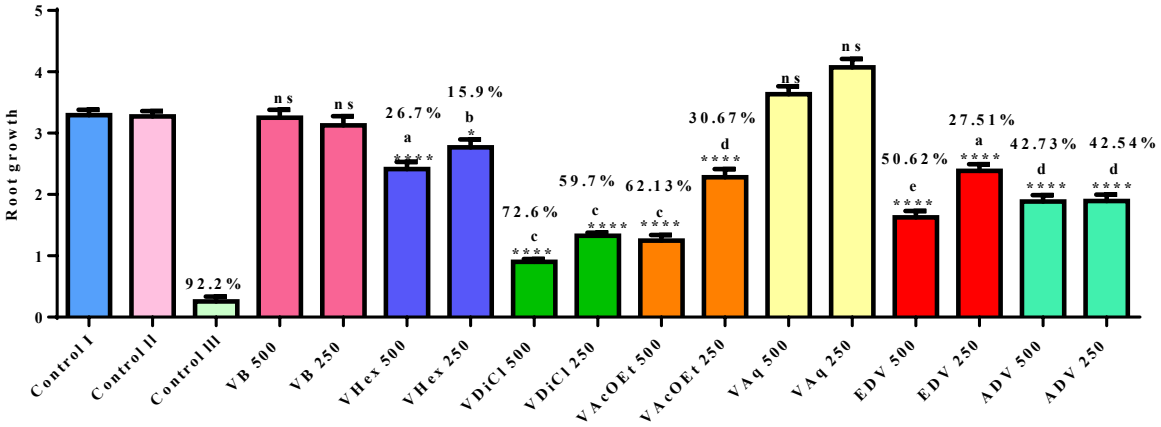

Fig. 5 Effect of crude extract and/or fractions from vinasse extract on L. sativa root growth [Control I = distilled water; Control II = 0.1% DMSO; Control III = Menadione (143 mgL^{-1})]

Note: Results are expressed by mean ± standard deviation. Significance is determined by ANOVA followed by Tukey's test. Different letters above the bars indicate significant differences between treatments according to Tukey's multiple comparison test ($p < 0.05$). Growth inhibition percentages are shown above the letters. (ns = non-significant compared to negative controls)

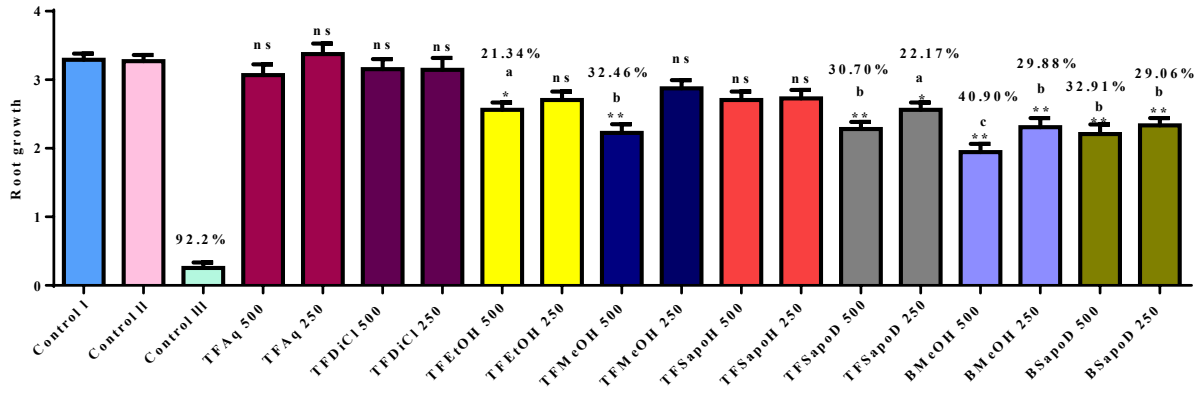

Fig. 6 Effect of extract of the filter cake and bagasse on L. sativa root growth [Control I = distilled water; Control II = 0.1% DMSO; Control III = Menadione (143 mgL^{-1})]

Note: Results are expressed by mean ± standard deviation. Significance is determined by ANOVA followed by Tukey's test. Different letters above the bars indicate significant differences between treatments according to Tukey's multiple comparison test ($p < 0.05$). Growth inhibition percentages are shown above the letters. (ns = non-significant compared to negative controls)

roots growth by approximately 50% at a concentration of 10000 mg L^{-1} (Sitthinoi et al. 2017). Comparing with our results, the residues from sugarcane industry had a much better effect, which reveals the great phytotoxic potential of them. The highest sensitivity of the roots resulted from their direct contact with phytotoxic substances (Chung et al. 2001). They are the first part of the plants to absorb these substances (Turk and Tawaha 2002), which are extremely active on the meristematic tissues of the growing root. As a result of this sensitivity, roots are considered to be better indicators of phytotoxic action for compounds with allelopathic potential (Ladhari et al. 2013). Fatty acids and phenolic compounds are commonly described in the literature as phytotoxic substances because they cause changes in the cell membrane and interfere in processes involving charge flow, respectively. It is believed that fatty acids form an ion channel in the plasma membrane, leading to cellular disorganization and, consequently, damage to the fundamental cellular processes. However, the toxicity of phenolics is provided by the presence of the aromatic ring that influences the photosynthesis, transport and ionic permeability of the cell membrane and the elimination of free radicals (Alamsjah et al. 2008; Wu et al. 2006; Macías et al. 2008). It seems, from our results, that the higher occurrence of oxylipins in VDiCl when compared to VAcOEt accounts for their greater phytotoxic activity.

A PCA model was constructed from the ESI (-) FT-ICR MS data of the 15 extracts. Fractions containing more intense oxylipin signals (VHex, VDiCl and VAcOEt), fractions originated from the filter cake, except TFAq, and more polar fractions from vinasse (VB, Vaq and EDV) formed different groups on the PCA score plot (Fig 7). Interestingly, when the results of phytotoxic activity were inserted in the data matrix for comparison (Fig 8), an analogous result was reached. Larger circles represent the most phytotoxic extracts on lettuce seeds.

Although BSapoD had the highest total phenol content (Table 1), VDiCl and VAcOEt contained oxylipins and were more phytotoxic. By including the signals of the major phenolic compounds (*m/z* 163, 167, 177, 193, 197, and 223) and the oxylipins (*m/z* 327 and *m/z* 329) for PCA construction, the extracts were grouped into two main blocks (Fig 9): Block A was composed of extracts with low or high phenolic signal intensities, but with low oxylipin intensities, and block B was formed by extracts with low or high phenolic signal intensities, but with high oxylipin intensities. These results confirm the importance of mixtures of fatty acids, especially oxylipins, and phenolic compounds for greater phytotoxic activity, the synergism between fatty acids and phenolic compounds is described in the study by Gomes et al. (2016), in which there was a significant improvement in the phytotoxic activity in samples with the mixture of these two classes.

Values of *L. sativa* root growth inhibition (phytotoxic activity) and values of total phenolic contents

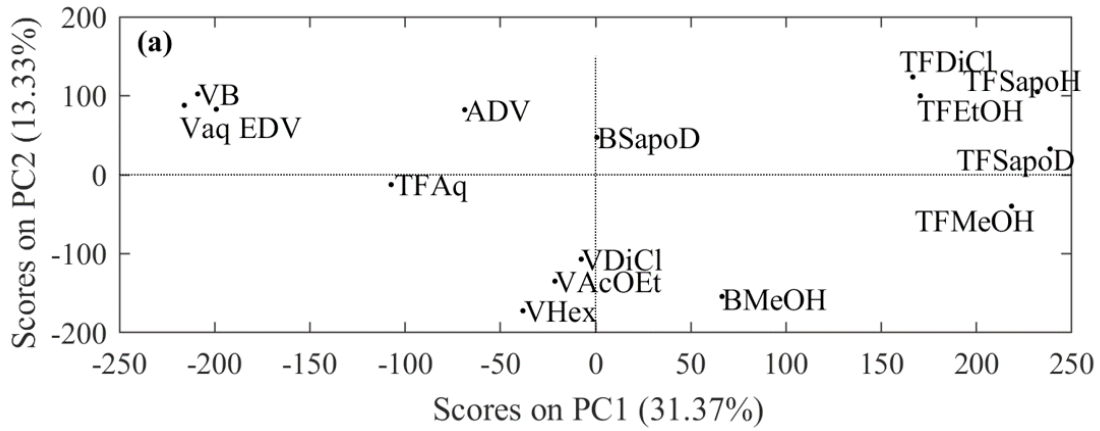

Fig. 7 Score plot of PC1 x PC2 of the 15 extracts obtained from ESI-FT-ICR MS data

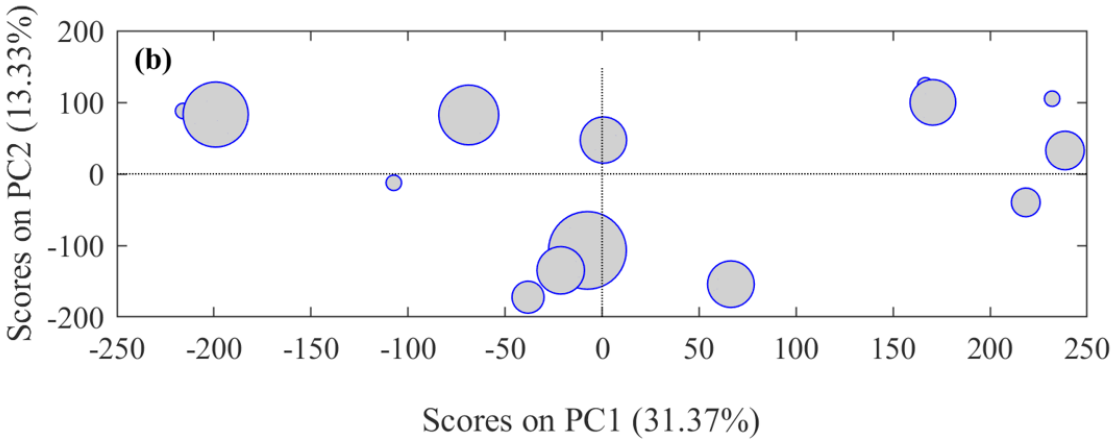

Fig. 8 Score plot of the 15 extracts. The size of the marker is proportional to phytotoxic in the extracts for lettuce seeds

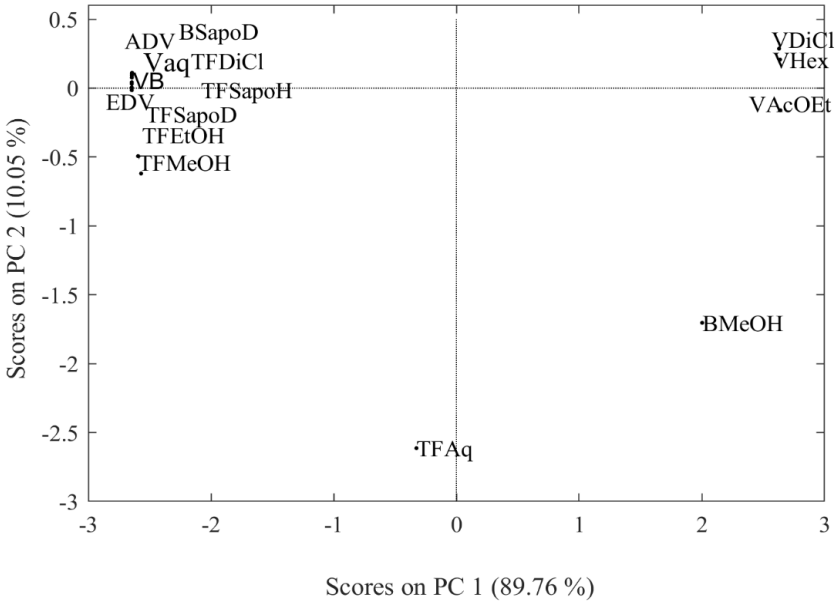

Fig. 9 Scores of the 15 extracts analyzed and 8 variables selected

of the 15 extracts were correlated in a multiple linear regression and showed an adjusted coefficient of determination (R^2_{adj}) of 0.20, indicating low linear relationship. When *m/z* signal intensity values of ions 329 and 327 were added to this correlation, the R^2_{adj} value increased to 0.48, with significant coefficients for the three variables, at a significance level of 5%. Since $[M-H]^-$ ion signal intensity values of individual phenolic compounds were added for linear regression calculation, the R^2_{adj} value continued to increase. If the phytotoxic activity was correlated only with signal intensity values of 329 and 327 ions (oxylipins), the R^2_{adj} value was 0.35. These results show that phytotoxic activity is related to the presence of oxylipins and phenolic acids in the extracts of sugarcane agroindustrial waste.

Conclusion

In the industrial processing of sugar cane, the residues: vinasse, filter cake and bagasse are produced on a large scale. Establishing an adequate way to manage agro-industrial waste is a major challenge for the sugar and alcohol industry , since its excessive disposal into the environment may cause serious pollution problemas. Brazil is the largest sugarcane producer in the world, and consequently vinasse, filter cake and bagasse are low cost raw materials and easily accessible there. Thus, considering the promising results observed in this work, through adequate technology, it is feasible that these residues, mainly vinasse, rich in phenolics and oxylipins, can be converted into natural herbicides, which adds value to industrial waste and also guarantees the development of more sustainable agriculture. To the best of our kwnowledge, it is the first work to compare the phytotoxic potential of different sugarcane residues and to identify oxylipins in *Saccharum officinarum* L.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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