Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 7 Issue 9 September 2023

Research Article

Sugarcane Filter Cake Waste as Natural Antibacterial Agent against *Xanthomonas citri subsp citri*

Guerra L[1], Nisoria-Santillán PE[1], Saguir FM[1,2] and Rodríguez-Vaquero MJ[1,2b]*

[1]*Institute of Microbiology, Faculty of Biochemistry, Chemistry and Pharmacy - National University of Tucumán (UNT), Argentina*

[2]*CONICET, Ayacucho, Tucumán, Argentina*

***Corresponding Author:** Rodríguez-Vaquero MJ, 1Institute of Microbiology, Faculty of Biochemistry, Chemistry and Pharmacy - National University of Tucumán (UNT) and CONICET, Ayacucho, Tucumán, Argentina.

DOI: 10.31080/ASAG.2023.07.1292

Received: May 29, 2023

Published: August 10, 2023

© All rights are reserved by **Rodríguez-Vaquero MJ., *et al.***

Abstract

The sugarcane filter cake waste (SFCW) is the main solid waste product generated during sugar production, without final disposition; and this work suggests that it could be reutilized to control *Xanthomonas citri subsp citri* (*X. citri*), the etiologic agent of citrus canker, in lemons. The aim of this work was the identification of phenolic compounds present in SFCW, and the evaluation of its antibacterial activity against *X. citri, in vitro* and *in vivo* using preventive and curative methods in lemons. Also, the antibacterial mode of action of SFCW on *X. citri* and its effect on *Xanthan* production were carried out. Results demonstrated the presence of phenolic compounds in SFCW and that quercetin, rutin, catechin and caffeic acids were the majority phenolic compounds. Phenolic extract had bactericidal action at high concentrations, inhibit the biofilm formation and reduce the exopolysaccharide production. The Inhibitory concentrations (IC_{50}, IC_{90}) and the Letal concentrations, LC_{50} and LC_{90} were determined. All individual phenolic compounds reduced the growth of *X. citri* and the effect increased with the concentration. In lemons, preventive and curative methods were effective to reduce *X. citri* survey. Moreover, our investigations showed that phenolic fraction of SFCW were effective to produce cellular death of *X. citri* in lemon. Our results demonstrated that SFCW could be reused as natural antibacterial agent against *X. citri*, etiologic agent of citrus canker in lemons.

Keywords: Sugarcane Filter Cake Waste; *Xanthomonas citri*; Phenolic Compounds; Canker; Lemons

Abbreviations

SFCW: Sugarcane Filter Cake Waste; X. citri: *Xanthomonas Citri Subsp Citri,* SFCW-PF: Phenolic Fraction of Sugarcane Filter Cake Waste

Introduction

Sugarcane is a lignocellulosic crop and the juice extracted from its stalks provides the raw material for 86% of sugar production [1]. Sugar is widely used as a source of energy and is one of the most important commodities in life as it is the basic element in food, beverages and pharmaceuticals [2,3]. The sugar production process generally consists of five successive steps: extraction of raw cane juice by crushing/milling of sugarcane; clarification/decoloration; concentration; crystallization and centrifugation/drying [4]. During this process also are produced a big amount of liquid waste, bagasse, molasses, and filter cake (In Argentine: Cachaza). Most sugar factories have problems with managing, handling, transporting, and disposing of sugarcane filter cake waste (SFCW), the main solid waste, because of its high water content, smell, and tendency to attract insects and other pestsdall of which adversely affect the factory and its surroundings. Establishing a suitable way to manage sugarcane filter cake residue, therefore, constitutes a challenge of some magnitude for the sugar industry [5].

On the other hand, Argentina leads lemon world production and industrialization generating 87% of the national production only from Tucumán province, processing 65% of this production in factories and commercializing 35% as fresh fruit, mainly for exports, which requires high quality and disease-free fruits [6]. An important problem in citrus plants is citrus canker disease caused by *Xanthomonas citri* subsp c*itri* (*X. citri*), which has significantly reduced citrus quantity and quality in many producing areas

worldwide [7]. It has worldwide distribution, it has the capacity to devastate citrus production and it is hard to eradicate [8]. Infected fruits have decreased commercial quality, compromising the acceptance by most markets [9]; but the most important economic impact is restriction of market access for fresh fruit entry into the canker-free European Union because *X. citri* is a quarantine pathogen [10]. The different strategies that *X. citri* have to colonize the plant tissue and thus promote the process of pathogenesis are the production of exopolysaccharides (natural long-chain macromolecules composed mostly of carbohydrate residues), extracellular enzymes and toxins, among others. *X. citri* is able to produce water-soluble exopolysaccharides that can sustain bacteria in poorly maintained environments. This polymer, which is also called xanthan, plays a role in biofilm formation and bacterial pathogenicity [11]. Actually, multiple applications of bactericidal copper sprays are required for canker control, but copper may induce copper resistance in bacterial populations after repeated exposure [12,13].

For these reasons, one of the greatest challenges that researchers have is the search of new natural antibacterial agent as alternative to control canker disease and a potential method to valorize the SFCW could be related to the recovery of phytochemicals with antibacterial activity against *X. citri*. At present there is no evidence about the phenolic or phytosterols concentration in SFCW or its antibacterial activity against *X. citri*. The aims of this study were to: 1) to extract, identify and characterize phenolic compounds in SFCW, 2) to investigate the antibacterial activity and the modifications in biofilm formation and *Xanthan* production by *X. citri* in media supplemented with SFCW extract, and 3) to determine the *X. citri* survey in lemons after SFCW treatment, applied to prevent the initial contamination.

Materials and Methods

Characterization of sugarcane filter cake waste (SFCW)

The SFCW samples were produced in a sugar mill in the Tucumán province located in the northwest region of Argentine (Geographical coordinates: Longitude O65°13'21.36" and latitude S26°49'26.9"). During sugar production, after the clarification process, the sugarcane juice was separated into two components: a clear juice, which rises to the top of the separator and goes to sugar manufacture, and a mud, which collects at the bottom of the separator. This mud or the filter cake was then filtered to separate the suspended matter and took to the laboratory in hermetic bags.

Phenolic compounds extraction.

SFCW was heated at 70°C for 72h, until complete drying, and then samples were mechanically powdered using a mortar. First, 100g of pulverized waste were placed into soxhlet extractor and petroleum ether was used as solvent during 4h at 60°C, solvent was evaporated and the fatty fraction present in sugarcane filter cake was conserved. Then, the defatted material was treated with ethanol 98% into soxhlet extractor during 3 h at 60°C. Solvent was evaporated and the phenolic compounds fraction present in sugarcane filter cake (SFCW-PF) was lyophilized. The yield of extraction was calculated using the following equation:

Yield = lyophilized material (g) x 100/pulverized waste (g)

Where

- **Lyophilized material (g):** Amount of powder in grams, obtained after lyophilization of the material obtained during extraction.
- **Pulverized waste (g):** Amount of each waste in grams, after the drying and spraying process.

Identification and quantification of phenolic compounds in SFCW.

Total phenolic compounds present in SFCW-PF were determined using a colorimetric determination based on the procedure of Singleton and Rossi (1965) [14]. Results are expressed as milligram per liter gallic acid equivalents (GAE). The flavonoids and Non-flavonoids compounds concentration was determined with 10.0 mL of the SFCW-PF mixed with 10.0 ml of diluted HCl (1:3) and 5.0 mL of an 8.0 mg mL^{-1} formaldehyde solution and incubated 24 h at room temperature in order to precipitate the flavonoid fraction [15] (Zoecklein, Fugelsang, Gump and Nury, 1990). The non-flavonoid phenol contents were determined in the filtrate using the procedure of Singleton and Rossi [14]. The flavonoid content was obtained by the difference between total phenol and non-flavonoid content. All determinations were carried out in triplicate. The phenolic fraction extract was resolved in 1 mL methanol/water (50:50, v/v) and 20 µL were analyzed by high-performance liquid chromatography-diode array detection (HPLC-DAD) as described Fanzone., *et al*. (2011) [16].

Antibacterial test

Bacterial strain and culture condition

The bacterial strain used as test organism was *Xanthomonas citri subsp*. *citri* (*X. citri*), isolated and identified from canker lemon lesions in the experimental agroindustrial station Obispo Colombres (EEAOC), Tucumán, Argentina. The bacterium was aerobically cultured at 28ºC in nutrient broth medium (containing in g L^{-1}: beef extract, 3; peptone, 5; sodium chloride, 8; the pH was adjusted at 7).

Screening of the antibacterial activity

The screening of the antibacterial activity was carried out using agar diffusion test according Rodríguez-Vaquero., *et al.* (2007) [17]. Soft agar nutrient medium (0.75% agar) was inoculated with liquid overnight culture to a cell density of 2.0 x 10^8 cfu mL^{-1}, and plates containing 10 mL of agar nutrient media (1,5% agar) were overlaid with 10 ml of this inoculated soft agar. Equidistant holes were made in the agar. A 30-µL volume of SFCW-PF (100 µg mL^{-1}) and individual phenolic compounds at a concentration of 50 µg mL^{-1} was pipetted into the agar wells. Copper oxychloride (3 µg mL^{-1}) was used as a positive control and the negative control was ethanol. After 36h incubation the diameter of the inhibition zones around the holes was measured with an accuracy of 0.5 mm using a calibrate ruler. All experiments were carried out at least in triplicate.

Inhibitory (IC_g) and Letal concentrations (LC) of SFCW-PF against *X. citri*

To determine the IC_g and LC of SFCW-PF on *X. citri*, XVM2 medium (composition: 20 mM NaCl, 10 mM $(NH4)_2SO_4$, 5 mM $MgSO_4$, 1 mM $CaCl_2$, 0.16 mM KH_2PO_4, 0.32 mM K_2HPO_4, 0.01 mM $FeSO_4$, 10 mM fructose, 10 mM sucrose, 0.03% Casamino Acids, pH 6.7) supplemented with different concentrations of SFCW-PF (10 to 1,000 µg mL^{-1}) was used. The media were inoculated with *X. citri* (1 x 10^5 cfu mL^{-1}) bacterial growth was followed by aerobically incubation for 36 h at 28ºC in shaker. Bacterial growth in different culture media with each waste dilution was measurement indirectly by measuring absorbance at 560 nm and directly counting the number of viable cells in solid medium. All experiments were carried out at least in triplicate. The IC_{g50} value is the concentration that produces the 50% of inhibition of the growth compared with the control and the IC_{g90} value is the concentration that produces an inhibition of 90% on the growth, compared with the control. The LC_{50} value is the concentration of waste that produces the death of half of inoculated cells and LC_{90} value is the concentration of waste that produces the death of 90% of the inoculated cells.

Influence of SFCW-PF on bacterial biofilm formation

The effect of SFCW-PF in X. citri biofilm formation was carried out using 200 µL of the overnight culture of X. citri (10^8 cfu mL^{-1}) in XVM2 medium supplemented with different concentrations of SFCW-PF (10, 50, 100, 200, 300 and 400 ***µg mL***$^{-1}$) or individual phenolic compounds (10, 50, 100, 200 and 400 ***µg mL***$^{-1}$). The culture was added to the wells of sterile flat bottom 96-well polystyrene microtiter plates and incubated for 56 h at 15°C to promote the biofilm production. Then, the wells were washed twice with distilled water in order to remove all non-adherent cells, and 200 µL of 0.01% (w/v) crystal violet (CV) were added to the wells for 30 min in darkness. The stained biofilm were rinsed with distilled water and extracted with 200 ***µ***L of 96% ethanol. The amount of biofilm was quantified by measuring the OD 595 nm of dissolved CV using the microplate reader. Controls of biofilm formation were carried out with addition of ethanol, and un-inoculated medium were also carried out.

Effect of different concentrations of SFCW-PF on *X. citri Xanthan* production

In order to determine the growth and the modifications in *Xanthan* production of *X. citri* in presence of SFCW-PF and individual phenolic compounds, SFCW-PF (10, 50, 100, 200, 300 and 400 µg mL^{-1}) and pure phenolic compounds (10, 50, 100, 200 and 400 µg mL^{-1}) were added to the apoplast-mimicking XVM2 medium. The media were inoculated with *X. citri* to obtain a final concentration of 10^5 ufc mL^{-1} with an overnight culture. Bacterial growth was followed by aerobically incubation for 36 h at 28ºC in shaker. Bacterial growth was measurement indirectly by measuring absorbance at 560 nm and directly counting the number of viable cells in solid medium.

The extraction and quantification of the *Xanthan* produced during the bacteria growth was carried out at 36h. The cultures were centrifuged at 10,000 xg, 4ºC for 10 min to eliminate the cell precipitates, followed by the removal of the proteins by the addition of 4% (w/v) trichloroacetic acid. The EPS were precipitated by addition of three volumes of cold ethanol and the mixture was maintained overnight. The precipitate was collected by centrifugation, and re-dissolved in distilled water in order to determine the yield by phenolic-sulfuric acid method. The glucose standard curve was prepared for the quantitative determination, according to Dubois., *et al.* (1956) [18] with some modifications. Briefly, different concentrations of glucose solutions (0.5 to 15.0 mg L^{-1}) and 0.1 ml of EPS samples were diluted to 2 mL with distilled water, then added with 1.0 mL of phenol 6% and 5.0 mL of sulfuric acid 95% (v/v) and shake up after 10 min standing, then absorbance was measured at 490 nm in UV- visible spectrophotometer. The concentration of EPS produced (mg mL^{-1}) was calculated using the standard curve and the EPS specific production was calculated by the formula

EPS specific production *(mg* cfu^{-1}*)* = [*EPS concentration (mg* mL^{-1}*)/number of cfu* mL^{-1}]

Survey of *X. citri* during lemon colonization process in presence of SFCW-PF

The survey of *X. citri* inoculated in lemons, before or after the SFCW-PF (400, 600, 800, 900 and 1000 µg mL^{-1}) application, was

tested using two methods to control the initial contamination in lemons, briefly

- Method A or preventive method: The preventive method objective is preventing the initial contamination by *X. citri* in lemons. First, an injury in free-disease yellow lemons (*Eureka* variety) was made using a sterile needle; then 20 µL of different concentrations of SFCW-PF, were added into the wound. After that, the lemons were inoculated with 10 µL of *X. citri* cells (10^5 cfu mL^{-1}) which were washed and re-suspended in sterile physiological solution, into the wound. The lemons were placed in hermetic sterile bags, incubated at 28 ºC for 8d and the number of viable cells was determined by successive dilution method.
- Method B or curative method: The curative method objective is producing the death of the initial contamination by *X. citri* in lemons. First, a wound was made in lemons with a sterilized needle and the lemons were inoculated with 10 µL of washed and re-suspended *X. citri* cells (10^5 cfu mL^{-1}). After that the lemons were placed in hermetic sterile bags and incubated at 28 ºC for 2 d. Then, 20 µL of different concentrations of SFCW-PF were added into the inoculated wound. And finally, lemons were incubated for 6 more days and the number of viable cells was determined by successive dilution method and the cfu mL^{-1} was determined.

Statistical analysis

All experiments were repeated three times with duplicate samples and viable plate counts from three replications. Data were analyzed by ANOVA using Minitab (Minitab Inc., PA, USA). Multiple means comparison was carried out by Duncan's multiple range tests ($p < 0.05$).

Results and Discussion

Identification and quantification of phenolic compounds in SFCW-PF

Our results demonstrated that phenolic compounds are present in SFCW-PF and the yield of the phenolic extraction was 20%, this result demonstrated that the method used was effective to extract the phenolic content present in this waste. The total phenolic compounds, flavonoid and non-flavonoids compounds concentrations and the main phenolic compounds present in SFCW-PF are shown in table 1. Ours results demonstrated that flavonoids compounds are majority compounds than phenolic acids compounds; and rutin, quercetin, catechin and trans-caffeic acid were the main phenolic compounds present in SFCW- PF. The chemical composition of SFCW (organics include starch, wax, amino acids, organic acids, phenolic compounds, etc.; inorganics include SiO_2, K_2O, P_2O_5, Fe_2O_3) was reported by Canilha., *et al.,* 2012 [19]. Other authors reported that sugarcane and its by-products contain high levels of phytochemicals such as phytosterols, terpenoids, flavonoids, fatty acids, and phenolic acids and that sugarcane juice's flavonoid content (0.6 mg mL^{-1}) was comparable to levels found in other food sources such as orange juice and black tea [20]. In concordance with ours results, the presence of caffeic acid in sugarcane was reported.

	SFCW-PF (Phenolic fraction)
	Total Phenolic concentration: 125.56 mg GAE/gr of SFCW Flavonoid compounds: 97.82 mg GAE/gr of SFCW Non-flavonoids compounds: 27.70 mg GAE/gr of SFCW
	Phenolic profile ($\mu g\ g^{-1}$)
	Gallic acid 0.40 ± 0.03
	Protocatechuic acid 0.60 ± 0.07
	Methyl gallate 0.60 ± 0.06
	Trans-caffeic acid 15.90 ± 1.10
	Ferulic acid 0.70 ± 0.80
	Trans-Caftaric acid *Nd*
	p-coumaric acid 0.40 ± 0.03
	Myricetin *Nd*
	Quercetin 20.50 ± 1.90
	Rutin 26.90 ± 2.00
	Catechin 14.50 ± 1.00
	Kaempferol *Nd*
	Resveratrol 7.90 ± 0.80

Table 1: Characterization of phenolic compounds present in SFCW. Nd: Not Detected.

Screening of the antibacterial activity, IC_g and LC values of SFCW-PF against *X. citri*

Table 2 shows the screening of the antibacterial effect of SFCW-PF against *X. citri* and the main pure phenolic compounds present in the studied waste. All samples showed an inhibition zone against the bacterium. SFCW- PF, the hydroxycinnamic acid (caffeic acid) and the flanovols (quercetin and its glycosidic form, rutin) produced a strong antibacterial effect, while the flavanol, catechin produced a moderate antibacterial effect against *X. citri.* Our results are in concordance of other authors [21], who evaluated 50 flavonoids for their antibacterial activities and reported that most of the flavonoids exhibited inhibitory effects against *Xanthomonas.*

Samples	Inhibition zone (mm)
SFCW-PF	+++
Caffeic acid	+++
Quercetin	+++
Rutin	+++
Catechin	++
Copper oxychloride	++++

Table 2: Screening of the antibacterial activity of SFCW-PF and major phenolic compounds present in SFCW extracts against *X. citri.*

Antibacterial activity: Inhibition zone <1 mm, nil (-); Inhibition zone 1-5 mm, weak (+).

Inhibition zone 6-11 mm, moderate (++); Inhibition zone 12-19 mm, high (+++).

inhibition zone >19 mm, strong (++++). Standard deviation ± 0.5 mm.

The IC and LC values of SFCW- PF against *X. citri* are presented in table 3. Results showed that SFCW- PF at 800 and 650 µg mL^{-1} produced the death of 90% and 50% of inoculated cells, respectively. Whereas, concentrations of 250 and 500 µg mL^{-1} of SFCW- PF reduced 50 and 90% the bacterial growth, respectively.

Samples	IC_{g50} (µg mL^{-1})	IC_{g90} (µg mL^{-1})	LC_{50} (µg mL^{-1})	LC_{90} (µg mL^{-1})
SFCW-PF	250 ± 10.00	500 ± 17.00	650 ± 20.00	800 ± 20.00

Table 3: IC_{50}, IC_{90}, $LC_{50,}$ LC_{90} of SFCW-PF against *X. citri*

Effect of SFCW-PF on bacterial growth, *xanthan* production and biofilm formation

To determine the effect on bacterial growth, the xanthan production, their specific production and the biofilm formation, lower concentrations were used. Figure 1(a) shows the effect different concentrations of SFCW-PF (10, 50, 100, 200, 300 and 400 µg mL^{-1}) on *X. citri* biofilm formation and EPS production, and Figure 1b shows the number of viable cells at the end of incubation and the EPS specific production. Our results demonstrated that in presence of the waste, the EPS production, the biofilm formation and the specific EPS production by *X. citri* decreased with the increase of the SFCW-PF concentration from 50 µg mL^{-1}, respect to control, but the number of viable cells was reduced from 100 µg mL^{-1}. The addition of 10 µg mL^{-1} of SFCW-PF did not affect bacterial viability, biofilm production or EPS production. From 300 µg mL^{-1} the EPS production and the specific EPS production was completely inhibited, with the concomitant inhibition on the biofilm formation.

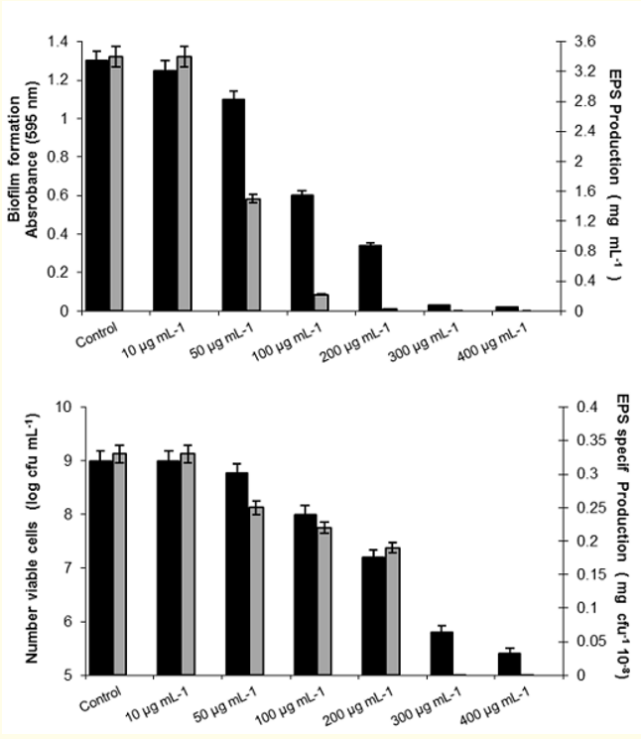

Figure 1: (a) Biofilm formation (■ absorbance at 595 nm) and EPS concentration (mg mL^{-1}) (■) produce by X. citri after 36 h incubation in XVM2 broth media supplemented with different concentrations of SFCW- PF (10, 50 , 100 , 200, 300 and 400 µg mL^{-1}). (b) Number of viable cells (log cfu mL^{-1}) (■) of X. citri after 36 h incubation at 28 ºC in XVM2 broth media supplemented with different concentrations of SFCW-PF and EPS specific production (mg mL^{-1}) (■) during its growth.

The number of viable cells of *X. citri* after 36 h incubation in presence of different concentrations (10, 50, 100, 200 and 400 µg mL^{-1}) of the main phenolic compounds present in SFCW-PF are shown in figure 2. The addition of 10 µg mL^{-1} of all individual phenolic compounds did not affect the bacterial viability. From the concentration of 50 µg mL^{-1}, all pure phenolic compounds produce a reduction in the viability of the bacteria, respect to the control. With the addition of caffeic acid, quercetin, rutin and catechin at 50 µg mL^{-1} a reduction in the growth of 0.8, 1.0, 0.6 and 0.3 log cycles of *X. citri* were observed, with respect to control, respectively. And with the highest concentration studied, 400 µg mL^{-1}, caffeic acid and quercetin produce the death of inoculated cells of *X. citri* by

0.6 and 1.6 log cycles respectively. Rutin maintained the number of cells inoculated into the culture media, and with the addition of catechin, *X. citri* growth only 0.3 log cycles at 36 h. Caffeic acid and Quercetin were the strongest antibacterial compounds against *X. citri*.

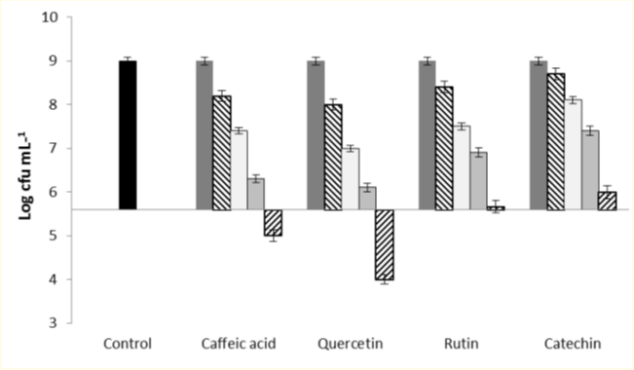

Figure 2: Number of viable cells of *X. citri* (log cfu mL^{-1}) after 36 h incubation at 28ºC in XVM2 broth media supplemented with pure commercial caffeic acid, quercetin, rutin and catechin at: (■) 0 µg mL^{-1} control; (■) 10 µg mL^{-1}, (▨) 50 µg mL^{-1}, (□) 100 µg mL^{-1} , (■) 200 µg mL^{-1} and (▨) 400 µg mL^{-1}. Each point represented the average value of four determinations.

Figure 3 (a) shows the effect of 100 µg mL^{-1} of caffeic acid, quercetin, rutin and catechin on *X. citri* biofilm formation and EPS production, and Figure 3b shows the number of viable cells at the end of incubation and the EPS specific production.

Our results demonstrated that all individual phenolic compounds produced a reduction of the EPS production by *X.citri* and a reduction in the biofilm formation. Rutin, quercetin and caffeic acid were the most effective to inhibit the growth, the viability, the EPS production and the biofilm formation. These results are in agreement with a previously work [17] that demonstrated the best antibacterial effect of these compounds against several bacteria, compared with catechin. This effect could be attributed to the difference in chemical structure of the compounds. Cushnie and Lamb [22], reported that the antibacterial mechanisms of action of various flavonoids could be attributed to inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function or inhibition of energy metabolism. The antibacterial effect of caffeic acid against several Gram-positive and Gram-negative bacteria was reported [17,23,24], and this effect could be attributed to their propenoic side chain, which might facilitate the transport of these molecules across the cell membrane.

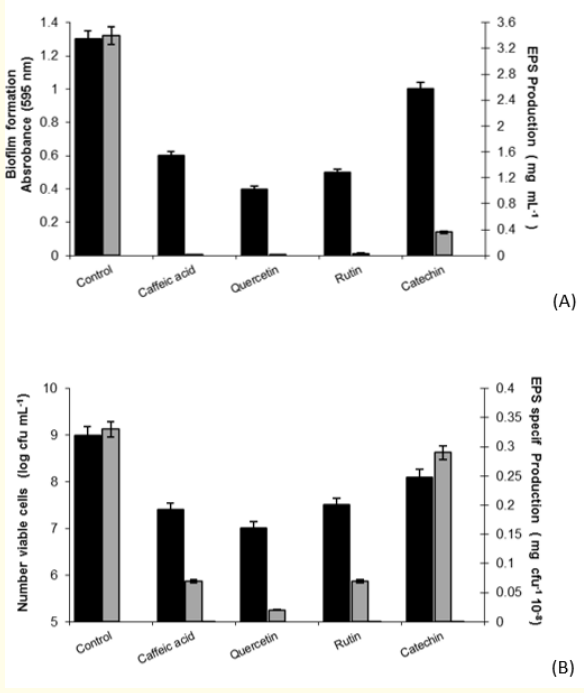

Figure 3: (a) Biofilm formation (■ absorbance at 595 nm) and EPS concentration (mg mL^{-1}) (■) produce by X. citri after 36 h incubation at 28 ºC in XVM2 broth media supplemented with different concentrations of pure commercial caffeic acid, quercetin, rutin and catechin (10, 50, 100, 200 and 400 µg mL^{-1}). (b) Number of viable cells (log cfu mL^{-1}) (■) of X. citri after 36 h incubation at 28 ºC in XVM2 broth media supplemented with different concentrations of SFCW- LMWPF and EPS specific production (mg mL^{-1}) (■) during its growth.

Survey of *X. citri* during lemon colonization process in presence of SFCW-PF

The antibacterial activity of different concentrations of SFCW-PF against *X. citri* was evaluated using lemons as fruit matrix and two applied methods (curative and preventive methods). figure 4 (a) shows the number of survivors in lemons during 8 days of incubation, after the application of treatment A, a preventive method, and figure 4 (b) after the application of treatment B, the curative methods, in the first colonization. It was demonstrated that preventive and curative method were both effective to reduce the number of viable cells of *X. citri* in lemons. In control lemons, the bacteria grew 3.9 log cycles after 8 days of incubation at 28ºC. The application of treatment A reduced the growth of *X. citri* using both methods at 400 and 600 µg mL^{-1}, compared to the control, a decreased in the number of viable cells and in the growth rate was observed. The application of 800 µg mL^{-1} inhibited completely the growth of

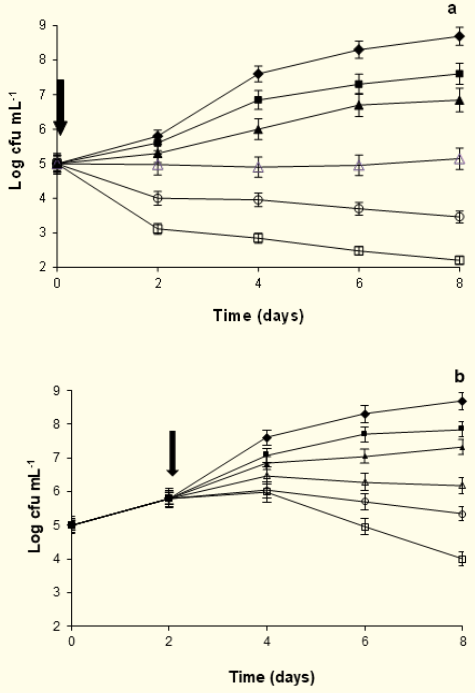

Figure 4: Number of viable cells of X. citri (log cfu mL^{-1}) in lemons during it growth with preventive (a) or curative (b) treatments, using SFCW- PF (400, 600, 800, 900 and 1000 µg mL^{-1}). (Control, 400 µg mL^{-1}, 600 µg mL^{-1}, 800 µg mL^{-1}, 900 µg mL^{-1} and 1000 µg mL^{-1}). The arrow indicates the moment of SFCW-PF application of treatments.

the bacterium using the preventive method and curative methods. With the application of the preventive method, from 900 µg mL^{-1} *X. citri a* cellular death was observed. However, using a curative method, with the addition of 900 µg mL^{-1} the growth of *X. citri* was completely inhibited; and with 1000 µg mL^{-1} the death of inoculated cells of *X. citri* was observed. Preventive method was more effective to reduce the number of viable cells in lemon than curative method.

In this investigation, the phenolic compounds presents in sugarcane filter cake waste were determined and the evaluation of the antibacterial activity against *X. citri* showed that the phenolic fraction of the waste was effective to inhibit the bacterial growth, its viability and the biofilm formation. Moreover, our results demonstrated that SFCW-PF was effective to produce cellular death of *X. citri* in lemon.

Conclusion

The present investigation is the first evidence among the presence of phenolic compounds in sugarcane filter cake waste, the main solid waste produced during sugar elaboration of our region; in this investigation the antibacterial activity against the etiologic agent of canker of citrus was demonstrated, as well as the inhibition in biofilm formation and EPS production. Our investigations concluded that phenolic extract had bactericidal effect against *X. citri* in lemons, inhibited the exopolysaccharide production and decreased the biofilm formation, a fundamental step in the development of plant diseases. The application of these extracts in lemons demonstrated that preventive method as curative methods were both effective to produce the death of *X. citri* at 8 days. The possible reuse of this industrial waste could be a good alternative in the formulation of a natural bio-input for agricultural application to the control of canker in lemons. On the other side, the possibility to transform a waste to raw material gives an environment friendly solution for waste final disposition.

Acknowledgements

The present study was supported by grants from National Agency for Scientific and Technological Promotion (PICT 2019-0834 Préstamo BID), PIP-CONICET, Research Council of the National University of Tucumán (PIUNT) and CONICET.

Bibliography

1. Ungureanu N., *et al*. "Sustainable Valorization of Waste and By-Products from Sugarcane Processing". Sustainability 14.17 (2022): 11089.

2. Meghana M and Shastri Y. "Sustainable valorization of sugar industry waste: status, opportunities, and challenges". *Bioresource Technology* 303 (2020): 122-929.

3. Eggleston G. "Positive aspects of cane sugar and sugar cane derived products in food and nutrition". *Journal of Agricultural and Food Chemistry* 66 (2018): 4007-4012.

4. Zhang H., *et al*. "Green production of sugar by membrane technology: How far is it from industrialization?" *Green Chemical Engineering* 2.1 (2021): 27-43.

5. Ochoa-George PA., *et al*. "Residue from sugarcane juice filtration (filter cake): energy use at the sugar factory". *Waste and Biomass Valorization* 1 (2010): 407-413.

6. Fogliata GM., *et al*. "Eficacia de tres estrobilurinas para el control de mancha rojiza (*Guignardia mangiferae*) y mancha negra (*Guignardia citricarpa*) en frutos de limón, en Tucumán, República Argentina". *Revista Industrial y Agrícola de Tucumán* 88.1 (2011): 37-45.

7. Long Y., *et al*. "Fluorescent Reporter-Based Evaluation Assay for Antibacterial Components Against *Xanthomonas citri subsp. Citri"*. *Frontiers in Microbiology* 13 (2022): 864963.

8. Gottwald TR., *et al*. "Citrus canker: the pathogen and its impact". *Online Plant Health Professionals* (2002).

9. Ference., *et al.* "Recent advances in the understanding of Xanthomonas citri ssp. citri pathogenesis and citrus canker disease management". *Molecular Plant Pathology* 19.6 (2018): 1302-1318.

10. Gottwald TR., *et al*. "The citrus epidemic in Florida: the scientific basis of regulatory eradication policy for an invasive species". *Phytopatology* 91 (2001): 30-34.

11. Pegos VR., *et al*. "Xanthan Gum Removal for 1H-NMR Analysis of the Intracellular Metabolome of the Bacteria *Xanthomonas Axonopodis* Pv. *Citri* 306". *Metabolites* 4.2 (2014): 218-231.

12. Behlau F., *et al*. "Monitoring for resistant populations of *Xanthomonas citri* subsp. *citri* and epiphytic bacteria on citrus trees treated with copper or streptomycin using a new semi-selective medium". *European Journal of Plant Pathology* 132 (2012): 259-270.

13. Favaro MA., *et al*. "Different Responses in Mandarin Cultivars Uncover a Role of Cuticular Waxes in the Resistance to Citrus Canker". *Phytopathology* 110.11 (2020): 1791-1801.

14. Singleton VL and Rossi JA. "Colorimetry of total phenolics with phosphomolybdic-Phosphotungstic acid reagents". *American Journal of Enology and Viticulture* 16 (1965): 144-158.

15. Zoecklein BW., *et al*. "Phenolic Compounds and wine Color. In: Production wine analysis". (129-168 pp.) Ed. Zoecklein, Bruce. Van Nostrand Reinhold. New York (1990).

16. Fanzone M., *et al*. "Phenolic composition of malbec grape skins and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning". *Journal of Agricultural and Food Chemistry*59.11 (2011): 6120-6136.

17. Rodríguez -Vaquero MJ., *et al*. "Antibacterial effect of phenolic compounds from different wines". *Food Control* 18 (2007): 93-101.

18. Dubois M., *et al*. "Colorimetric methods for determination of sugars and related substances". *Analytical Chemistry* 28 (1956): 350-356.

19. Canilha L., *et al*. "Bioconversion of sugarcane biomass into ethanol: An overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation". *Journal of Biomedicine and Biotechnology* (2012): 989572.

20. Atif Khurshid Wani., *et al*. "Advances in safe processing of sugarcane and bagasse for the generation of biofuels and bioactive compounds". *Journal of Agriculture and food Research* 12 (2023).

21. An-Ping Li., *et al*. "Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria". *Pesticide Biochemistry and Physiology* 188 (2022): 105221.

22. Cushnie and Lamb., *et al*. "Antimicrobial activity of flavonoids". *International Journal of Antimicrobial Agents* 26 (2005): 343-356.

23. Rodríguez-Vaquero MJ., *et al*. "Antibacterial, antioxidant and antihypertensive properties of polyphenols from argentinean red wines varieties". *Open Journal of Pharmacology and Pharmacotherapeutics* 5.1 (2020): 001-006.

24. Vallejo CV., *et al*. "Antibacterial mechanism of strawberry polyphenols against *Listeria monocytogenes* and *Salmonella* typhimurium". *Journal of the Science Food and Agriculture* 101 (2021): 441-448.