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Quality and safety of fresh sugarcane juice sold by street vendors: a growing public health concern

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Sugarcane juice is a nutrient-rich beverage with numerous health benefits. However, in Morocco, the safety and quality and of fresh sugarcane juice have not been adequately assessed. To bridge this gap, a total of 100 sugarcane juice samples sold by street vendors in the city of Beni Mellal, Morocco, were collected between February and June 2024 and analyzed for key quality and safety parameters, including the evaluation of the microbiological quality and safety of fresh sugarcane juice sold in Morocco, the analysis of its physicochemical properties, and the assessment of the potential health risks linked with its consumption. Physicochemical analysis revealed a pH of 5.23 ± 0.09, total titratable acidity (TTA) of 0.16 ± 0.02% (g citric acid equivalent/100 mL), and total soluble solids (TSS) of 18.48 ± 0.69 °Brix. The juice exhibited notable bioactive properties, with total phenolic content (TPC) of 90.25 ± 8.02 mg GAE/100 mL, total flavonoid content (TFC) of 46.83 ± 8.79 mg QE/100 mL, vitamin C content of 11.25 ± 1.55 mg/100 mL, and DPPH scavenging activity with IC_{50} of 72.45 ± 7.07 g/L. Microbiological assessment indicated contamination with Total Aerobic Mesophilic Flora, total coliforms, fecal coliforms, yeasts and molds, staphylococci at a level of 5.79 ± 0.36, 3.78 ± 0.33, 1.67 ± 0.34, 3.73 ± 0.23, and 2.81 ± 0.23 log CFU/mL, respectively. Additionally, *Escherichia coli* was detected in 77% of samples and *Staphylococcus aureus* was found in 7% of samples, while *Salmonella* and *Listeria monocytogenes* were no detected. Antimicrobial susceptibility revealed high resistance of *E. coli* and *S. aureus* to ampicillin, penicillin, streptomycin, and tetracycline, with multidrug-resistant (MDR) profiles identified in 40.26% of *E. coli* and 42.86% of *S. aureus* isolates. Notably, 24.67% of *E. coli* and 42.86% of *S. aureus* isolates exhibited a multiple antibiotic resistance (MAR) index > 0.2, indicating a high-risk contamination source. Moreover, conventional PCR analysis revealed the presence of the *sea* gene in one *S. aureus* isolate (14.28%). Additionally, the *stx1* and *stx2* genes were detected in 8 (10.39%) and 5 (6.49%) *E. coli* isolates, respectively. In contrast, the *hlyA* gene was not detected in any of the *E. coli* isolates. Our results underscore a serious public health concern, emphasizing the urgent need for improved hygienic practices and regulatory monitoring fresh juice sold by street vendors in Morocco.

KEYWORDS

***Saccharum officinarum* L., microbial contamination, antimicrobial resistance, virulence, *Escherichia coli*, *Staphylococcus aureus*, food safety**

1 Introduction

Food safety has become a growing interest for both national and international authorities, as well as the global population. Consumers are increasingly demanding safe and high-quality food products (Sorbo et al., 2023). Additionally, challenges such as climate change, pollution, and the overuse of pesticides and synthetic products in agriculture have created significant obstacles. Consequently, ensuring the safety and nutritional quality of food has become a key priority for international regulatory bodies (Agrimonti et al., 2021; Ed-Dra et al., 2025). Indeed, fruit and vegetable juices are recognized as sustainable and nutrient-rich food sources, providing essential vitamins, minerals, fiber, and antioxidants (Butu and Rodino, 2019). Their role in enhancing wellbeing and reducing the risk of illnesses has further heightened their importance in global food systems (Renda and Söhretoglu, 2024). However, various studies have reported the implication of vegetables and fruits in many cases of foodborne outbreaks (Aiyedun et al., 2021). In fact, the contamination of fruits and vegetables can occur during farming practices, especially when using contaminated irrigation water (Steele and Odumeru, 2004; Gurtler and Gibson, 2022). Moreover, the contamination can be linked to harvest practices like contact with contaminated hands of farmers, post-harvest manipulation as well as the pressing and preparation of juices (Lenzi et al., 2021). Therefore, final products, including Fruit and vegetable juices may harbor harmful bacteria, including fecal contaminants and disease-causing pathogens such as *Escherichia coli*, *Salmonella*, and others (Lee et al., 2021; Nan et al., 2022; Neggazi et al., 2024).

Sugarcane juice, extracted from the stem of *Saccharum officinarum* L., is a widely consumed natural beverage appreciated worldwide for its distinctive sweetness and aroma (Dhansu et al., 2023). It contains a high concentration of beneficial bioactive molecules, including phenolic acid derivatives (glucosyringic acid, dihydromelilotoside, malaysin A, 2-O-caffeoylglucarate, and coumaric acid), flavones (diosmetin, luteolin, apigenin, and tricine derivatives), and dilignols (Duarte-Almeida et al., 2011; Rodrigues et al., 2021), making it a nutritionally valuable beverage with significant health benefits (Hewawansa et al., 2024). Traditionally, sugarcane juice has been widely applied in therapeutic practices for managing conditions including anuria, jaundice, dysuria, bleeding disorders, and urinary complications (Chinnadurai, 2017). However, its preparation and sale often occur in informal markets under unhygienic conditions, raising concerns about safety and microbial contamination (Panigrahi et al., 2021). This is particularly concerning in the street markets of developing countries, where the risk of contamination is significantly higher. Existing literature suggests that improper hygiene standards during processing contribute significantly to sugarcane juice contamination, the utilization of contaminated raw sugarcane, inadequate cleaning of processing equipment (e.g., presses and knives), unclean contact surfaces, vendors' hands and clothing, and airborne particles (da Costa Messias et al., 2024). These factors collectively pose a significant risk to sugarcane juice safety, threatening consumer health.

Importantly, a survey study on sugarcane juice sold in the streets of Punjab, Pakistan, revealed antibiotic-resistant enterobacteriaceae including *E. coli*, *Shigella* spp., and *Salmonella* spp., presents considerable public health challenges regarding product safety (Amjad et al., 2022). Similarly, a study in Brazil showed a wide contamination of sugarcane juice with total coliforms (100%) and *E. coli* (19.05%), with a sample was detected positive for the presence of *Salmonella* Typhimurium (da Costa Messias et al., 2024). The excessive and inappropriate application of antimicrobial compounds, especially in animal husbandry practices, has substantially contributed to the emergence of resistant microbial strains (Mandal and Mandal, 2018). These studies underscore the significant risk of microbial contamination in fresh sugarcane juice sold by street vendors.

The presence of antimicrobial-resistant and virulent pathogens in fresh products such as sugarcane juice is a major public health concern, posing significant challenges for food safety authorities. Antimicrobial resistance (AMR) allows microorganisms to withstand antimicrobial treatments, creating serious risks, particularly for vulnerable populations (Ed-Dra et al., 2019; Salam et al., 2023). Microorganisms can develop resistance after exposure to sublethal concentrations of antimicrobial agents in different sectors. In this regard, the overuse and misuse of antimicrobial agents, particularly in livestock farming, have accelerated development of resistance (Khmaissa et al., 2024). Additionally, exposure to environmental stressors and synthetic compounds, including pesticide residues, and environmental pollutants, can further induce AMR (Wang et al., 2023; Ifedinezi et al., 2024; Karwowska, 2024; Murray et al., 2024). Alarming research forecasts position antibiotic resistance as potentially the foremost cause of mortality worldwide within three decades, with direct AMR-related deaths potentially reaching 1.91 million per year by 2050 (95% UI, 1.56–2.26 million) and associated with 8.22 million deaths (95% UI, 6.85–9.65 million) globally (Naghavi et al., 2024). Therefore, enhanced surveillance is urgently needed, particularly for fresh food products such as juices, to help policymakers in implementing effective strategies to tackle the development and dissemination of antimicrobial resistant and virulent pathogens through the food chain.

In Morocco, street food markets have experienced significant growth in recent years, playing a crucial socio-economic role by providing affordable and accessible foods to urban populations while supporting local livelihoods, particularly for low-income vendors. These vendors are often concentrated in densely populated areas, serving vulnerable groups such as children, the elderly, and low-income families. However, inadequate hygiene practices, unsafe food handling, and poor sanitation pose serious health risks, increasing the likelihood of severe foodborne illnesses, especially among immunocompromised population. Street-vended sugarcane juice has rapidly gained popularity in Morocco; however, despite its widespread consumption, no studies have assessed its microbiological quality, physicochemical properties, or associated health risks. This lack of data hinders the ability of public health authorities and consumers to make informed decisions regarding its safety. To address this gap, our study provides the first comprehensive evaluation of the quality and safety of fresh sugarcane juice sold in Morocco. The findings aim to support improved food safety regulations and enhance consumer protection.

2 Materials and methods

2.1 Sample collection

Sampling was carried out in Beni Mellal city, located in central Morocco, between February and June 2024. Sampling took place in the afternoon in crowded areas, including "Al Massira," "Safaa," "University Campus," and "Central Bus Station" sites. A sample size of 100 samples was obtained for laboratory examination, with 25 samples from each site over the study period, corresponding to approximately five samples per site per month. Street vendors were randomly selected based on their presence at the time of sampling. Most vendors were mobile, except for those at "Al Massira" site, who were stationary. Each sample (~200 mL) was collected in a sterile bag and transported within 30 min at 4°C using a cooler to the Higher School of Technology of Beni Mellal. Upon arrival at the laboratory, samples were analyzed immediately, or, if necessary, stored at 4 ± 1°C and analyzed within 24 h.

2.2 Physicochemical analysis

To characterize the physiochemical properties of sugarcane juice samples, various parameters were assessed, including pH, total titratable acidity (TTA), and total soluble solids (TSS; °Brix). The pH was calculated using a digital pH meter (Milwaukee MW150, Romania) according to the standard method (ISO 1842, 1991). Before use, pH meter was calibrated using buffer solutions at pH 4.00 and pH 7.00, then a sufficient volume of the sample was prepared in a 100 mL beaker to ensure proper electrode immersion. The TSS were measured using a digital refractometer (Milwaukee MA883, Romania) following the producer's guidelines. However, TTA was determined according to the standard method AOAC 942.15 (AOAC, 2016) and expressed in percentage using the following formula:

$$\%\ TTA = \frac{Vt \times M \times meq}{Vs} \times 100$$

Here, *Vt* denotes the volume of the titrant (NaOH), *M* is the molarity of NaOH (0.1 M), *meq* refers to the citric acid equivalence factor (0.070), and *Vs* indicates the volume of the sample analyzed. The total acidity was reported as grams of citric acid equivalents per 100 mL of sample (g CAE/100 mL).

2.3 Total phenolic, flavonoids, and vitamin C contents

Quantification of total phenolic content (TPC) in sugarcane juice was performed through the Folin-Ciocalteu assay, following established spectrophotometric protocols (Habibi et al., 2024). The results were reported as milligrams of gallic acid equivalents per 100 mL of juice (mg GAE/100 mL), reflecting the total phenolic content of the samples. The total flavonoid content (TFC) was determined using aluminum chloride colorimetric method, as described previously (Wu et al., 2021), and expressed in milligrams of quercetin equivalent per 100 mL of juice (mg QE/100 mL). To ensure the reliability of the results, the Folin–Ciocalteu method used for the determination of total phenolic content and the aluminum chloride colorimetric method used for the determination of total flavonoid content were validated through the preparation of standard calibration curves using gallic acid and quercetin, respectively, both exhibiting high linearity ($R^2 > 0.99$). However, the vitamin C content was determined directly from juice samples using iodine titration method (Feszterová et al., 2023), Data are presented as vitamin C equivalents (mg VC/100 mL ± SD), derived from triplicate measurements of each sample.

2.4 Antioxidant activity

DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging assay was used to determine the antioxidant activity, following a previously published method (Ed-Dra et al., 2021). DPPH solution was prepared by dissolving 0.04 of DPPH (HiMedia, India) in 1L of methanol (HiMedia, India). Concurrently, serial 2-fold dilutions of sugarcane juice were prepared using methanol. Following this, 0.5 mL of each diluted sample was combined with an equal volume of 0.04 g/L DPPH methanolic solution and vortexed thoroughly. The reaction mixtures were then incubated in darkness at the ambient temperature (25 ± 2°C) for 30 min. Post-incubation, the absorbance (A) was recorded at 517 nm using a visible spectrophotometer (ONDA V-10 PLUS, Italy). The DPPH scavenging activity (%) was determined using the following equation:

$$\%inhibition = \frac{(A\ blank - A\ sample)}{A\ blank} \times 100$$

L-ascorbic acid served as the reference antioxidant standard. The half-maximal inhibitory concentration (IC_{50}) was derived by non-linear regression analysis of the dose-response curve (percentage inhibition vs. sample concentration), generated using Microsoft Excel (Version 16, Microsoft Corp., USA).

2.5 Microbiological analysis

To assess the microbiological quality of the analyzed samples, serial decimal dilutions were prepared by transferring 1 mL of each sample into 9 mL of sterile physiological saline solution (NaCl 9 g/L; HiMedia, India). Total Aerobic Mesophilic Flora (TAMF) was quantified by spreading 1 mL of each dilution onto Plate Count Agar (PCA; Biokar, Beauvais, France) and incubating at 30 °C for 72 h in accordance with ISO 4833-1 (2013). Total and fecal coliforms were enumerated using Violet Red Bile Agar (VRBA; Biokar), with incubation at 30 °C for 24 h for total coliforms and at 44°C for 24 h for fecal coliforms, following (ISO 4832, 2006). Yeasts and molds were counted on Chloramphenicol Glucose Agar (CGA; Biokar), with incubation at 25°C for 3–5 days as per (ISO 21527-1, 2008). Enumeration of *E. coli* was performed using Tryptone Bile X-glucuronide (TBX; Biokar) agar, incubated at 44°C for 24 h (ISO 16649-2, 2001). Staphylococci were assessed by surface spreading of 0.1 mL from each dilution on Baird-Parker Agar (BP; Biokar)

followed by incubation at 36 °C for 48 hours according to ISO 6888-1 (2021). In addition, the presence of *Listeria monocytogenes* and *Salmonella* spp. was investigated in 25 mL of each sample following standard detection protocols previously described (Ed-Dra et al., 2017; Bouymajane et al., 2021).

2.6 Biochemical confirmation of *E. coli* and *S. aureus* isolates

Presumptive *E. coli* colonies, characterized by their blue-green appearance on Tryptone Bile X-glucuronide (TBX) agar, were subcultured onto Tryptone Soy Agar (TSA; Biokar, Beauvais, France) for purification. For confirmation, a single colony was transferred into indole-free peptone water (Biokar) and incubated at 36°C for 24 h. Following incubation, 0.5 mL of Kovac's reagent was added to the culture. The development of a red ring at the liquid surface indicated indole production, confirming the identity of the isolates as *E. coli*. Similarly, suspected *Staphylococcus aureus* isolates, initially identified by their black or gray colonies with clear halos on Baird-Parker agar, resulting from tellurite reduction and lecithinase activity were purified on TSA. For confirmation, a single colony was inoculated into Brain Heart Infusion (BHI) broth and incubated at 36°C for 24 h. Then, 0.1 mL of the culture was mixed with 0.3 mL of rabbit plasma (Biokar), and the tube was examined for coagulation after 4–6 h and again at 24 h. A positive coagulase reaction confirmed the isolates as *S. aureus* (Ed-Dra et al., 2018). The confirmed *E. coli* and *S. aureus* isolates were stored in 25% glycerol at −20°C until further use. The reliability of indole production test was confirmed by using *E. coli* ATCC 25922 as positive control and *Salmonella* Typhimurium ATCC 14028 as negative control; however, the reliability of coagulase test was confirmed by using *S. epidermidis* ATCC 12228 as negative control and *S. aureus* ATCC 29213 as positive control.

2.7 Antibiotic susceptibility assay

The isolated *E. coli* and *S. aureus* strains were tested against a panel of antimicrobial agents (Supplementary Tables S2, S3) using disc diffusion method on Muller-Hinton agar (Biokar, Beauvais, France), following the guideline of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2024), and the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2020). Isolates with intermediate susceptibility were considered resistant for the purpose of analysis. Those exhibiting resistance to antibiotics from more than two different classes were designated as multidrug-resistant (MDR). The Multiple Antibiotic Resistance (MAR) index was computed by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics evaluated (Ed-Dra et al., 2018). A MAR index >0.2 indicates that the bacterial strain likely originates from a high-risk source with frequent antibiotic exposure. In contrast, a MAR index of 0.2 or below suggests origin from environments with limited antibiotic use. For quality control purposes, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were employed as reference strains in this study.

2.8 Virulence genes detection

Conventional PCR was used to detect specific virulence genes in the isolated E. coli and S. aureus strains. Boiling method was used for DNA extraction, involving three repeated cycles of boiling at 100°C for 10 min and freezing at −20°C for 15 min, followed by centrifugation at 12,000 × g for 5 min. The resulting supernatant containing genomic DNA was quantified using a NanoDrop spectrophotometer (Berthold Colibri LB 915, Germany). Detection of the sea gene, which encodes enterotoxin production in S. aureus isolates, as well as the stx1 (Shiga-like toxin 1), stx2 (Shiga-like toxin 2) and the hlyA (enterohemolysin) genes in E. coli isolates, was performed according previously published protocols (Table 1). PCR amplification products were analyzed by electrophoresis on a 1.2% agarose gel pre-stained with ethidium bromide. DNA fragment sizes were estimated by comparison with a molecular weight marker (GeneRuler 1 kb Plus DNA Ladder, Thermo Scientific). To ensure the reliability of virulence gene detection, sterile distilled water was used as a negative control, while E. coli ATCC 43895, which harbors the stx1, stx2, and hlyA genes, and S. aureus ATCC 13565, which harbors the sea gene, were used as positive controls.

2.9 Data analysis

Data were compiled and statistically analyzed using Microsoft Excel (New York, USA), with results expressed as mean ± standard deviation (SD). Graphical illustrations and Pearson correlation analysis (significance set at $p < 0.05$) were conducted using GraphPad Prism software (version 9; GraphPad, San Diego, CA, USA).

3 Results

3.1 Physicochemical characterization

The physicochemical properties of sugarcane juice, including pH, TTA, and TSS, were analyzed, and the results are summarized in Supplementary Table S1. The analyzed sugarcane juice samples had an average pH of 5.23 ± 0.09, with minimum and maximum values of 4.98 and 5.54, respectively, indicating a slightly acidic nature. The TTA, expressed as citric acid equivalents, averaged 0.16 ± 0.02% (g CAE/100 mL), with a minimal value of 0.11% and a maximal value of 0.21%. Additionally, the TSS content, measured in °Brix, averaged 18.48 ± 0.69 °Brix, with values ranging between 17.1 and 19.9 °Brix.

3.2 Determination of bioactive compounds

Biochemical analysis demonstrated that sugarcane juice is rich in bioactive compounds (Supplementary Table S1). The samples contained an average TPC of 90.25 ± 8.02 mg GAE/100 mL, ranging from 77.08 to 110.2 mg GAE/100 mL. Additionally, the average TFC was 46.83 ± 8.79 mg QE/100 mL, with values between 30.08 and 63.25 mg QE/100 mL. Vitamin C concentrations averaged 11.25 ± 1.55 mg/100 mL, with a range between 7.92 and

TABLE 1 Primer sequences used for the amplification of targeted virulence genes.

Genes	Primers (5′-3′)	Size (bp)	Function	References
sea	F: TGCAGGGAACAGCTTTAGGCAA	500	Enterotoxin	Sallam et al., 2015
	R: GATTAATCCCCTCTGAACCTTCC			
stx1	F: ACACTGGATGATCTCAGTGG	614	Shiga-like toxin 1	Fagan et al., 1999
	R : CTGAATCCCCCTCCATTATG			
stx2	F: GTTTTTCTTCGGTATCCTATTCC	484	Shiga-like toxin 2	Sarimehmetoglu et al., 2009
	R: GATGCATCTCTGGTCATTGTATTAC			
hlyA	F: GCATCATCAAGCGTACGTTCC	534	Enterohemolysin	Srivani et al., 2017
	R: AATGAGCCAAGCTGGTTAAGCT			

14.08 mg/100 mL. These bioactive compounds contribute to the antioxidant activity of sugarcane juice, as demonstrated by the DPPH scavenging assay, which yielded an average IC_{50} of 72.45 ± 7.07 g/L (ranged between 62.18 and 85.69 g/L). In comparison, the IC_{50} of the standard (ascorbic acid) was significantly lower at 0.045 ± 0.02 g/L. Notably, a lower IC_{50} value indicates higher antioxidant potential.

Additionally, Pearson correlation analysis revealed a strong correlation between the TPC and antioxidant activity (measured by IC_{50}) ($r = -0.92$; 95% CI: −0.94 to −0.88; $R^2 = 0.84$; $p < 0.0001$), as well as between TFC and antioxidant activity ($r = -0.92$; 95% CI: −0.94 to −0.88; $R^2 = 0.84$; $p < 0.0001$) (Figures 1A, B).

3.3 Microbiological assessment

This study investigated the microbiological quality of sugarcane juice samples, with the quantitative findings illustrated in Figure 2. The mean count of Total Aerobic Mesophilic Flora (TAMF) was 5.79 ± 0.36 log CFU/mL, with observed values ranging from 4.90 to 6.43 log CFU/mL, indicating a substantial microbial load. Total coliforms (TC) exhibited an average concentration of 3.78 ± 0.33 log CFU/mL, within a range of 2.30 to 4.22 log CFU/mL. Fecal coliforms (FC) were present at an average of 1.67 ± 0.34 log CFU/mL, with counts varying between 0.70 and 2.32 log CFU/mL. Fungal contamination, represented by yeast and mold counts, showed a relatively narrow distribution, with a mean of 3.73 ± 0.23 log CFU/mL and values between 3.18 and 3.24 log CFU/mL. Staphylococci were detected at an average level of 2.81 ± 0.23 log CFU/mL, ranging from 1.78 to 3.24 log CFU/mL. Among the tested samples, *E. coli* was identified in 77 cases, with a mean count of 1.31 ± 0.26 log CFU/mL, and concentrations ranging from 0.48 to 1.81 log CFU/mL. Conversely, *S. aureus* was detected in only 7 samples, with a mean count of 1.94 ± 0.16 log CFU/mL and a range of 1.70 to 2.18 log CFU/mL. These findings underscore significant variability in microbial contamination, suggesting potential lapses in hygienic practices and the need for enhanced quality control in the preparation and distribution of sugarcane juice.

3.4 Antimicrobial susceptibility profiles

The isolated bacterial strains were subjected to antimicrobial susceptibility testing against a panel of antibiotics representing various classes. The outcomes of these tests are detailed in Table 2, with specific results for *E. coli* and *S. aureus* presented in Supplementary Tables S2, S3, respectively.

Among the *E. coli* isolates ($n = 77$), antimicrobial susceptibility testing showed a high prevalence of resistance to ampicillin (80.52%), followed by streptomycin (75.32%), tetracycline (42.86%), and trimethoprim-sulfamethoxazole (23.38%), as detailed in Table 2. All isolates were fully susceptible to cefoxitin, ceftriaxone, cefotaxime, and imipenem. The isolates were distributed across 17 distinct AMR profiles, with 31 isolates (40.26%) classified as multidrug-resistant (MDR) due to resistance to more than two antibiotic classes (Table 3). MAR index evaluation revealed that 19 isolates (24.67%) had an index >0.2, suggesting exposure to environments with frequent antibiotic usage and indicating a considerable public health risk (Supplementary Table S2).

As for the *S. aureus* isolates ($n = 7$), resistance was most frequently observed against penicillin (85.71%), followed by streptomycin (71.43%) and tetracycline (57.14%). All isolates were susceptible to oxacillin, cefoxitin, gentamicin, kanamycin, fusidic acid, erythromycin, vancomycin, trimethoprim-sulfamethoxazole, and clindamycin (Table 2). Each of the seven isolates exhibited a unique AMR profile, with 3 isolates (42.86%) identified as MDR (Table 4). Additionally, 3 isolates (42.86%) recorded MAR indices above 0.2, indicating a potential link to high-risk contamination sources (Supplementary Table S3).

3.5 Virulence genes

Virulence genes detection was performed using conventional PCR, and the results are summarized in Supplementary Tables S2, S3. Among the *S. aureus* isolates, the *sea* gene was detected in only one isolate (14.28%). For the *E. coli* isolates, screening for Shiga toxin genes (*stx1*, *stx2*) and *hlyA* revealed that 10 isolates carried at least one virulence gene. Specifically, *stx1* was detected in 8 isolates (10.39%) and *stx2* in 5 isolates (6.49%), while hlyA was not detected in any of the examined isolates (Figure 3). Furthermore, three *E. coli* isolates (3.89%) were found to harbor both *stx1* and *stx2* genes.

4 Discussion

In recent years, the consumption of fruit juices has markedly increased, driven by their high nutritional value and the

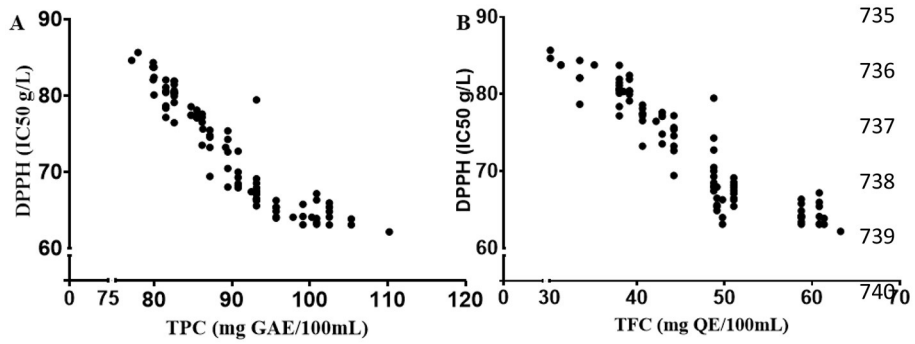

FIGURE 1
Pearson correlation between TPC and antioxidant activity **(A)** and between TFC and antioxidant activity **(B)**.

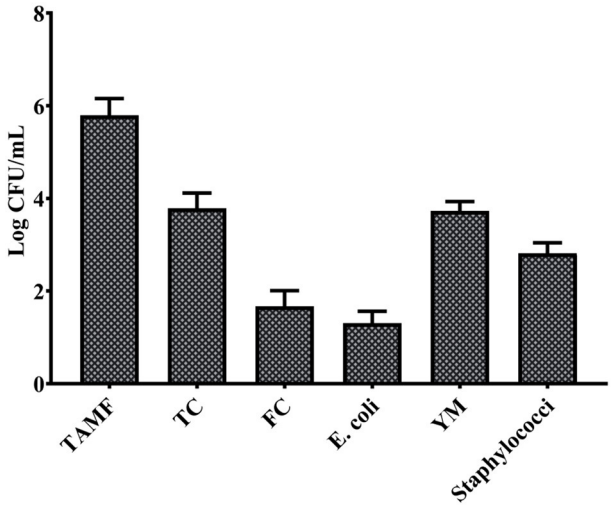

FIGURE 2
Microbiological contamination of sugarcane juice samples. The average rate and standard deviation of *E. coli* and *S. aureus* were determined based on only positive samples (77 samples for *E. coli* and 7 samples for *S. aureus*).

growing public perception of their associated health benefits. In Morocco, sugarcane juice is a popular beverage typically extracted mechanically from cane stalks, filtered, and sold fresh by street vendors without undergoing any prior treatment. However, the absence of suitable treatment may increase the risk of microbial contamination, posing potential food safety hazards to consumers. Despite these concerns, no studies in Morocco have yet evaluated quality and safety of fresh sugarcane juice. In this regard, we conducted this study to assess the quality and safety of sugarcane juice distributed by street vendors, providing critical data to inform public health measures.

In this study, we evaluated the physicochemical characteristics of sugarcane juice sold by street vendors in Beni Mellal, Morocco. The results revealed that the analyzed samples had pH values ranging from 4.98 to 5.54, indicating a slightly acidic nature. These values align with findings from India and Pakistan, where sugarcane juice exhibited a pH of 5.2 (Kamble et al., 2021; Irshad et al., 2024), as well as with studies from Bangladesh, where pH values ranged between 4.97 and 5.35 (Akter et al., 2024). A study conducted in Malaysia showed slightly higher pH values with an average of 5.8 ± 0.05 (Adulvitayakorn et al., 2020), while a study carried out in Tanzania reported slightly lower pH values, between 4.8 and 4.9 (Issa-Zacharia and Rwabunywenge, 2023). Additionally, our findings indicated that the TTA varied from 0.11% to 0.21%, which is consistent with the results of Kamble et al. (2021) in India (0.2%) and Akter et al. (2024) in Bangladesh (0.22–0.37%).Our study showed higher TTA values than that reported in Tanzania (average of 0.08%) (Issa-Zacharia and Rwabunywenge, 2023) and Malaysia (average of 0.032 ± 0.006%) (Adulvitayakorn et al., 2020), but lower than that found in Pakistan (average of 0.31%) (Irshad et al., 2024). Furthermore, the analyzed samples exhibited TSS values between 17.1 and 19.9 °Brix, with an average of 18.48 ± 0.69 °Brix. These values are comparable to those reported in India (18 °Brix) by Kamble et al. (2021), and Pakistan (18.5 °Brix) by Irshad et al. (2024). However, they are higher than those observed in Bangladesh (between 7.58 and 12.99 °Brix) (Akter et al., 2024) and Malaysia (10.8 ± 0.12 °Brix) (Adulvitayakorn et al., 2020). A study from Tanzania found TSS values between 12.2 and 22.1 °Brix (Issa-Zacharia and Rwabunywenge, 2023). These results indicate that sugarcane juice is rich in TSS, has a slightly acidic pH, and contains low TTA. While it can be considered a nutritious source for consumers, its physicochemical properties may also create a favorable environment for the growth of spoilage and pathogenic microorganisms.

The quantification of bioactive compounds revealed that sugarcane juice is rich in TPC, with values ranging from 77.08 to 110.2 mg GAE/100 mL (average of 90.25 ± 8.02 mg GAE/100 mL), and TFC, with values between 30.08 and 63.25 mg QE/100 mL (average of 46.83 ± 8.79 mg QE/100 mL). Additionally, notable amounts of vitamin C were detected, ranging from 7.92 to 14.08 mg/100 mL (average of 11.25 ± 1.55 mg/100 mL). Previous studies have also highlighted the abundance of bioactive compounds in sugarcane juice, though reported values vary. For instance, Irshad et al. (2024) documented a TPC of 48.5 mg/100 mL and a TFC of 37.61 mg/100 mL in fresh sugarcane juice. While other studies reported an average value of 374 mg/100 mL for TPC (Adulvitayakorn et al., 2020), and 2.60 mg/100 mL for TFC (Duarte-Almeida et al., 2011). Notably,

TABLE 2 Antimicrobial susceptibility of *E. coli* and *S. aureus* isolates to the tested antimicrobial agents.

Antimicrobial agents	Code	*E. coli* ($n = 77$)		*S. aureus* ($n = 7$)	
		S (%)	R (%)	S (%)	R (%)
Amoxicillin–clavulanic acid (20–10 μg)	AMC	71 (92.21%)	6 (7.79%)	NT	NT
Ampicillin (10 μg)	AMP	15 (19.48%)	62 (80.52%)	NT	NT
Penicillin (10 U)	P	NT	NT	1 (14.29%)	6 (85.71%)
Oxacillin (5 μg)	OX	NT	NT	7 (100%)	0 (0%)
Cefoxitin (30 μg)	FOX	77 (100%)	0 (0%)	7 (100%)	0 (0%)
Cefuroxime (30 μg)	CXM	71 (92.21%)	6 (7.79%)	NT	NT
Ceftriaxone (30 μg)	CRO	77 (100%)	0 (0%)	NT	NT
Cefotaxime (30 μg)	CTX	77 (100%)	0 (0%)	NT	NT
Streptomycin (10 μg)	S	19 (24.68%)	58 (75.32%)	2 (28.57%)	5 (71.43%)
Gentamicin (10 μg)	GN	76 (98.70%)	1 (1.30%)	7 (100%)	0 (0%)
Kanamycin (30 μg)	K	74 (96.10%)	3 (3.90%)	7 (100%)	0 (0%)
Fusidic acid (10 μg)	FD	NT	NT	7 (100%)	0 (0%)
Nalidixic acid (30 μg)	NA	75 (97.40%)	2 (2.60%)	NT	NT
Ciprofloxacin (5 μg)	CIP	75 (97.40%)	2 (2.60%)	6 (85.71%)	1 (14.29%)
Enrofloxacin (5 μg)	ENR	75 (97.40%)	2 (2.60%)	6 (85.71%)	1 (14.29%)
Erythromycin (15 μg)	E	NT	NT	7 (100%)	0 (0%)
Tetracycline (30 μg)	TE	44 (57.14%)	33 (42.86%)	3 (42.86%)	4 (57.14%)
Doxycycline (30 μg)	DXT	NT	NT	5 (71.43%)	2 (28.57%)
Vancomycin (30 μg)	VA	NT	NT	7 (100%)	0 (0%)
Chloramphenicol (30 μg)	C	72 (93.51%)	5 (6.49%)	6 (85.71%)	1 (14.29%)
Trimethoprim–sulfamethoxazole (1.25 μg–23.75 μg)	SXT	59 (76.62%)	18 (23.38%)	7 (100%)	0 (0%)
Clindamycin (2 μg)	CD	NT	NT	7 (100%)	0 (0%)
Imipenem (10 μg)	IPM	77 (100%)	0 (0%)	NT	NT

NT, not tested; S, susceptible; R, resistant.

the levels of vitamin C detected in this study were lower compared to the average concentration of 49.97 mg/100 mL reported by Irshad et al. (2024). Additionally, we report a strong correlation ($p < 0.05$) between TPC, TFC, and antioxidant activity, measured via DPPH scavenging activity, reinforcing the functional potential of sugarcane juice. The presence of these bioactive compounds' positions sugarcane juice as a nutrient-rich, health-promoting beverage with growing global demand (Arif et al., 2019; Hewawansa et al., 2024). Indeed, variability in bioactive compound concentrations of sugarcane juice may be attributed to factors such as environmental conditions, soil properties, extraction methods, and sugarcane varieties (Duarte-Almeida et al., 2011; Rodrigues et al., 2021; Hewawansa et al., 2024). Furthermore, studies indicate that bioactive compounds can degrade during storage (Irshad et al., 2024), necessitating the development of effective preservation techniques to maintain their nutritional quality for large-scale industrial applications (Adulvitayakorn et al., 2020; Tarafdar et al., 2021; Irshad et al., 2024).

Microbiological analysis showed that the sugarcane juice samples were highly contaminated with TAMF, TC, FC, yeasts, and molds. Additionally, 77 samples (77%) were contaminated with *E. coli* and 7 samples (7%) were contaminated with *S. aureus*. However, none of the samples studied were contaminated with *Salmonella* or *L. monocytogenes*. This contamination reflects poor hygienic conditions during the preparation and sale of the juice (da Costa Messias et al., 2024). The presence of FC and *E. coli* indicates poor hygienic practices by street vendors. Additionally, the raw material (sugarcane) may be contaminated with fecal bacteria, likely due to the use of polluted irrigation water (Akinde et al., 2016; Amjad et al., 2022), raising concerns about the potential for fecal-oral transmission of pathogens. Previous studies have reported high levels of fecal bacteria, such as *E. coli*, in irrigation water (Tahri et al., 2021). Consequently, the presence of fecal-origin microorganisms can lead to foodborne illnesses, typically causing self-limiting diarrhea in immunocompetent individuals but potentially severe disease in immunocompromised persons (Castro-Rosas et al., 2012; Chen et al., 2022). Moreover, *S. aureus* is recognized as a major foodborne pathogen that can secrete pathogenic enterotoxins like A, B, C, D, E, and others (Argudín et al., 2010; Ed-Dra et al.,

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TABLE 3 Antimicrobial resistance profiles of *E. coli* isolates ($n = 77$).

No. of antimicrobials	Resistance profile	No. of Isolates	MAR*
0	–	8	0
1	AMP	9	0.06
	S	5	0.06
2	AMP, S	22	0.13
	S, TE	2	0.13
3	AMP, S, TE	11	0.19
	AMP, TE, SXT	1	0.19
4	AMP, S, TE, SXT	8	0.25
	AMP, S, K, TE	1	0.25
5	AMC, AMP, CXM, S, TE	1	0.31
	AMC, AMP, S, TE, SXT	1	0.31
6	AMC, AMP, S, TE, C, SXT	2	0.38
	AMP, CXM, S, TE, C, SXT	1	0.38
	AMP, CXM, S, GN, K, TE, SXT	1	0.38
	AMC, AMP, CXM, S, TE, SXT	2	0.38
7	AMP, S, NA, CIP, ENR, TE, SXT	1	0.44
9	AMP, CXM, S, NA, CIP, ENR, TE, C, SXT	1	0.56

*MAR, Multiple Antibiotic Resistance index; AMP, ampicillin; AMC, amoxicillin-clavulanic acid; S, streptomycin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; K, kanamycin; GN, gentamicin; CXM, cefuroxime; C, chloramphenicol; NA, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin.

TABLE 4 Antimicrobial resistance profiles of *S. aureus* isolates ($n = 7$).

No. of antimicrobials	Resistance profile	No. of isolates	MAR*
0	–	1	0
1	P	1	0.06
2	P, S	1	0.13
3	P, S, TE	1	0.19
4	P, S, TE, C	1	0.25
	P, S, TE, DXT	1	0.25
6	P, S, CIP, ENR, TE, DXT	1	0.38

*MAR, Multiple Antibiotic Resistance Index; P, penicillin; S, streptomycin; TE, tetracycline; DXT, doxycycline; C, chloramphenicol; CIP, ciprofloxacin; ENR, enrofloxacin.

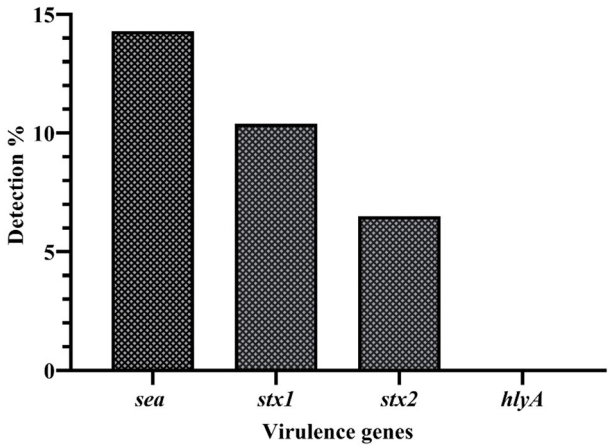

FIGURE 3
Distribution of virulence genes among *S. aureus* (*sea* gene) and *E. coli* (*stx1*, *stx2*, and *hlyA* genes).

2018; Grispoldi et al., 2021), characterized by rapid infection where symptoms like vomiting, stomach pain, and diarrhea appear after 2–6 h (Ünüvar, 2018). Thus, the detection of *S. aureus* in fresh sugarcane juice is a major concern for public health. Furthermore, yeast contamination can contribute to spoilage, as yeasts metabolize the sugars in the juice, leading to alcoholic fermentation and the low of organoleptic properties (Bevilacqua et al., 2013). Molds contaminations are also concerning, as they may result in mycotoxins production during storage, posing a significant public health risk (Nan et al., 2022; Zhang et al., 2025).

Antimicrobial susceptibility assessment revealed that both *E. coli* and *S. aureus* exhibit high resistance to penicillin-class antimicrobials (ampicillin and penicillin), streptomycin, and tetracycline. These findings align with previous studies conducted in Morocco and other regions (Tan et al., 2014; Ed-Dra et al., 2018; El Ftouhy et al., 2023), where the prolonged use of these older antimicrobial agents in human and veterinary medicine has contributed to the emergence of bacterial resistance (Xu et al., 2021). The misuse of antimicrobials, particularly in veterinary settings for prophylaxis, therapy, and growth promotion, has further exacerbated AMR (Odey et al., 2023; Tang et al., 2023). Additionally, bacterial exposure to environmental stressors can drive resistance through co-resistance mechanisms (Li et al., 2022; Wang et al., 2023). Consequently, resistant isolates may disseminate into the environment, contaminating soil, water, crops, and ultimately plant-based foods (Jung and Rubin, 2020). Our results also indicate a high prevalence of MDR, with 40.26% (31/77) of *E. coli* and 42.86% (3/7) of *S. aureus* isolates exhibiting MDR profiles. This poses significant public health concern and severely limits treatment options. Furthermore, 24.67% (19/77) of *E. coli* and 42.86% (3/7) of *S. aureus* isolates had a MAR index >0.2, suggesting that they originated from high-risk contamination sources.

Furthermore, the detection of virulence genes showed the detection of the *sea* gene in one *S. aureus* isolate (14.28%). This gene encodes staphylococcal enterotoxin A, a toxin characterized by its superantigenic properties, which induce a massive immune response (Hu et al., 2021; Zhu et al., 2023). In addition, it is highly stable under heat treatment and resistant to proteolytic enzymes,

allowing it to remain active even after food processing (Zhu et al., 2023). Consequently, the presence of such toxins in food products is a significant public health concern, as they can lead to rapid and severe intoxication in consumers (Grispoldi et al., 2021). We also detected the presence of Shiga toxin-encoding genes *stx1* and *stx2* in 8 (10.39%) and 5 (6.49%) *E. coli* isolates, respectively, with both genes (*stx1* and *stx2*) detected in 3 (3.89%) isolates. These genes are recognized as major virulence factors of Shiga toxin-producing *E. coli* (STEC), primarily associated with *E. coli* O157:H7 (Sarimehmetoglu et al., 2009), but also found in various non-O157 serogroups such as O26, O103, O111, and O145, among others (Hoyle et al., 2021). The presence of *E. coli* strains harboring these virulence factors in food products, particularly fresh juice, poses serious public health risks due to their potential to cause severe illness, including hemolytic uremic syndrome (Freedman et al., 2023).

Given these findings, sugarcane juice may serve as a key transmission route for MDR and virulent bacterial pathogens to humans. To mitigate this risk, effective measures must be implemented to eliminate these pathogens while preserving the nutritional quality of the product. These measures can include:

- Implementation of strict hygiene regulations and regular inspections for street vendors, including mandatory food safety training.
- Ensuring the quality of raw sugarcane by promoting the use of clean, treated irrigation water and monitoring agricultural practices to minimize fecal contamination at the source.
- Requiring vendors to use clean, sanitized equipment and store sugarcane and juice under proper refrigeration to limit microbial growth. In this regard, public awareness campaigns can help educate both vendors and consumers about the risks of unsafe juice consumption.
- Implementation and integration of routine monitoring for AMR in foodborne pathogens into national food safety programs to prevent the spread of resistant strains through the food chain.

5 Conclusions

This study demonstrated that sugarcane juice is rich in nutritional compounds, marked with the abundance of TSS, TTA, TPC, TFC, and vitamin C. Its slightly acidic pH further enhances its appeal as a favorable beverage for consumers. However, microbiological analysis revealed contamination with various microorganisms. The presence of fecal coliforms indicates poor respect for good hygienic practices and suggests potential fecal contamination sources. Moreover, the detection of MDR and virulent *E. coli* and *S. aureus* poses significant public health concerns, underscoring the need for stricter food safety measures and improved hygiene practices among street vendors. Consequently, the consumption of fresh sugarcane juice may pose a risk of foodborne illnesses, highlighting the necessity for sustainable preservation methods that reduce microbial contaminants while retaining their nutritional quality. Although our study provides valuable insights, certain limitations should be noted. Future research should broaden the scope to evaluate both the quality and microbiological safety of raw plant materials utilized in sugarcane juice preparation, alongside assessing street vendors' compliance with hygienic practices, to better identify potential risk factors associated with contamination by highly pathogenic microorganisms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

AE-D: Validation, Investigation, Formal analysis, Software, Data curation, Writing – original draft, Methodology, Conceptualization, Resources. AA: Validation, Project administration, Resources, Funding acquisition, Conceptualization, Writing – review & editing, Supervision. EA: Data curation, Writing – review & editing, Formal analysis. LN: Writing – review & editing, Investigation, Conceptualization, Methodology, Formal analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1628211/full#supplementary-material

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