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Study of two sugarcane by-products as source of secondary metabolites and heat-induced compounds with potential bioactive applications

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A crucial step in the engineering of bioactive materials from sugarcane by-products is understanding their physical, chemical, and biological characteristics, particularly their molecular composition and biological activities. This study aimed to characterize the physicochemical properties of methanolic and aqueous extracts from sugarcane molasses and vinasses, determine their antioxidant capacity, and identify key compounds of biological interest; specifically phenolic compounds (PCs) and heat-induced compounds (HICs). Through non-targeted analytical approaches, we identified a diverse range of PCs and HICs in the extracts. In vitro tests revealed significant antioxidant effects in both aqueous and methanolic fractions, with the methanolic extracts showing superior free radical scavenging capacity. This bioactivity was linked to PCs such as *p*-coumaric acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, chlorogenic acid, and schaftoside, as well as HICs like 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP); 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF); 2,6-dimethoxyphenol; and 1,6-anhydro-*β*-D-glucopyranose. These findings underscore the potential of sugarcane molasses and vinasses as sources of bioactive compounds, which can be engineered into new materials with promising biological properties for health, pharmacological, and food industry applications. Furthermore, our research highlights the integration of bioengineering, material science, and sustainable practices within the sugarcane industry by promoting the valorization of by-products, contributing to resource efficiency and industrial innovation under circular economy principles.

Keywords Molasses and vinasses, Phenolic compounds, Maillard reaction products, Bioactivity, Antioxidant capacity, Sustainable innovation

In an era increasingly focused on sustainable development, resource optimization, and minimizing environmental impacts, applied research has made substantial strides in addressing challenges related to the industrial transformation of raw materials and the inevitable generation of waste[1,2]. The agro-industrial sector, particularly the sugarcane industry, generates significant amounts of by-products, including molasses and vinasses, which, if not properly managed, can pose considerable economic and environmental concerns[3–5]. Molasses, a viscous by-product produced during the crystallization of sugarcane juice, and vinasses, a liquid residue from ethanol production, have been traditionally used in animal feed and soil fertilization[6,7]. However, these uses do not fully

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exploit their real value, especially considering that these by-products contain a wide variety of compounds that could be repurposed as bioactive materials for health, pharmacology, and food industry applications[8–10].

One of the most extensively studied groups of compounds in this context is phenolic compounds (PCs), a class of secondary metabolites naturally synthesized by plants during growth. PCs play crucial physiological roles in the adaptive response to various stress factors[11]. Many of these phytochemicals have been found in sugarcane extracts and are associated with medicinal properties, demonstrating positive relationships with several cellular effects, including anti-inflammatory, cytoprotective, immunomodulatory, antimicrobial, and antiproliferative activities[8,12–14]. In addition, industrial transformation processes applied to plant-based or agricultural products, such as sugarcane, can lead to the formation of additional compounds, specifically heat-induced compounds (HICs), resulting from interactions among naturally occurring components during thermal treatments. Among the most studied HICs are Maillard reaction products (MRPs), formed through reactions between reducing sugars and free amino groups of peptides and proteins[15]. Particularly for sugarcane, MRPs can be generated throughout its industrial processing, beginning at harvest, but they are primarily formed during juice clarification and evaporation[8]. While many of these compounds have been widely described for their contribution to the flavor, color, and aroma of various food products[15,16], some studies have also explored the bioactive potential of certain HICs formed during heat treatment, highlighting their antioxidant capacity and other biological activities[17–20], thus making them promising candidates for use in engineering bioactive materials.

In processed materials derived from agricultural products, such as sugarcane, it is essential to consider not only the presence of plant secondary metabolites but also their potential coexistence with processing-induced compounds, as both may influence the capacity of these materials to exert specific biological effects. Although processing can alter the composition and structure of certain PCs, this does not necessarily lead to a reduction in the overall bioactive potential of the materials. On the contrary, thermal treatments may promote the formation of new compounds, such as HICs, that can retain or even enhance biological activity, either through additive, synergistic, or protective interactions[21–23]. Despite the biological relevance of both compound classes, few studies have addressed their independent characterization[23–25]. This study contributes to filling that gap by evaluating PCs and HICs separately, providing insight into their individual presence and bioactive capacity in sugarcane molasses and vinasses.

In the specific context of sugarcane-derived products, the potential of some products, by-products, and waste materials as sources of bioactive compounds has been documented[26–28]. However, this potential has mainly been attributed to secondary metabolites such as terpenes and phenolic compounds, with less attention given to compounds formed during the manufacturing process. By evaluating the physicochemical characteristics, antioxidant properties, and chemical profiles of separately obtained extracts, this study provides insights into the presence, diversity, and potential bioactivity of PCs and HICs in sugarcane molasses and vinasses, thereby contributing to a deeper understanding of these compounds in such by-products. Consequently, the main objective of this research was to independently characterize both PCs and HICs in sugarcane molasses and vinasses using non-targeted analytical approaches. This comprehensive analysis could open new avenues for the valorization of these two by-products and support sustainable innovation in the sugarcane industry. It also aims to identify key molecules that could serve as the basis for developing novel bioactive materials, potentially applicable as functional ingredients or prototypes for nutraceutical and pharmaceutical uses. A simplified visual summary of the study's experimental strategy and main findings is presented in Fig. 1.

Materials and methods

Materials and reagents

Folin–Ciocalteu reagent; 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); 2,2-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS); 2,4,6-tripyridyl-s-triazine (TPTZ); and the standards of gallic acid and Trolox were sourced from Sigma-Aldrich. HPLC-grade solvents like methanol and acetonitrile were purchased from VWR Chemicals. Ultrapure water was obtained from a Millipore system. All other chemicals and reagents utilized were of analytical grade.

Sample preparation

For this study, two by-products of the sugarcane industry were used: molasses and vinasses. The molasses samples were obtained from Ingenio San Carlos, and the vinasses from Ingenio Mayagüez, both located in the Valle del Cauca region in Colombia. These facilities operate under continuous and standardized industrial processes and were selected to ensure consistency across the analyzed samples. Once received, all the samples were refrigerated (approx. 4 °C) until use. Before each test, the samples were diluted in distilled water to appropriate concentrations to facilitate processing and data collection. The respective dilution factor was considered for each analysis, and the results were expressed in dry matter. All diluted samples were homogenized and filtered through a filtering aid (Celite® 545—Merck Millipore) to remove any interfering agents.

General characterization of by-products

The molasses and vinasses were characterized regarding dry matter content (DMC—%), total soluble solids (TSS—°Brix), pH, and density. DMC was determined using a gravimetric method with vacuum drying for 24 h (65 °C ± 2 °C, 13 kPa)[29].TSS were quantified by measuring the refractive index at 20 °C using a refractometer with electronic temperature control and converting the values to °Brix. pH readings were taken using a digital pH-meter. The density was calculated based on the ratio of mass to volume of the samples, both measured at the time of performing the dilutions required to facilitate data collection.

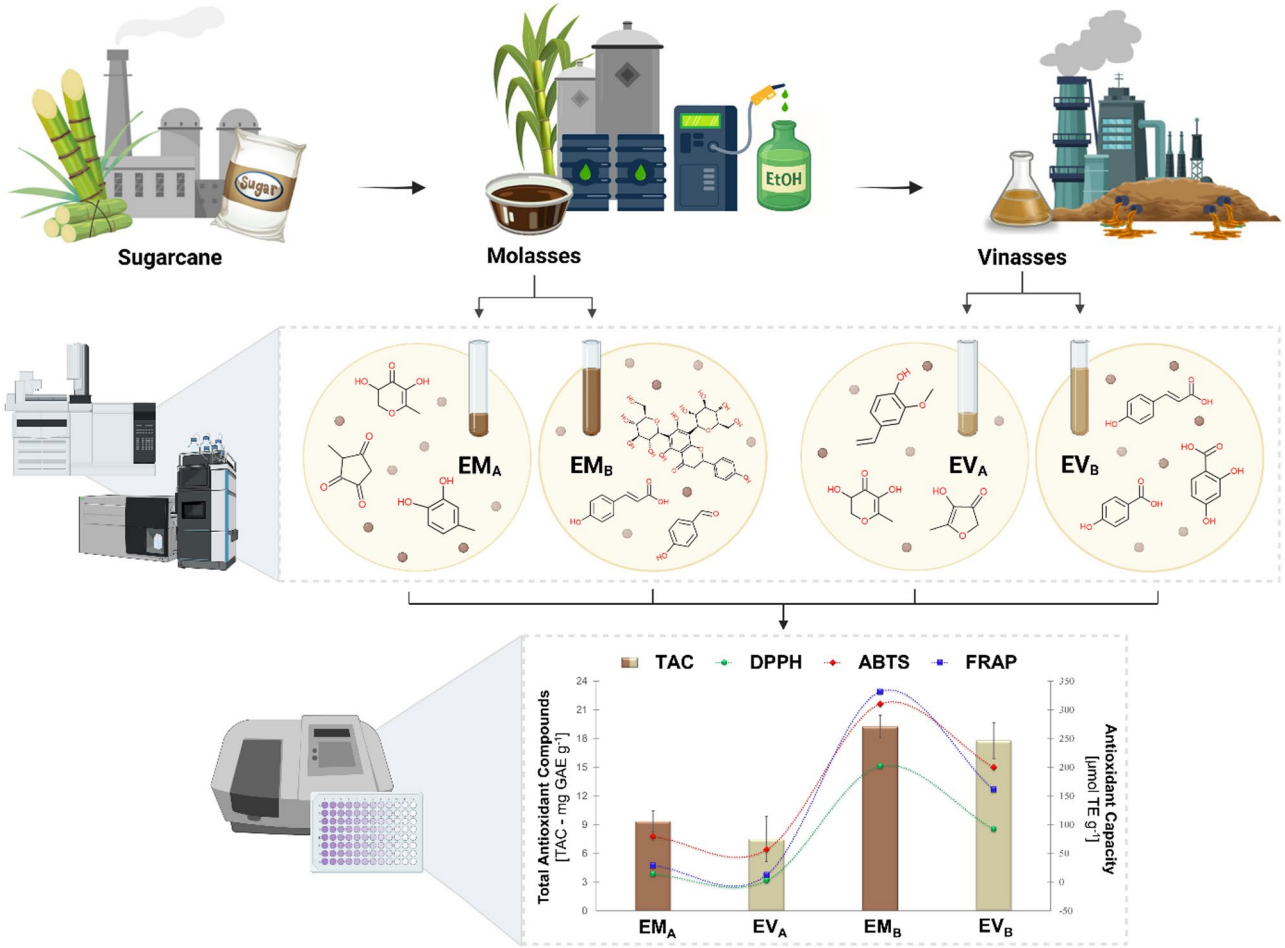

Fig. 1. Schematic overview of the experimental workflow and main findings for sugarcane molasses (EM) and vinasses (EV) extracts. Subscripts A and B indicate aqueous and methanolic extracts, respectively, for each by-product.

Extraction of compounds of biological interest

Two main groups of molecules were considered as compounds of biological interest in this study: PCs, mostly originated during the secondary metabolism of sugarcane; and HICs, formed during the thermal processing applied in the industrial transformation of sugarcane. HICs included MRPs and other volatile and low molecular weight compounds. The extracts were obtained following a solid-phase extraction (SPE) procedure implemented by Caderby et al.[24] with some adaptations[30,31]. The molasses and vinasse samples were diluted in acidified water (pH 2.0 ± 0.2 with 37% HCl) at a 1:10 ratio. These solutions were shaken in an ultrasonic bath for 15 min and then centrifuged at 10,000×*g* for 10 min. The resulting supernatants were vacuum filtered through a 0.45 µm cellulose nitrate membrane to remove solid particles. The recovered filtrates were injected into cartridges packed with reverse-phase polymeric sorbent (Oasis® HLB, 3 mL, 60 mg—Waters) previously conditioned by sequentially passing 3 mL each of methanol and acidified water (pH 2.0). In this step, the PCs were retained on the sorbent while the HICs passed through and remained in the filtrate. To maximize the extraction of the HICs, the cartridges were washed with 3 mL of acidified water at a drop-wise flow rate (approx. 0.1 mL min^{-1}), obtaining an aqueous filtrate presumably rich in HICs (extracts A). Subsequently, the PCs retained on the cartridges were eluted with methanol in two applications of 3 mL each at slow flow rate, generating a methanolic fraction potentially rich in PCs (extracts B). Once extracts A and B were obtained for both the molasses samples (EM$_A$ and EM$_B$) and the vinasse samples (EV$_A$ and EV$_B$), they were neutralized to pH 7.0 ± 0.2 and subjected to ultrafiltration using regenerated cellulose centrifuge filters with a nominal molecular size cutoff of 3 kDa (Amicon® Ultra 2 mL, 3 K—Merck Millipore). The manufacturer's recommendations regarding time and relative centrifugation force were followed for this process. The filtered fractions were recovered for each type of sample and stored under refrigeration (approx. 4 °C) in dark conditions until further analysis.

Quantification of total antioxidant compounds (TAC)

The TAC content of both the molasses and vinasse samples, as well as their respective extracts, was quantified spectrophotometrically using the Folin-Ciocalteu test, following the protocol of Singleton et al.[32] with some modifications[33]. Gallic acid (GA) was used as the standard, and distilled water as the blank sample. In a 1.5 mL

Eppendorf tube, 600 µL of distilled water were mixed with 10 µL of the standard solution, the blank sample, or the sample to be analyzed. Immediately, 50 µL of Folin-Ciocalteu reagent was added, the solution was shaken and allowed to react for 1 min. After this time, 150 µL of a 20% (w/v) Na_2CO_3 solution was added, the mixture was stirred, and 190 µL of distilled water were added to complete a volume of 1 mL. After 120 min of incubation in the dark and at room temperature (approx. 23 °C), 300 µL of the mixture were transferred into a 96-well microplate spectrophotometer reader, and the absorbance at 760 nm was measured. Based on the absorbance values of the standard solution at five different concentrations, a calibration curve was constructed, allowing the calculation of the total concentration of antioxidant compounds in the analyzed samples. The TAC content was expressed as mg equivalents of GA per g of dry matter (mg GAE g^{-1}).

Determination of antioxidant capacity

The antioxidant capacity of the extracts was evaluated based on their potential for free radical scavenging. Three in vitro assays were applied: DPPH, ABTS, and FRAP (Ferric-Reducing Antioxidant Power).

DPPH assay

The procedure proposed by Brand-Williams et al.[34] was applied with some modifications[35,36], using Trolox as the standard and methanol as the blank sample. Briefly, 300 µL of a 0.1 mM DPPH radical methanolic solution were transferred to a 96-well plate and mixed with 15 µL of the standard solution, the blank sample, or the extract to be analyzed. The plate was kept in the dark for 30 min at room temperature, after which the solution absorbance was measured at 517 nm. The antioxidant activity was expressed as the inhibition percentage (*I*) according to Eq. (1):

$$I\,[\%] = \frac{A_0 - A}{A_0} \times 100 \tag{1}$$

where A_0 and A are the absorbance values of the blank sample and the extracts studied, respectively. Based on the inhibition percentages of the standard solution at five different concentrations, a calibration curve was constructed to calculate the Trolox Equivalent Antioxidant Capacity (TEAC) of the extracts, which was expressed in micromoles of Trolox Equivalents (TE) per gram of dry matter (µmol TE g^{-1}). Additionally, the results were expressed in terms of the IC_{50} value, which refers to the concentration of the extract required to achieve a 50% inhibition of the radical. This parameter was calculated from the function that relates the percentages of inhibition reached by the extracts at different concentrations[35,37].

ABTS assay

The ABTS assay was performed following the method described by Re et al.[38] with specific modifications[27,39,40]. Trolox served as the standard, and ethanol was used as the blank sample. The ABTS radical cation ($ABTS^{\cdot+}$) was generated by mixing a 2.45 mM solution of $K_2S_2O_8$ and a 7 mM solution of the ABTS reagent in a 1:1 volumetric ratio. This mixture was allowed to react in the dark at room temperature for 16 h to form the $ABTS^{\cdot+}$. The resulting radical solution was then diluted with ethanol to achieve an absorbance of 0.7 ± 0.02 at 753 nm. For the assay, 300 µL of the diluted $ABTS^{\cdot+}$ solution were transferred into a 96-well plate, followed by 4 µL of the standard solution, the blank sample, or the extract to be analyzed. The plate was incubated in the dark at room temperature for 6 min. After incubation, the absorbance was measured at 753 nm. In the same way as in the DPPH assay, the results of this test were expressed as TEAC (µmol TE g^{-1}) and IC_{50}.

FRAP assay

The adaptations of the Benzie & Strain method[41,42] suggested by Pulido et al.[43] and other authors[44,45] were followed. Trolox and $FeSO_4$ were used as standards, and distilled water was used as the blank sample. Prior to each test, the FRAP reagent was prepared and kept in a water bath at 37 °C throughout the process. The FRAP reagent was made by mixing the following solutions in a 10:1:1 volume ratio: 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3$, and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl. In a 96-well plate, 285 µL of freshly prepared FRAP reagent were mixed with 15 µL of the standard solution, the blank sample, or the extract to be analyzed. This mixture was incubated at room temperature for 30 min in the dark, after which the absorbance was measured at 593 nm. Calibration curves were constructed based on the absorbance values of Trolox and $FeSO_4$ standard solutions at five different concentrations. These curves were used to calculate the FRAP value of the extracts by interpolating from their absorbance values. The antioxidant capacity was expressed as FRAP value in equivalent micromoles of Trolox and ferrous sulfate per gram of dry matter (µmol TE g^{-1} and µmol $FeSO_4$ g^{-1}, respectively).

Identification of PCs and HICs present in the extracts

The profile of main compounds present in the molasses and vinasse extracts was obtained. PCs contained in the methanolic fractions (extracts B) were analyzed by liquid chromatography (LC), while HICs in aqueous fractions (extracts A) underwent analysis using gas chromatography (GC). In both cases, these separation techniques were coupled to high-resolution tandem mass spectrometry (MS/MS).

Characterization of the phenolic profile of extracts by LC–MS analysis

The methanolic extracts of molasses and vinasses (EM_B and EV_B) were analyzed using an ultra-high-performance liquid chromatography system (Agilent 1290—Agilent Technologies) coupled to a quadrupole time-of-flight mass spectrometer (Agilent 6540—Agilent Technologies) equipped with an electrospray ionization source (Agilent Jet Stream—Agilent Technologies) (UHPLC-ESI-QTOF-MS). The protocol suggested by Ballesteros-

Vivas et al.[46] was followed. For chromatographic separation, a Zorbax® Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm particle diameter—Agilent Technologies) was used. The separation was performed at 40 °C with a gradient elution program. The mobile phases consisted of 0.01% (v/v) formic acid in water (eluent A) and 0.01% (v/v) formic acid in acetonitrile (eluent B). The injection volume of the samples was fixed at 2 µL with a flow rate of 0.5 mL min^{-1}. The gradient elution was applied as follows: 0–30% B in 0–7 min, 30–80% B in 7–9 min, 80–100% B in 9–11 min, 100% B in 11–13 min, 0% B in 13–14 min, followed by a 3-min conditioning cycle under the same conditions for the next analysis. To obtain the MS and MS/MS spectra, the mass spectrometer was operated in negative ion mode. The parameters for MS analysis were as follows: capillary voltage of 3000 V, nebulizer pressure of 40 psi, drying gas flow rate of 8 L min^{-1}, gas temperature of 300 °C, skimmer voltage of 45 V, and fragmentor voltage of 110 V. The MS and auto MS/MS modes were set to acquire *m/z* values ranging between 50–1100 and 50–800, respectively; at a scan rate of 5 spectra per second. Agilent MassHunter Workstation Qualitative analysis software (version 10.0) was used for post-acquisition data processing. Accurate mass data, isotopic patterns, MS/MS fragmentation patterns, MS databases search, and bibliographic search were employed for the tentative identification of PCs present in the analyzed extracts.

Characterization of the volatile profile of extracts by GC–MS analysis

The aqueous extracts of molasses and vinasses (EM_A and EV_A) were analyzed using a gas chromatography system (Agilent 7890B—Agilent Technologies) coupled to a quadrupole time-of-flight mass spectrometer (Agilent 7200—Agilent Technologies) equipped with an electronic ionization interface (GC-EI-QTOF-MS). The protocol suggested by Ballesteros-Vivas et al.[46] was followed with some modifications. For chromatographic separation, a DuraGuard capillary column (DB-5 ms, 30 m + 10 m, 250 µm internal diameter, 0.25 µm film thickness—J&W Scientific, Agilent Technologies) was used. Helium served as the carrier gas at a constant flow rate of 0.8 mL min^{-1}. The splitless mode was used for injecting 1 µL of the extract, with the injection temperature maintained at 250 °C. The GC oven temperature program was initially set at 60 °C for 1 min, then increased gradually at a rate of 10 °C min^{-1} to 325 °C, and held at this temperature for 10 min. The MS detector was operated in full-scan acquisition mode over a mass range of 50–600 Da, with a scan rate of 5 spectra per second. The MS parameters were as follows: electron impact ionization at 70 eV, transfer line temperature of 290 °C, mass analyzer temperature of 150 °C, and ionization source temperature of 250 °C. Tentative identification of the compounds was performed by systematic deconvolution of the mass spectra from the chromatographic signals using the Agilent MassHunter Unknowns Analysis tool (version 10.2) linked to the NIST MS database (version 20).

Statistical analysis

All measurements were performed in triplicate, and the results were expressed as mean values ± standard deviation. Statistical validation was conducted using a completely randomized analysis of variance (ANOVA), followed by post hoc analysis to identify specific differences among groups. Statistical significance was set at $p < 0.05$. Pearson's correlation coefficient was used to examine the relationships between the studied variables. All statistical analyses were conducted using SPSS software (PASW Statistics 18, version 18.0.0).

Results and discussion

Physicochemical characterization of sugarcane molasses and vinasses and their extracts

Table 1 presents the physicochemical characterization of the molasses and vinasses samples analyzed in this study. The pH and density values for both materials fall within the typical ranges documented in literature[47–49]. Specifically, the DMC and TSS values found in the molasses comply with the limits defined by the Colombian Technical Standard NTC 587/1994[50]. In contrast, the DMC and TSS values for vinasse were lower than those reported in previous studies[49,51,52]. This variability is not unusual, as the composition of organic and inorganic matter in vinasse can fluctuate based on variables such as the type of raw material used and the specific processing conditions applied during its generation[24,47].

Regarding TAC, these were measured using the Folin-Ciocalteu test. Although this test is typically used to estimate total PCs, it is well known that the Folin-Ciocalteu reagent reacts with any reducing molecule. Therefore, this test quantifies the total antioxidant agents present in a sample[26,39,53]. Based on this, the analyzed vinasses revealed a significantly higher concentration of TAC compared to molasses ($p < 0.05$). This result is consistent with previous findings[28]. However, a discrepancy was observed between the TAC content of the vinasse samples in this study and the values reported in the literature[24,28].

Even recognizing these differences, sugarcane molasses and vinasses have been highlighted as by-products of special interest due to their content of molecules with bioactive properties derived from their antioxidant power. Evidence suggests that the concentration of these compounds can increase in some of the products and

Parameter	Molasses samples	Vinasses samples
Dry matter content (DMC—%)	95 ± 1	6.1 ± 0.6
Total soluble solids (TSS—°Brix)	91.0 ± 0.9	6.6 ± 0.1
pH	5.55 ± 0.02	4.2 ± 0.1
Density (g mL^{-1})	1.50 ± 0.04	1.02 ± 0.03
Total antioxidant compounds (TAC—mg GAE g^{-1})	20.0 ± 1.6	25.3 ± 1.1

Table 1. Physicochemical characterization of sugarcane molasses and vinasses samples.

by-products obtained from sugarcane during the industrial processing[26,28,54]. Considering that various HICs with antioxidant activity (e.g., MRPs) are synthesized during sugarcane processing, it is likely that their presence adds to that of compounds from the plant's secondary metabolism (including PCs), potentially contributing to the increase in the overall concentration of TAC in some factory by-products, particularly molasses and vinasses[55,56]. Additionally, since these types of compounds are not consumed by yeasts during fermentation and are not degraded in the distillation processes involved in ethanol production, their concentration is expected to be higher in vinasses[24,28].

From the SPE process applied to the molasses and vinasse samples, two extracts were obtained for each by-product: one aqueous (EM_A and EV_A) and one methanolic (EM_B and EV_B). Figure 2 illustrates the TAC concentration of the extracts relative to that of the initial samples. For both by-products, the aqueous fractions exhibited a significantly lower TAC concentration compared to the methanolic fractions ($p < 0.05$). Specifically, the EM_A and EV_A extracts contributed 47% and 30%, respectively, of the TAC relative to the initial samples, whereas the EM_B and EV_B extracts showed higher TAC contributions of 96% and 70%. Additionally, statistical analysis indicated that the type of by-product from which each fraction was derived did not significantly affect the TAC values ($p > 0.05$), as the TAC levels for each type of fraction remained within relatively similar ranges.

A review of the published literature about TAC in sugarcane molasses and vinasses extracts, revealed some limitations that complicate direct comparisons of results. These limitations include variability in sample origin and differences in extraction and measurement protocols. Despite these challenges, this study highlights the potential of sugarcane by-products as significant sources of antioxidant molecules. The TAC values observed in this study are comparable to those reported for other agro-industrial waste, such as grape pomace (ranging from 21.4 to 11.6 mg GAE g^{-1})[57], litchi seeds (17.9 mg GAE g^{-1})[58], cauliflower waste (9.2 mg GAE g^{-1})[59], and garlic waste (6.91 mg GAE g^{-1})[60]. Additionally, sugarcane by-products exhibit higher TAC values than other by-products, such as potato peels (5.40 mg GAE g^{-1})[59], banana peels (3.8 mg GAE g^{-1})[58], and Kinnow seed (3.68 mg GAE g^{-1})[58].

Antioxidant capacity of the extracts

Table 2 presents the results corresponding to the ability of the extracts to scavenge free radicals, assessed by three in vitro tests: DPPH, ABTS, and FRAP. From the results obtained, it was evident that the antioxidant capacity values expressed in TE were generally higher with the ABTS method, while the DPPH method yielded the lowest values. These findings align with expectations, as the DPPH method is more selective in scavenging free radicals via hydrogen atom transfer (HAT mechanism), given that the radical does not react with aromatic acids containing only one hydroxyl group[61]. In contrast, the ABTS method generates a higher antioxidant response due to a synergistic effect, where molecules with low-volume aromatic ring systems have better access to the radical site, reacting quickly by single-electron transfer (SET mechanism), while antioxidants with more complex structures react more slowly via HAT[53,62]. The FRAP method, unlike the previous two, exclusively measures active compounds through SET, resulting in similar outcomes to the ABTS method. However, due to the acidic conditions required during the test to protect iron solubility, FRAP values in TE are usually lower than those from the ABTS assay[28,53].

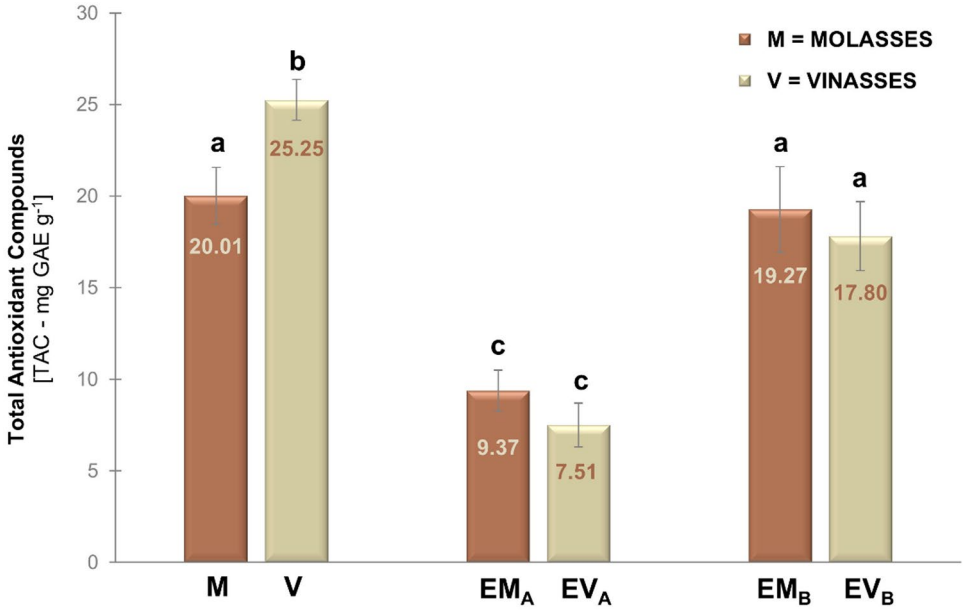

Fig. 2. Concentration of total antioxidant compounds (TAC) of the molasses (M) and vinasse (V) samples and their aqueous (EM_A and EV_A) and methanolic (EM_B and EV_B) extracts. (**a**, **b**, **c**) Bars with different letters are significantly different ($p < 0.05$).

Sample	DPPH		ABTS		FRAP	
	TEAC (μmol TE g^{-1})	IC_{50} (mg mL^{-1})	TEAC (μmol TE g^{-1})	IC_{50} (mg mL^{-1})	FRAP value (μmol TE g^{-1})	FRAP value (μmol $FeSO_4$ g^{-1})
EM_A	14.24 ± 3.35[a]	49.73 ± 3.78[a]	79.96 ± 07.91[a]	20.32 ± 1.63[a]	29.23 ± 1.91[a]	54.86 ± 3.62[a]
EV_A	3.09 ± 0.91[b]	77.01 ± 6.71[b]	56.95 ± 14.43[b]	22.83 ± 2.44[a]	12.91 ± 1.10[b]	22.80 ± 2.17[b]
EM_B	202.20 ± 18.93[c]	2.95 ± 0.06[c]	310.38 ± 11.70[c]	4.88 ± 0.17[b]	331.92 ± 14.53[c]	626.44 ± 27.75[c]
EV_B	92.50 ± 14.19[d]	7.74 ± 1.03[d]	200.27 ± 27.38[d]	8.63 ± 0.31[c]	161.43 ± 06.06[d]	304.26 ± 11.59[d]
Trolox*	–	0.14 ± 0.07[e]	–	0.37 ± 0.01[d]	–	6860.05 ± 474.64[e]

Table 2. Antioxidant capacity of molasses (EM_A and EM_B) and vinasse (EV_A and EV_B) extracts analyzed by different in vitro tests. * The results for Trolox are presented as a reference sample [a,b,c,d,e] Values with different superscript letters in the same columns are significantly different ($p < 0.05$).

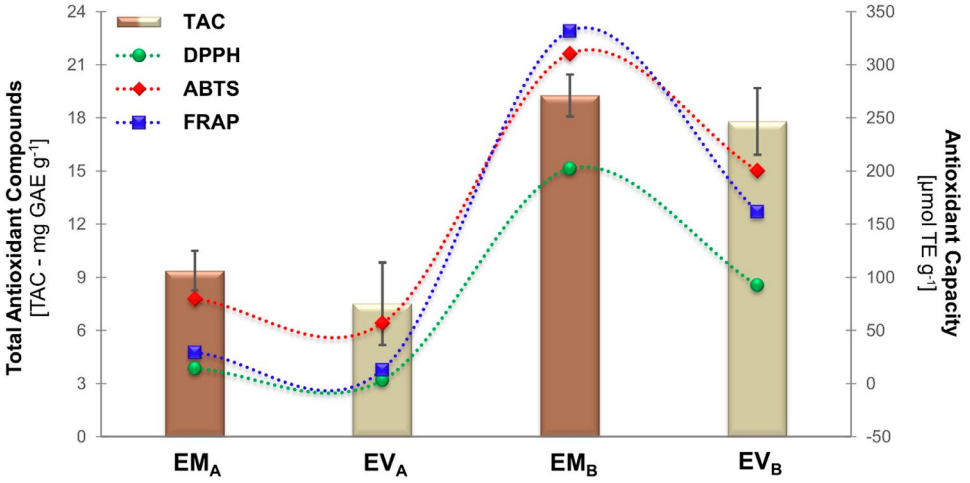

Fig. 3. Total antioxidant compounds (TAC) and antioxidant capacity of sugarcane molasses (EM_A and EM_B) and vinasse (EV_A and EV_B) extracts.

As shown in Table 2, regardless of the test applied, the molasses extracts (EM_A and EM_B) demonstrated higher antioxidant activity compared to the vinasse extracts (EV_A and EV_B), as indicated by significantly higher TEAC and FRAP values ($p < 0.05$) and lower IC_{50} values. Additionally, when comparing the two fractions extracted from each sample, it was found that, for both molasses and vinasse, the methanolic extracts (EM_B and EV_B) exhibited superior free radical scavenging performance compared to the aqueous extracts (EM_A and EV_A). These results are consistent with the findings from TAC quantification, indicating a strong correlation between these two variables ($0.863 < r < 0.949$; $p < 0.05$) (Fig. 3).

Although the potential of certain products, by-products, and waste from the sugarcane industry as sources of antioxidant compounds has been documented[26–28,39], the separation of PCs and HICs from these materials as independent fractions has not been widely explored. In fact, only one study has been known in which sugarcane vinasse samples revealed a behavior opposite to that previously described, where sugarcane vinasse samples demonstrated that the contribution to the overall antioxidant activity was more significant in the HIC-rich fractions (specifically MRPs) than in the PC-rich fractions[24]. Considering that these two types of compounds present in sugarcane by-products have notable antioxidant potential but originate from different processes (PCs from the plant's secondary metabolism and HICs generated during thermal industrial processes), these differences are interesting, since they provide an opportunity to continue exploring the separation of these fractions and their characteristics.

Characterization of the extracts in terms of their compounds of biological interest

The extracts obtained from molasses and vinasse samples were subjected to chromatographic separation and MS/MS analysis. Tentative identification of the compounds was achieved by comparing MS data (exact mass, isotopic distribution, and fragmentation pattern) with information from the literature and specialized databases (*e.g.*, Metlin, HMDB, and NIST). For LC–MS, acceptance criteria included a maximum difference of 5 ppm between theoretical and experimentally measured molecular ions and an ion abundance threshold of 1000 counts. For GC–MS, compounds were accepted based on a coincidence factor greater than 70%. Profiles of the tentatively identified compounds in each extract are described below.

Profile of PCs identified in methanolic extracts of molasses and vinasses (EM_B and EV_B)

The UHPLC-ESI-QTOF-MS/MS analysis of EM_B and EV_B extracts identified a total of 18 compounds in the EM_B extract and 19 in the EV_B, predominantly phenolic acids, and some flavonoids, both in free and conjugated forms. Detailed information about these compounds is provided in Table 3. Some compounds showed multiple correspondences, indicating the presence of different isomers with the same exact mass.

Comparing the PCs profiles, 15 compounds were identified in both extracts, of which 67% exhibited similar relative abundance values with no statistically significant differences ($p > 0.05$). Among these, compounds such as an isomer of feruloylquinic acid (B10), *p*-coumaric acid (B17), and schaftoside (B18) were particularly relevant. Specifically, *p*-coumaric acid was the most abundant PC in the EM_B extract and the second most abundant in EV_B, accounting for 14.9% and 17.6% in each case. On the other hand, the remaining compounds showed a different pattern, displaying significant differences in relative abundance between the two extracts ($p < 0.05$). In this category, compounds B01, B04, B05, B07, B13, B14, B16, and B19 were more abundant in the EM_B extract. Explicitly, 4-hydroxybenzaldehyde (B13); one isomer of chlorogenic acid (B04); 2,4-dihydroxybenzoic acid (B14); and 4-hydroxybenzoic acid (B05) had relative abundances of 13.9%, 8.5%, 8.0%, and 7.6%, respectively. In contrast, the EV_B extract exhibited higher relative abundances of compounds B03, B04, B05, B07, B09, B11, B13, B14, and B22, with 2,4-dihydroxybenzoic acid (B14); 4-hydroxybenzoic acid (B05); 4-hydroxybenzaldehyde (B13); and one isomer of chlorogenic acid (B04) being the most prominent, with respective abundances of 22.4%, 11.9%, 4.6%, and 4.4%. To be precise, B14 was the most predominant compound in the EV_B extract.

Multiple studies have cataloged sugarcane and its derivatives as significant sources of various phenolic acids and flavonoids, primarily in glycoside forms[26,54,63,64]. It is well-established that the phenolic structures in sugarcane stalks are progressively released during industrial processing, leading to higher concentrations of PCs in the by-products[28,55,56]. This phenomenon is attributed to milling processes that induce enzymatic reactions, promoting the methylation of caffeic acid to ferulic acid and facilitating the hydrolysis of lignin and hemicellulose in the cell wall, thereby releasing PCs such as vanillin and *p*-coumaric acid[26,54,56]. According to the above, the obtained results align with previous reports on molasses, which have documented the presence of these and other PCs identified in this study. Specifically, *p*-coumaric acid, ferulic acid, 4-hydroxybenzaldehyde, schaftoside, and several quinic acid derivatives have been identified as significant components[26,35,54,63,65]. Regarding vinasses, although their phenolic profile has not been extensively studied, several of the compounds identified here align closely with those previously reported for this by-product[24].

After examining the PCs profile of the extracts, the similarity in TAC observed between EM_B and EV_B ($p > 0.05$) (Fig. 2) can be attributed to the common presence of conjugated double bonds and multiple hydroxyl groups in the identified compounds, which confer optimal chemical properties for free radical scavenging[28,66,67]. Many of

#	Ret. time [min]	Tentative identification	Molecular formula	Adduct	Monoisotopic mass	$[M-H]^-$ (m/z)	Error [ppm]	EM_B		EV_B	
								Relative abundance*	(%)	Relative abundance*	(%)
B01	1.979	Dihydroferulic acid 4-sulfate	$C_{10}H_{12}O_7S$	$[M-H]^-$	276.0304	275.0231	−0.7	123,709 (3.5%)	2.4%	–	–
B02	2.048	Quinic acid	$C_7H_{12}O_6$	$[M-H]^-$	192.0634	191.0560	−0.1	140,664 (6.3%)[a]	2.7%	124,977 (7.4%)[a]	2.0%
B03	2.529	Pyrocatechol	$C_6H_6O_2$	$[M-H]^-$	110.0368	109.0280	−1.6	–	–	85,889 (13.6%)	1.4%
B04	2.943	Chlorogenic acid isomer I	$C_{16}H_{18}O_9$	$[M-H]^-$	354.0951	353.0880	0.5	439,841 (10.1%)[a]	8.5%	266,110 (0.5%)[b]	4.4%
B05	3.271	4-Hydroxybenzoic acid	$C_7H_6O_3$	$[M-H]^-$	138.0317	137.0220	−3.6	395,370 (1.5%)[a]	7.6%	728,116 (18.5%)[b]	11.9%
B06	3.437	Coumaroylquinic acid isomer I	$C_{16}H_{18}O_8$	$[M-H]^-$	338.1002	337.0930	0.3	81,027 (0.2%)[a]	1.6%	111,728 (21.5%)[a]	1.8%
B07	3.649	Chlorogenic acid isomer II	$C_{16}H_{18}O_9$	$[M-H]^-$	354.0951	353.0880	0.5	188,224 (2.8%)[a]	3.6%	119,351 (20.4%)[b]	2.0%
B08	3.755	Chlorogenic acid isomer III	$C_{16}H_{18}O_9$	$[M-H]^-$	354.0951	353.0880	0.5	193,023 (27.7%)[a]	3.7%	263,128 (4.3%)[a]	4.3%
B09	3.758	Vanillin	$C_8H_8O_3$	$[M-H]^-$	152.0473	151.0401	0.2	–	–	154,895 (0.7%)	2.5%
B10	3.841	Feruloylquinic acid isomer I	$C_{17}H_{20}O_9$	$[M-H]^-$	368.1107	367.1030	−1.2	354,965 (1.4%)[a]	6.9%	365,918 (32.5%)[a]	6.0%
B11	3.874	Vanillic acid/Homogentisic acid	$C_8H_8O_4$	$[M-H]^-$	168.0423	167.0330	−1.7	–	–	85,195 (2.4%)	1.4%
B12	3.941	Caffeic acid	$C_9H_8O_4$	$[M-H]^-$	180.0423	179.0330	−1.5	80,021 (10.4%)[a]	1.5%	79,644 (9.3%)[a]	1.3%
B13	4.057	4-Hydroxybenzaldehyde	$C_7H_6O_2$	$[M-H]^-$	122.0368	121.0262	−3.2	722,799 (4.9%)[a]	13.9%	281,355 (15.9%)[b]	4.6%
B14	4.060	2,4-Dihydroxybenzoic acid	$C_7H_6O_4$	$[M-H]^-$	154.0266	153.0195	0.9	414,494 (5.2%)[a]	8.0%	1,369,233 (12.1%)[b]	22.4%
B15	4.308	Coumaroylquinic acid isomer II	$C_{16}H_{18}O_8$	$[M-H]^-$	338.1002	337.0930	0.3	263,871 (1.3%)[a]	5.1%	235,335 (7.7%)[a]	3.9%
B16	4.696	Feruloylquinic acid isomer II	$C_{17}H_{20}O_9$	$[M-H]^-$	368.1107	367.1030	−1.2	242,759 (10.6%)	4.7%	–	–
B17	4.802	p-Coumaric acid	$C_9H_8O_3$	$[M-H]^-$	164.0473	163.0380	−1.9	770,213 (19.8%)[a]	14.9%	1,071,451 (24.1%)[a]	17.6%
B18	5.041	Schaftoside	$C_{26}H_{28}O_{14}$	$[M-H]^-$	564.1479	563.1400	−1.1	373,169 (8.4%)[a]	7.2%	342,420 (27.0%)[a]	5.6%
B19	5.094	Apigenin-6,8-C-diglucoside	$C_{27}H_{30}O_{15}$	$[M-H]^-$	594.1585	593.1503	−1.5	103,895 (4.4%)	2.0%	–	–
B20	5.137	3,4-Dihydrobenzaldehyde	$C_7H_6O_3$	$[M-H]^-$	138.0317	137.0230	−0.8	129,093 (9.6%)[a]	2.5%	140,294 (7.9%)[a]	2.3%
B21	5.190	Methoxyluteolin-8-C-glucoside	$C_{22}H_{22}O_{11}$	$[M-H]^-$	462.1162	461.1078	−2.5	164,625 (20.9%)[a]	3.2%	128,851 (6.1%)[a]	2.1%
B22	5.327	Ferulic acid	$C_{10}H_{10}O_4$	$[M-H]^-$	194.0579	193.0500	−2.3	–	–	146,810 (15.8%)	2.4%

Table 3. Compounds tentatively identified in methanolic extracts of sugarcane molasses and vinasses (EM_B and EV_B) by UHPLC-ESI-QTOF-MS/MS analysis. * Relative abundance values are accompanied by their respective relative standard deviation (RSD—%). Superscript letters ([a,b]) indicate statistically significant differences for the same row ($p < 0.05$).

these compounds are well-documented for their antioxidant activity. Nonetheless, the specific characteristics of certain compounds in each extract may account for the observed differences in antioxidant responses ($p < 0.05$) (Table 2), since as is known, antioxidant activity increases with the degree of hydroxylation[66,67]. Accordingly, the molecular structures of compounds such as dihydroferulic acid 4-sulfate (B01), chlorogenic acid (B04), and apigenin-*6,8-C*-diglucoside (B19) may enhance the antioxidant capacity of the EM_B extract due to a higher hydroxyl content. In contrast, the simpler structures of the compounds identified in EV_B might contribute to the lower antioxidant performance observed in this extract across the in vitro assays conducted in this study (Fig. 4).

Profile of HICs identified in aqueous extracts of molasses and vinasses (EM_A and EV_A)

GC–MS analysis of the EM_A and EV_A extracts revealed several volatile compounds, including some phenols, acids, pyranones, furanones, and alcohols. Following the established acceptance criteria, 17 compounds were tentatively identified in the EM_A extract and 19 in the EV_A extract. Among the identified molecules, only six compounds were common to both extracts, indicating significant differences in the volatile profiles of EM_A and EV_A. Table 4 summarizes the compounds tentatively identified in sugarcane molasses and vinasses extracts via GC–MS. To provide a more comprehensive evaluation of the results, the relative abundance of each compound was analyzed based on the percentages of their respective chromatographic peak areas.

Six compounds were identified in both extracts (A07, A11, A22, A24, A25, and A30). Among these, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) (A07) was the predominant compound in EM_A (22.94%) and the third most abundant in EV_A (7.60%). Another notable compound in the EM_A extract is 4-methylcatechol (A10), which has been associated with inducing apoptosis in various cancer cells[68–70]. Interestingly, the EV_A extract was more outstanding in terms of molecules with relevant biological activities. For instance, 2-methoxy-4-vinylphenol (A09) is a recognized aromatic compound with proven anti-inflammatory properties[71–74]. Additionally, compounds such as the 2,6-dimethoxyphenol (A13); 2,4-di-*tert*-butylphenol (A17); and 1,6-anhydro-*β*-D-glucopyranose (A18) have been previously described as molecules with antioxidant functionality[75–78].

Comparing the results of this study with existing literature reveals that several compounds tentatively identified in the EM_A and EV_A extracts have also been detected in various sugarcane industry derivatives. Notable examples include 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (A03); DDMP (A07); 2-methoxy-4-vinylphenol (A09); 2,6-dimethoxyphenol (A13); 2,4-di-*tert*-butylphenol (A17); and 3,4-dimethoxyphenol (A29). These compounds have been documented in the volatile profiles of sugarcane derivatives such as clarified

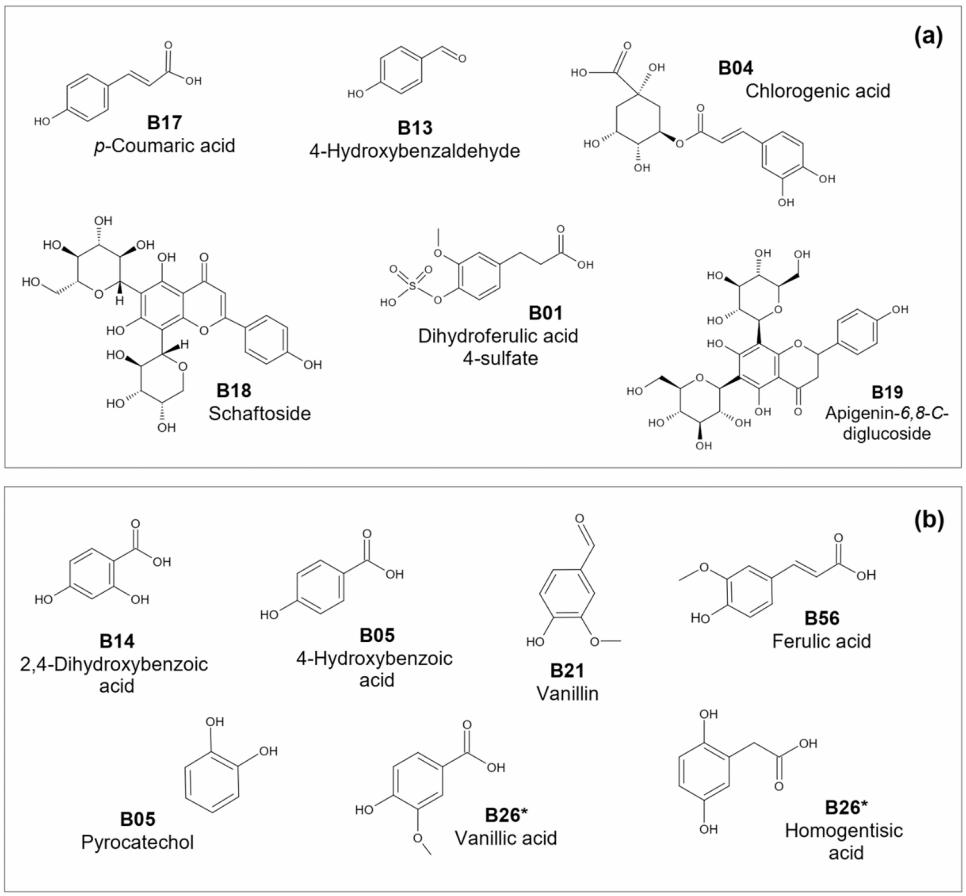

Fig. 4. Representative chemical structures of some of the main compounds tentatively identified in the methanolic extracts of sugarcane molasses (**a**) and vinasses (**b**).

juices, brown sugar, and non-centrifugal sugarcane (NCS)[39,79–82], as well as in by-products like molasses and vinasses[83–85]. These findings align with established expectations, as the specialized literature indicates that these types of compounds are commonly produced during the thermal processing of sugarcane juices[79]. Given that sugarcane is rich in reducing sugars and free amino acids, the temperature and pH conditions prevalent in juice clarification and evaporation processes are conducive to Maillard reactions, which are known to generate a range of volatile compounds, including those identified in this study[8]. Among the molecules found in the analyzed extracts, HDMF (A03); DDMP (A07); and 2,4-di-*tert*-butylphenol (A17) are recognized MRPs[17,18,86,87]. These compounds not only contribute to the sensory attributes of thermally processed products (such as aromas, flavors, and colors), but also exhibit significant biological properties, particularly as antioxidants[17,18,87–89]. While the Maillard reaction is complex and generates a diverse range of products, the understanding of factors influencing the biological effects of MRPs remains limited. However, in vitro studies suggest that some MRPs exert their bioactive effects by reducing oxidative damage caused by reactive oxygen species (ROS), acting as reducing agents and metal chelators[15,90].

Other molecules identified that could be considered HICs are 2,6-dimethoxyphenol (A13) and 1,6-anhydro-*β*-D-glucopyranose (A18). These compounds, found in the EV_A extract, have been studied for their antioxidant potential[77,78]. They are thought to result from the thermal decomposition of polymers like lignin and cellulose and are commonly used as distinctive markers of intensive biomass heating[91,92].

Despite the statistically similar TAC contents of the EM_A and EV_A extracts ($p > 0.05$) (Fig. 2), the differential composition of HICs may partially account for the observed differences in their antioxidant responses ($p < 0.05$) (Table 2). However, the superior in vitro free radical scavenging activity of the EM_A extract compared to EV_A cannot be solely attributed to their number of molecules, as the EM_A extract contained fewer compounds than EV_A. This suggests that the bioactive potential of the extracts is not exclusively dependent on the quantity or concentration of identified compounds but also on their chemical nature[93]. Structural factors, such as molecular

#	Ret. time [min]	Tentative identification	Molecular formula	Monoisotopic mass	Match factor	Peak area (%)*	
						EM_A	EV_A
A01	6.130	2,2-Dimethyl-4-decene	$C_{12}H_{24}$	168.1878	73	–	5.28
A02	6.410	2,2'-Bi-1,3-dioxolane	$C_6H_{10}O_4$	146.0579	82	2.73	–
A03	6.688	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)	$C_6H_8O_3$	128.0473	70	–	1.42
A04	6.733	3-Methylpiperazine-2,5-dione	$C_5H_8N_2O_2$	128.0585	72	4.60	–
A05	7.003	3-Methylcyclopentane-1,2,4-trione	$C_6H_6O_3$	126.0316	80	16.16	–
A06	7.833	Glycerol	$C_3H_8O_3$	92.0473	73	7.50	–
A07	8.087	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP)	$C_6H_8O_4$	144.0422	90	22.94	7.60
A08	9.935	1-(2-Methyl-1,3-oxathiolan-2-yl) ethanone	$C_6H_{10}O_2S$	146.0401	72	4.90	–
A09	10.293	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.0680	87	–	4.15
A10	10.365	4-Methylcatechol	$C_7H_8O_2$	124.0524	77	2.61	–
A11	10.371	2-Methyl-1,3-dioxolane	$C_4H_8O_2$	88.0524	87	10.75	4.11
A12	10.656	1-Deoxy-*d*-arabitol	$C_5H_{12}O_4$	136.0735	87	–	8.25
A13	10.767	2,6-Dimethoxyphenol	$C_8H_{10}O_3$	154.0629	89	–	5.78
A14	11.504	1-Butoxy-2-propanol	$C_7H_{16}O_2$	132.1150	74	–	7.10
A15	11.505	1,2,3-Benzenetriol	$C_6H_6O_3$	126.0316	81	3.74	–
A16	11.678	5-Decanol	$C_{10}H_{22}O$	158.1670	71	3.98	–
A17	12.747	2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.1670	91	–	3.15
A18	12.837	1,6-Anhydro-*β*-D-glucopyranose	$C_6H_{10}O_5$	162.0528	83	–	5.79
A19	13.102	4-Butyl-2-methoxyphenol	$C_{11}H_{16}O_2$	180.1150	80	–	3.32
A20	13.425	1,5-Anhydro-*d*-mannitol	$C_6H_{12}O_5$	164.0684	78	–	3.57
A21	14.097	2-(1-Ethoxyethoxy)-2-(2-oxiranyl) ethanol	$C_8H_{16}O_4$	176.1048	71	–	5.89
A22	15.073	1-Hydroxycyclohexyl phenyl ketone	$C_{13}H_{16}O_2$	204.1150	92	3.08	3.91
A23	15.579	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.2089	88	2.70	–
A24	17.699	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.2402	88	2.40	5.07
A25	17.938	3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)propionic acid	$C_{17}H_{26}O_3$	278.1881	88	2.58	3.54
A26	19.349	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282.2558	90	–	11.26
A27	19.602	Octadecanoic acid	$C_{18}H_{36}O_2$	284.2715	84	2.20	–
A28	21.312	Eicosanoic acid	$C_{20}H_{40}O_2$	312.3028	79	2.68	–
A29	23.526	3,4-Dimethoxyphenol	$C_8H_{10}O_3$	154.0629	85	–	6.73
A30	30.126	Tris (2,4-di-*tert*-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662.4463	72	4.45	4.08

Table 4. Compounds tentatively identified in aqueous extracts of sugarcane molasses and vinasses (EM_A and EV_A) by GC-EI-QTOF-MS analysis using the NIST 20 MS database. * The percentage of the area of the respective chromatographic peak of each compound was considered as its relative abundance within each extract.

size and the types of active functional groups in the identified compounds in each extract (Fig. 5), likely play a significant role in the observed differences in antioxidant activities between EM_A and EV_A[90,94].

Given that the characterization of EM_A and EV_A extracts was conducted tentatively, the precise identification of some compounds detected in the chromatographic analysis remains unresolved. Thus, further studies using different methodological approaches are necessary to accurately identify all compounds present in each extract. Additionally, due to the complexity of the analyzed samples, isolating, purifying, and evaluating each compound individually to identify specific antioxidants presents significant challenges. Therefore, the pragmatic approach adopted in this study confirmed the antioxidant and bioactive potential of the aqueous extracts of molasses and vinasses by associating it with the combined presence of various volatile molecules. Despite these limitations, our results are consistent with existing evidence, as they highlight the presence of HICs also found in other diverse sources such as roasted mustard seed oil[76], maple syrup extracts[95], dehydrated tamarind pulp[77], heated potato fiber[96], and dark malt extracts[90]. These sources, like sugarcane molasses and vinasses, share the common characteristic of undergoing industrial transformation processes involving similar thermal treatments.

Discussion

To explore the potential of sugarcane molasses and vinasses as viable bioactive materials, this research focused on studying the in vitro antioxidant properties of two specific groups of compounds extracted from these sources: PCs, as products of the secondary metabolism of the sugarcane, and the HICs formed during thermal processing. Both groups of compounds were selectively recovered from the sugarcane molasses and vinasses in methanolic and aqueous fractions and were then evaluated comparatively in terms of chemical composition and antioxidant capacity.

The results indicated that both TAC and antioxidant activity were significantly higher in the methanolic extracts (EM_B and EV_B) compared to the aqueous extracts (EM_A and EV_A) ($p < 0.05$), regardless of the by-product analyzed. This disparity can be attributed to the differential composition of each fraction. As expected, the methanolic extracts contained a higher abundance of PCs, including various phenolic acids and flavonoids, while the aqueous fractions comprised a diverse mixture of molecules, including some MRPs. In this sense, these differences in chemical composition are likely responsible for the observed variations in radical scavenging capacities between the extracts.

It is well-established that the antioxidant properties of a substance are influenced by specific structural factors, such as the degree of unsaturation, the presence and positioning of functional groups (e.g., hydroxyl, methoxyl, and sulfhydryl), and the substance's ability to restore itself after neutralizing free radicals[66,93,94,97]. Although the volatile compounds identified in the aqueous extracts have molecular structures with antioxidant-active groups, the PCs found in the methanolic extracts have more extensive polyunsaturated conjugated systems and high degrees of hydroxylation. These structural features make them more prone to neutralizing free radicals through electron or hydrogen transfer mechanisms[98,99].

Although the antioxidant activity of PCs and HICs present in molasses and vinasses was assessed in separate extracts, both compound groups were obtained from the same sugarcane-derived matrices. This coexistence

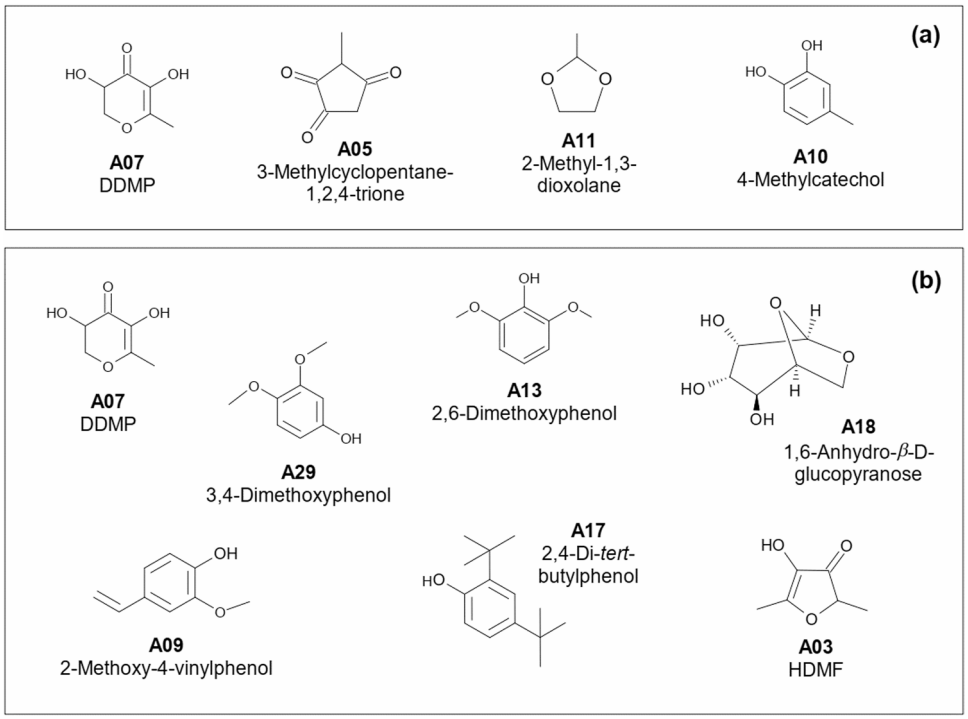

Fig. 5. Representative chemical structures of some of the main compounds tentatively identified in the aqueous extracts of sugarcane molasses (**a**) and vinasses (**b**).

reflects the compositional complexity of these by-products and suggests that the combined presence of PCs and HICs may enhance their bioactive properties through complementary mechanisms. Molecules with diverse functional groups and physicochemical characteristics may act at multiple levels of radical neutralization, potentially broadening the overall bioactive effect[99]. PCs typically exert their antioxidant effects by donating hydrogen atoms or electrons to neutralize free radicals and by chelating metal ions[53,93,94]. In turn, HICs, such as MRPs, can also scavenge radicals and chelate metals through distinct functional groups, including heterocyclic nitrogens and reductones[15,25,100]. This functional complementarity reinforces the value of sugarcane by-products as promising sources for antioxidant recovery.

While bioactive potential is often investigated by evaluating one analyte at a time, the non-targeted approach applied in this study provided a more comprehensive view of the bioactive properties of sugarcane by-products. This approach not only enabled the identification of molecules previously reported for their significant biological activities but also revealed unnoticed compounds that, while requiring further validation, could broaden the spectrum of biologically relevant molecules and help address unresolved phenomena. This perspective enhances our understanding of how sugarcane by-products can be harnessed as sources for engineering of bioactive materials, offering new possibilities for future applications.

Beyond the findings presented in this study, it is important to consider that, as with most agricultural-origin products, the composition and bioactivity of sugarcane molasses and vinasses may be influenced by factors such as variety, cultivation conditions, or industrial processing parameters[47,101,102]. Although these aspects were not the focus of the present study, they represent relevant directions for future research. Still, the results obtained here under controlled conditions offer a solid basis for understanding the contribution of PCs and HICs to the antioxidant potential of sugarcane by-products.

The antioxidant effects observed between the methanolic and aqueous extracts underscore the value of sugarcane molasses and vinasses as notable sources of bioactive compounds. Given that antioxidant activity is often correlated with other bioactive properties[99], these findings provide a solid foundation for further research into broader bioactivities that could be harnessed across multiple application fronts. Future work should include evaluating additional biological properties of these extracts, such as their antiproliferative potential in specific cellular contexts, and assessing their suitability for integration into functional systems. Potential applications could include functional food development, nutraceutical formulation, pharmacological prototypes, and bio-based materials design; particularly in strategies where oxidative stress modulation plays a central role. These perspectives further support the role of sugarcane by-products in the development of sustainable bioactive materials, with relevance across multiple sectors such as healthcare, nutrition, and materials science.

Conclusions and future work

The results of this study confirm the potential of sugarcane molasses and vinasses as sources of bioactive compounds. Both methanolic and aqueous extracts showed similar TAC contents, suggesting that the type of by-product did not significantly influence their TAC levels. Notably, the methanolic fractions (EM_B and EV_B) demonstrated superior free radical scavenging activity across various in vitro mechanisms, which is closely related to their higher TAC concentrations. Chromatographic separation followed by MS analysis revealed common features in the molasses and vinasses samples, including a significant presence of PCs such as *p*-coumaric acid, schaftoside, chlorogenic acid, and feruloylquinic acid in the methanolic extracts (EM_B and EV_B); whereas the aqueous extracts (EM_A and EV_A) prominently featured DDMP as one of the most prevalent HICs.

Overall, the results of this study suggest that the antioxidant responses observed in molasses and vinasses extracts are related to the diverse natural compounds derived from the secondary metabolism of sugarcane, as well as various compounds formed during its industrial processing. The coexistence of PCs and HICs in these by-products may enhance their antioxidant properties due to the contribution of distinct but complementary mechanisms, potentially leading to additive or even synergistic biological responses. This functional interaction reinforces the value of sugarcane molasses and vinasses as promising materials for obtaining bioactive compounds that could support innovation in the development of bioactive materials. Such relevance aligns with current trends in waste valorization, indicating that these by-products may serve as sources of effective agents with notable potential in sustainable bioproduct design. Accordingly, it is crucial to explore whether these antioxidant properties are associated with additional biological activities related to oxidative stress regulation, such as cell proliferation inhibition. Investigating these connections could open new avenues for future health-related and pharmacological applications, including the development of targeted therapies for chronic diseases like cancer. Future studies should aim to elucidate the molecular mechanisms by which these compounds exert their bioactivity, particularly those associated with oxidative stress pathways and the regulation of cell proliferation. Gaining such insights is essential for translating the antioxidant potential of sugarcane molasses and vinasses into practical and therapeutically relevant applications, and fully leveraging their value in the development of bioactive materials. These research directions are currently being pursued in complementary studies derived from this investigation.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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