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Research Article

Phytochemical and Antioxidant Dynamics of the Soursop Fruit (*Annona muricata* L.) in response to *Colletotrichum* spp.

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This work evaluates the effect of the pathogens *Colletotrichum siamense* and *C. gloeosporioides* on the response of soursop fruits. The bioactive compounds (total phenols, flavonoids, anthraquinones, coumarins, steroids, terpenoids, alkaloids, and saponins) were evaluated qualitatively in soursop pulp. Positive phytochemicals and antioxidant activity (DPPH•, ABTS•+, and FRAP) were quantified at day zero, one, three, and five. Fruits treated with *C. gloeosporioides* showed higher disease severity ($P < 0.05$). Early fruit response (day one) was observed with both pathogens, increased the concentration of saponins and repressed the production of quercetin 3-O-glucoside ($P < 0.05$). Likewise, *C. siamense* decreased total soluble phenols and flavonoids and increased antiradical activity DPPH•. Besides, *C. gloeosporioides* decreased the levels of kaempferol 3-O-rutinoside and ferulic acid ($P < 0.05$). Regarding the late response (day three), both pathogens decreased the concentration of saponins and increased flavonoids and phytosterols ($P < 0.05$). Nevertheless, *C. siamense* increased the levels of total soluble phenols, p-coumaric acid, kaempferol, and antiradical activity FRAP ($P < 0.05$). Also, *C. gloeosporioides* repressed the production of quercetin 3-O-glucoside at day five ($P < 0.05$). Soursop fruits had a response to the attack of *Colletotrichum* during ripening at physicochemical and oxidative levels, which is associated with the production of compounds related to the development inhibition of pathogens. Even so, soursop fruits showed higher susceptibility to *C. gloeosporioides* and higher sensitivity to the attack of *C. siamense*.

1. Introduction

The soursop (*Annona muricata* L.) is classified as multiple fruit, and its consumption is mainly fresh. The highest production of this fruit is located in Venezuela, Brazil, Colombia, and Mexico. Mexico is the largest producer and consumer of soursop [1, 2]. Soursop fruit has excellent marketing potential, since it has a good taste for the consumer [3]. In Mexico, Nayarit is the leading producer of this fruit with a production of 21,810.86 t in 2017 [2].

The rapid softening during postharvest storage is one of the most critical problems that avoid the commercialization

of this fruit. The most used method to preserve soursop and prevent its softening is refrigeration, with which the shelf life has been extended up to 8 days [4]. Moreover, other factors have also reduced the marketing to international markets such as irregular production, low fruit quality, and pathogen attack [5]. In this regard, fruits are attacked by the fungi *Colletotrichum* spp., which produce a disease called anthracnose [6]. Álvarez et al. [7] identified several species of *Colletotrichum,* such as *C. theobromicola, C. tropicale, C. siamense,* and *C. gloeosporioides,* among others in different tissues of soursop by PCR using the sequences of the ITS regions.

On the other hand, the plants have generated defense mechanisms that allow them to tolerate the damages caused by pathogens [8]. However, when the organisms exceeded the structural defense mechanisms of the plant, a second defense barrier associated with the translation of the pathogen-induced signals are activated in messages that trigger defense strategies specific to plant immunity [9]. One of these mechanisms is the induction of systemic defense that includes the production of secondary metabolites such as phenols, flavonoids, tannins, saponins, anthocyanins, acetogenins, lignins, proteinase inhibitors, lectins, defensins, volatile, phytoalexins (including isoflavonoids), terpenoids, and alkaloids [10], which have an essential role in biotic interactions such as chemical defense against herbivores and pathogens [11]. Nevertheless, few reports exist that evaluate the secondary metabolisms as an anthracnose resistance defense mechanism in the soursop fruit-pathogen interaction.

Due to the abovementioned, the objective of the present work was to evaluate the pathogenic damage and the effect of the pathogens *C. siamense* and *C. gloeosporioides* on the response of soursop fruits in the phytochemical content and antioxidant activity.

2. Materials and Methods

2.1. Experimental Site and Plant Material. Soursop fruits (*Annona muricata* L.) were harvested at physiological maturity from a commercial orchard located in the Ejido Puerta de la Lima, Compostela, Nayarit, Mexico (21° 05′ N; −105° 11′ W; 30 m.a.s.l.). Soursop fruits are multiple fruits, which are derived from several separate individual flowers whose fertilized ovaries are fused to join a single structure. Each of the fruits is derived from a berry. The soursop fruits were ovoid. They are covered with scattered spine-like structures. The pulp of the soursop fruits is soft, white, fibrous, and fleshy [12]. Fruits without mechanical, physical, or/and phytopathological damages were selected. After, the fruits were disinfected by immersion in sodium hypochlorite at 1.5% for two minutes and then rinsed with sterile distilled water [13].

2.2. Microorganisms. Colletotrichum gloeosporioides and *C. siamense* strains were used for this investigation. These pathogens were previously sequenced and analyzed by BLAST in the NCBI database (National Center for Biotechnology Information), showing a high identity (99%) with *C. gloeosporioides* (KP238317.1) and *C. siamense* (KR445677.1).

2.3. Fruit Inoculation. Soursop fruits were inoculated by immersion for one min in a spore suspension (1×10^{5} esp./mL) of the fungus *C. gloeosporioides* or *C. siamense* [14]. The fruits were stored at $28 \pm 2°C$ with a relative humidity of 95%. Fruits were further evaluated at 0, 1, 3, and 5 days of storage.

2.4. Infection Severity Assessment. The percentage of pathological damage in the fruit cuticle was used to determine the severity of the infection on day 3, that is, when the fruits reached the maturity of consumption.

2.5. Phytochemical Analysis

2.5.1. Sample Preparation. One gram of soursop pulp was homogenized with distilled water using an Ultraturrax (T8 IKA® Staufen, Germany) and then centrifuged (Z326 K Hermle, Wehingen, Germany) at 10410 g for 15 min at 4°C. Finally, the supernatant was recovered [15].

2.5.2. Qualitative Analysis of Phytochemical Compounds. To determine the presence of total soluble phenols, flavonoids, anthraquinones, coumarins, steroids, terpenoids, alkaloids, and saponins in soursop treatments, the standard methods described by Sofowara [16], Harborne [17], and Evans [18] were used. The results were expressed with the presence (+) or absence (−) of the phytochemical compound evaluated. These techniques are described below.

2.6. Phenols and Tannins. Three hundred microliters of the extract were added to a reaction tube; afterward, a few drops of ferric chloride ($FeCl_3$) at 10% were added. The appearance of a blue or green precipitate indicated the presence of phenols and tannins [18].

2.7. Flavonoids. Three hundred microliters of the extract were added to a reaction tube; then, 1 mL of 10% ammonia (as NH_4OH) and 1 mL of concentrated H_2SO_4 were added. The disappearance of the yellow color indicated the presence of flavonoids [18].

2.8. Coumarins. Three hundred microliters of the extract was added to a reaction tube. Next, a filter paper strip previously soaked in an alkaline solution of NaOH (0.06 g/mL) was placed inside the tube without touching the extract. Then, the tube was capped and heated to vapor evolution. The presence of fluorescent spots indicated that the sample was positive [18].

2.9. Anthraquinones. One milliliter of benzene, 1 mL of 10% ammonia (as NH_4OH), and 300 μL of extract were added in

a reaction tube. The test is positive if a pink, red, or violet color appears in the low phase ammonia [16].

2.10. Steroids and Terpenoids.
Three hundred microliters of extract was placed in a tube; next, 1 mL of chloroform was added. After, the tubes were cooled on ice. Then, a few drops of sulfuric acid were added and evaluated. The generation of reddish-brown precipitate at the tube bottom indicated the presence of steroids. The formation of a reddish-brown phase at the top indicated the presence of terpenoids [17].

2.11. Alkaloids.
Three hundred microliters of extract were placed in a reaction tube; next, 300 μL of 2N hydrochloric acid (HCl) 2N and 300 μL of Mayer's reagent were added. The presence of a pale precipitate indicated the presence of the alkaloids [16].

2.12. Saponins.
Three hundred microliters of extract were added to a reaction tube; next, 2 mL of distilled water was added. Then, the mix water-extract was vigorously stirred for 10 s. The test was positive if the foam is persistent and stable [16].

2.13. Quantitative Analysis of Phytochemical Compounds.
The content of total soluble phenols, flavonoids, steroids, and saponins was determined according to the procedures described next.

2.14. Total Soluble Phenols.
The content of total soluble phenols was determined with the method proposed by Singleton et al. [19]. The determinations were performed at 760 nm in a spectrophotometer (BioTek Synergy HT, USA), and a calibration curve from 0 to 400 mg·L^{-1} with gallic acid was carried out. The results were expressed in mg equivalents of gallic acid per 100 g of fresh weight (mg EAG/100 g FW).

2.15. Total Flavonoids.
The total flavonoid content was determined by adding 1.4 mL of deionized water and 0.5 mL of the flavonoid reagent to 0.1 mL of the extract. Later, it was allowed to stand for 30 min in the dark, and then, absorbance was measured at 430 nm in a spectrophotometer (BioTek Synergy HT, USA). A calibration curve with routine in a concentration range of 0–10 g·L^{-1} was performed. The total flavonoid content was expressed as mg routine equivalents per 100 g of fresh weight (mg RE/100 g FW). The flavonoid reagent was prepared as follows: first, 70 mL of methanol, 25 mL of distilled water, and 5 mL of acetic acid were mixed. Next, 133 mg of aluminum trichloride and 400 mg of sodium acetate were added and mixed [20].

2.16. Total Steroids.
The total steroid content was determined using the Lieberman–Buchard method [21] at a wavelength of 550 nm (BioTek Synergy HT, USA). The total steroid content was quantified through a calibration curve from 0 to 1000 mg·L^{-1} using cholesterol as standard. The results were expressed in mg equivalents of cholesterol per 100 g of fresh weight (mg EC/100 g FW).

2.17. Total Saponins.
The total content of saponins was determined according to the indirect method described by Soto et al. [22]. This method is based on the determination of reducing sugars by the 3, 5 dinitrosalicylic acid (DNS) method [23]. A calibration curve was developed with triterpene saponin of *Quillaja saponaria* (20–35% saponin) with concentration ranges from 0 g·L^{-1} to 10 g·L^{-1}. The results were expressed in mg equivalent to saponin of *Quillaja saponaria* on 100 g of fresh weight (mg ESQS/100 g FW).

2.18. Determination of Antioxidant Capacity by the DPPH Method.
To determine antioxidant capacity by the DPPH method, the protocol proposed by Brand-Williams et al. [24] was used. The change in absorbance at 517 nm was measured in a spectrophotometer (BioTek Synergy HT, USA). Antioxidant activity was determined using a calibration curve with ascorbic acid with concentration ranges from 0 to 100 mg·L^{-1}. The results were expressed in mg equivalents of ascorbic acid on 100 g of fresh weight (mg EAA/100 g FW).

2.19. Determination of Antioxidant Capacity by the ABTS Technique.
The determination of antioxidant capacity by the ABTS method was performed using the method described by Re et al. [25]. The absorbance was measured in a spectrophotometer (BioTek Synergy HT, USA) at 734 nm. Antioxidant activity was determined using a calibration curve with ascorbic acid with values from 0 to 150 mg·L^{-1}. The results were expressed in mg equivalent of ascorbic acid on 100 g of fresh weight (mg EAA/100 g FW).

2.20. Antioxidant Capacity of Iron Reduction (FRAP).
The antioxidant capacity of iron reduction (FRAP) was determined by the method described by Yen and Chen [26]. Absorbance was measured at 700 nm using a spectrophotometer (BioTek Synergy HT, USA). The reducing activity was determined with a calibration curve using ascorbic acid concentrations from 0 to 30 mg·L^{-1}. The results were expressed in mg equivalents ascorbic acid on 100 g of fresh weight (mg EAA/100 g FW).

2.21. Analysis and Identification of Phenolic Soluble Compounds by UPLC-ESI-QTOF MS^{E}.
Phytochemical profile was analyzed using an ultra-performance liquid chromatography system (UPLC), Acquity UPLC ™ H-Class (Waters, Manchester, Reino Unido), coupled to a mass spectrometer (MS QTof) with an atmospheric pressure electrospray ionization (ESI) interface (Vion, Waters Co, MA, USA). The column used was an Acquity BEH C18 (100 × 2.1 mm, 1.7 um) at 35°C. The elution gradient was performed with a binary system consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The following gradient was

applied at a flow rate of 0.4 mL/min: 0 min, 5% B and 22 min, 95% B, held for 5 min, followed by a rebalance step for 3 min. The injection volume was 2 μL, and the sample temperature was set at 10°C [27]. The conditions of the mass spectrometer were as follows: the temperature of the source was adjusted to 120°C, and nitrogen was used as the desolvation gas (800 L/h) at a temperature of 450°C. The sampling cone and capillary voltages were 40 V and 3.5 kV, respectively. Data acquisition was performed using the high definition MSE negative ionization mode with a mass range of 50–2000 Da. Leucine-enkephalin (50 pg/mL) at 10 mL/min was used for mass correction. Peak identification was carried out by identifying the exact mass of the pseudomolecular ion (confirmation of elemental composition with <10 ppm mass error), analysis of the fragmentation pattern, and isotopic distribution of the pseudomolecular ion. Data acquisition was performed with the UNIFI Scientific Information System (Waters Co).

2.22. Statistical Analysis.

A completely randomized design was used for statistical analysis of the severity of the infection. Data were analyzed using Analysis of Variance (ANOVA) with a 5% significance level. The comparison of means was carried out using the Tukey test. The experimental unit was one fruit with six replicates on different fruits.

A completely randomized block design was used to analyze the rest of the variables measured. The blocks were the days of storage. All experiments were analyzed by Analysis of Variance (ANOVA) with a 5% significance level. The comparison of means was carried out using the LSD test when ANOVA showed significant differences. The experimental unit was one fruit with six replicates on different fruits.

3. Results and Discussion

3.1. Severity of the Pathogenicity.

Figure 1 shows the damage of soursop fruits in different treatments (control, *C. siamense,* and *C. gloeosporioides*) stored for three days after inoculation at 28 ± 2°C. The fruit damage indicates the virulence of the pathogens and the sensitivity from the fruits to strains evaluated. The control and treated fruits with *C. siamense* showed no significant statistical differences in the pathogenesis ($P < 0.05$), as it is observed in Figure 2. Arauz [28] mentions that *Colletotrichum* spp. cause latent infections, and once the phytopathogenic fungi found the right conditions, the disease is generated.

The control and treated fruits with *C. gloeosoporioides* showed significant statistical differences in the pathogenesis ($P < 0.05$). Fruits treated with *C. gloeosporioides* showed greater severity of the disease. However, treated fruits with *C. siamense* showed no significant statistical differences in the severity of the disease with the fruits treated with *C. gloeosporioides* or with the control fruits ($P > 0.05$) (Figure 2).

C. gloeosporioides is considered the principal causative agent of anthracnose in soursop [29], while *C. siamense* has

been described recently for this fruit [7]. Possibly, this is because *C. gloeosporioides* has developed strategies to avoid the defense mechanisms of the fruit. In contrast, *C. siamense* has not yet fully developed these abilities, which may cause soursop fruits to be more sensitive to the presence of this fungus, triggering biochemical responses and the production of different types of metabolites, as it is shown in later results.

3.2. Phytochemical Evaluation of Soursop Fruits

3.2.1. Qualitative Analysis of Phytochemicals Compounds.

The phytochemical profile results were positive for the presence of total soluble phenols, saponins, flavonoids, and phytosterols in the three evaluated treatments (control fruits, fruits inoculated with *C. gloeosporioides*, and fruits inoculated with *C. siamense*) (Table 1). The presence of phenols, saponins, flavonoids, and phytosterols has been reported in ethanolic and aqueous extracts of soursop fruits [30]. These compounds have been previously reported by their antibacterial and antifungal activities [31]. The quantitative analysis of the compounds is shown in the following sections.

3.2.2. Quantitative Analysis of Phytochemicals Compounds

(1) Total Soluble Phenols. Total soluble phenols decreased in the first days of the experiment in the three treatments. ANOVA showed significant differences ($P < 0.05$) for the fruits inoculated with *C. siamense* on day 1 (77.10 mg EAG/100 g FW) compared with the fruits inoculated with *C. gloeosporioides* (98.62 mg EAG/100 g FW) and control (101.35 mg EAG/100 g FW) (Figure 3(a)). Therefore, we observed an early response of the fruits to the presence of this fungus.

Pérez-Márquez et al. [32] reported that when the tissue cell is healthy and intact, polyphenol oxidase (PPO) enzyme and its substrates (phenols) are found in separate compartments (chloroplasts and vacuoles, respectively). Nonetheless, when the cell is infected, the PPO enzyme and the substrates come into contact, and an oxidation reaction occurs, generating the formation of quinones, which explains the decrease in the phenolic compounds of the fruits treated with *C. siamense*.

On the other hand, the fruits treated with *C. siamense* showed a significant increase ($P < 0.05$) on day 3 (118.08 mg EAG/100 g FW) in comparison to *C. gloeosoporioides* and the control (93.55 and 91.03 mg EAG/100 g FW, respectively) that did not show significant differences between them ($P > 0.05$) indicating a late response of the fruits to the presence of *C. siamense* (Figure 3(a)).

Subsequently, an increase in the concentration of total phenols for all treatments on day five was recorded. However, the fruits with the presence of pathogens showed a significantly higher concentration ($P < 0.05$) of phenols with values of 164.68 mg EAG/100 g FW for *C. siamense* and 159.49 mg EAG/100 g FW for *C. gloeosporioides* compared to the control 134.36 mg EAG/100 g FW. That indicates that the
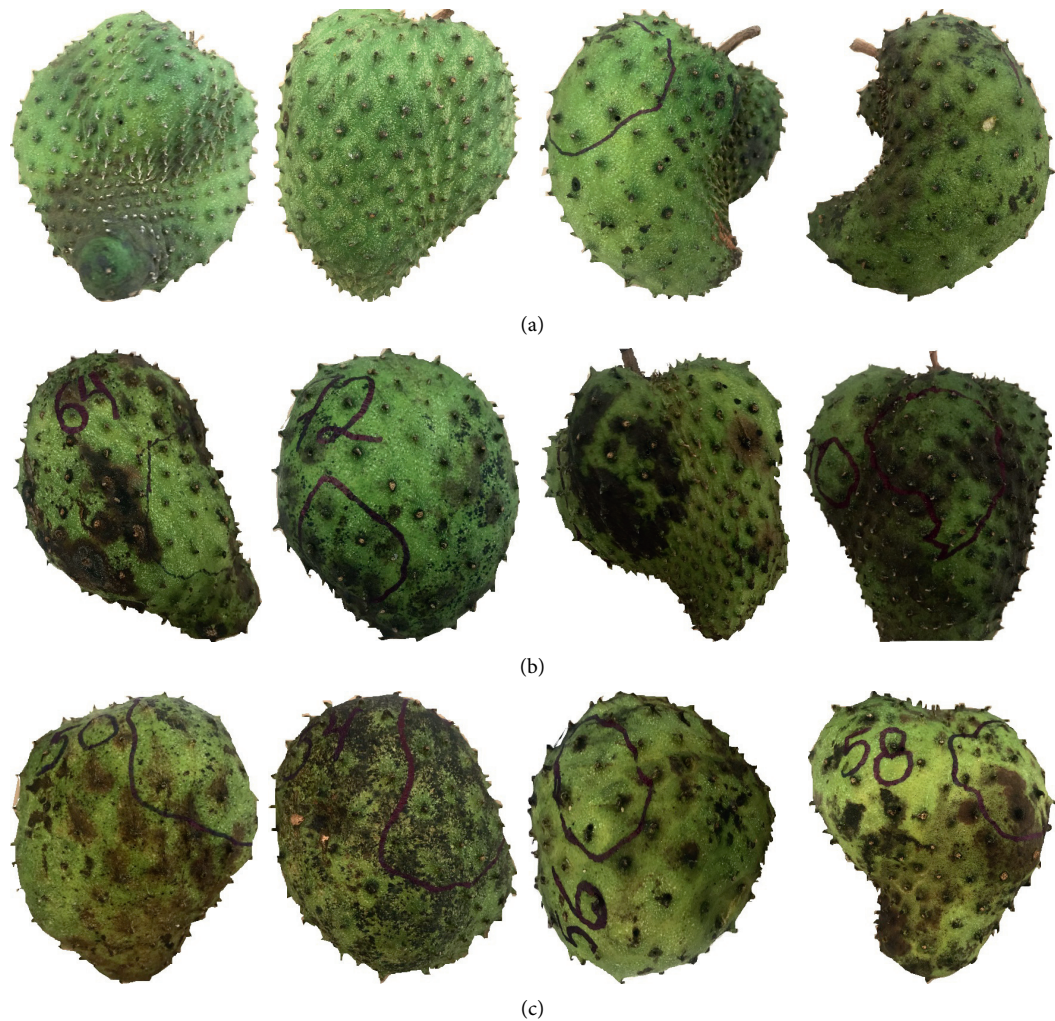
(a)

(b)

(c)

FIGURE 1: Soursop fruits stored for three days at $28 \pm 2^{\circ}$C and 95% RH. Control fruits (a) and fruits treated with *C. siamense* (b) and *C. gloeosporioides* (c).
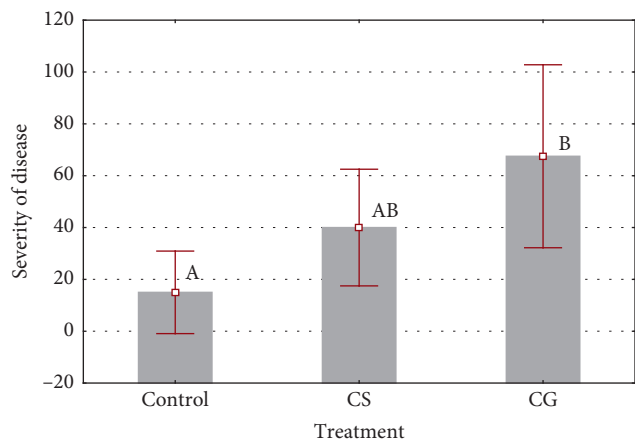

FIGURE 2: Infection severity of control fruits and fruits treated with *Colletotrichum siamense* (CS) and *Colletotrichum gloeosporioides* (CG).

fruits treated with either *C. siamense* or *C. gloeosporioides* showed a late response at day five of storage.

Balois-Morales et al. [15] mentioned that when abiotic factors stress the vegetative tissues, exists an accumulation of phenolic compounds. The high values obtained in this investigation indicate that the increase in the concentration of phenols is a response to stress, which can be abiotic or biotic [33], in this work by the presence of the pathogens. That involves the production of biochemical inhibitors (phenolic compounds) for the damage caused by pathogens in soursop fruits. Kim et al. [34] mentioned that phenols content could be associated with the infection process of the pathogen and the characteristics of defense materials such as phytoanthropin.

The increase in the concentration of phenolic compounds in soursop fruits can cause cell shrinkage, integrity loss of cell wall and cell membrane and the inhibition of the activity of essential enzymes in the metabolism of pathogens [35].

(2) Total Flavonoids. A decrease in the concentration of flavonoids ($P < 0.05$) was observed in the fruits inoculated with *C. siamense* (11.24 mg RE/100 g FW) in comparison

TABLE 1: Phytochemical compounds determined in the soursop fruit treatments.

Compound	Fruits control	Fruits inoculated with *C. gloeosporioides*	Fruits inoculated with *C. siamense*
Total phenols	+	+	+
Flavonoids	+	+	+
Anthraquinones	−	−	−
Coumarins	−	−	−
Steroids	+	+	+
Terpenoids	+	+	+
Alkaloids	−	−	−
Saponins	+	+	+

(+), presence; (−), absence.

with the fruits treated with *C. gloeosporioides* (20.24 mg RE/100 g FW) and the control treatment at day one (20.35 mg RE/100 g FW), which indicates an early response for the fruits treated with this pathogen.

On the other hand, the fruits treated with both pathogens showed a late response, increasing their concentration at day three compared to the control ($P < 0.05$). The fruits inoculated with *C. siamense* had a concentration of 16.77 mg RE/100 g FW, while fruit treated with *C. gloeosporioides* had the highest level of flavonoids (17.18 mg RE/100 g FW). In contrast, the control fruits had the lowest value of flavonoids (13.21 mg RE/100 g FW) (Figure 3(b)).

Flavonoids belong to the phenolic compounds [36], so that they can be affected by the polyphenol oxidase enzyme and decrease in its concentration in the early stages of a fungal infection, such occurs with the total soluble phenolic compounds of the fruits inoculated with *C. siamense*.

Defense and pigmentation are some of flavonoids functions [36]. The antioxidant activity of flavonoids results from a combination of its chelating properties of iron and retention of reactive oxygen species (ROS). Besides the inhibition of some enzymes such as lipoxygenase, cyclo-oxygenase, myeloperoxidase, and NADPH oxidase [37]. These activities provide them bactericidal, fungicidal, and antiviral properties [38]. For this reason, the increase in flavonoid concentrations may be a response to the presence of the pathogens on day 3.

(3) Total Phytosterols. On day one of storage, no significant differences between treatments ($P > 0.05$) were found. However, a late response to pathogenesis was observed on day three due to the increase in the concentration of phytosterols in the treatments with both pathogens. The fruits inoculated with *C. siamense* showed the highest concentration (1155.11 mg EC/100 g FW), followed by the fruits inoculated with *C. gloeosporioides* (882.84 mg EC/100 g FW), in comparison to the control fruits (18.45 mg EC/100 g FW) (Figure 3(c)).

One of the defense mechanisms against biotic or abiotic stress of the plant is the production of phytosterols, which act as molecular signals within the plant or between the plant and its environment (environmental conditions or presence of microorganisms) [39]. Steroids are widely distributed in plants and function as signal molecules involved in processes such as stem elongation, vascular differentiation, male fertility, senescence and flowering time, leaf development, and resistance to biotic and abiotic stress [40].

Likewise, Burčová et al. [41] found that a Norway spruce bark hexanoic extract, whose main components were β-sitosterol, γ-sitosterol, and kampesterol, was able to inhibit the development of filamentous fungi such as *Alternaria alternata*, *Mucor racemosus*, *Penicillium purpurogenum*, and *Rhizopus oryzae*. Besides, Briceño et al. [42] found that suspension-cultured cells of *Solanum lycopersicum* cv. Micro-Tom was able to accumulate extracellularly isofucosterol and β-sitosterol as response to methyl jasmonate, which induces resistance in plants. These authors also found the same phenomenon with the application of cyclodextrins as potential elicitors of the defense response induction.

Therefore, the fruits inoculated with the pathogens showed a delayed response due to the synthesis of secondary metabolites related to plant defense, such as phytosterols and/or their precursors.

(3) Total Saponins. An early response was observed in pathogens treatments where the concentration of saponins for both treatments increased ($P < 0.05$), highlighting the fruits inoculated with *C. siamense*, with a concentration of 1612.12 mg ESQS/100 g FW followed by the fruits inoculated with *C. gloeosporioides* (1049.90 mg ESQS/100 g FW) compared to the control treatment at day one, which had a concentration of 470.10 mg ESQS/100 g FW.

Regarding the late response, it was observed that the treatments with the pathogens decreased the concentration of saponins (463.00 ± 80 mg ESQS/100 g FW) on day three of storage compared to the control, which increased its concentration to 1522.14 mg ESQS/100 g FW (Figure 3(d)).

Apaza et al. [43] declared that plant species present defense mechanisms against pathogens such as fungi, bacteria, viruses, and nematodes. Among these mechanisms is the production of saponins, due to these acts as a biofungicide.

The ability to form complexes with sterols, proteins, and membrane phospholipids represents the primary mechanism of antifungal activity of saponins [44]. The saponins are within the constitutive defenses of the plants, which represent a chemical barrier [45]. The above could explain the reason for the early response in the soursop fruits inoculated with the pathogens.
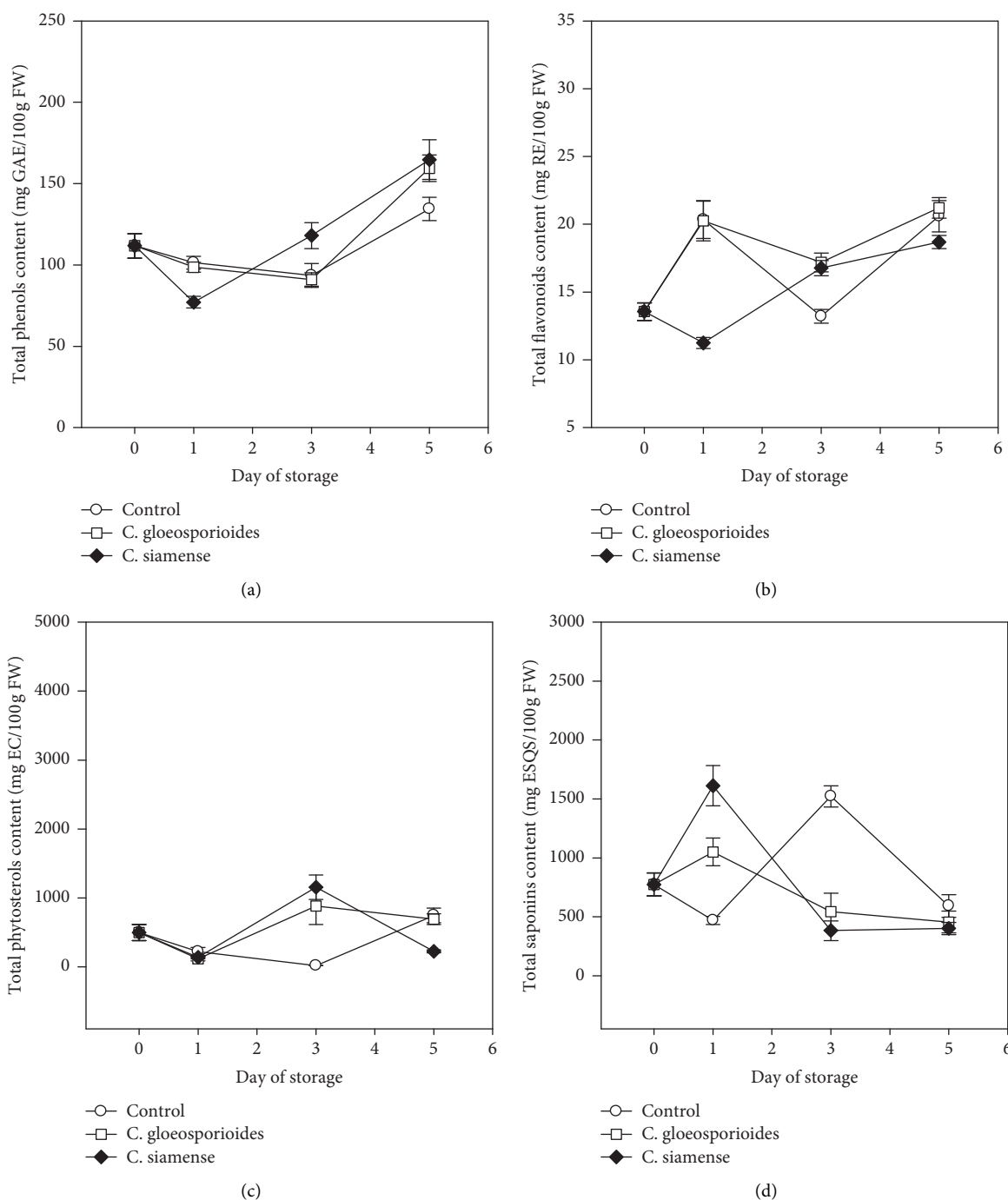
FIGURE 3: Total soluble phenols (a), total flavonoids (b), total phytosterols (c), and total saponins (d) content in soursop fruits with three different treatments. Control (C), *C. gloeosporioides*, and *C. siamense*.

Likewise, the use of saponins to form complexes with other molecules as response to pathogen attack could decrease their concentration in the fruit regarding control on day 3.

Antioxidant activity: DPPH•, ABTS•+, and FRAP

The results of this investigation showed an early response in the fruits inoculated with *C. siamense*, increasing its antiradical activity DPPH• up to 194.09 mg EAA/100 g FW ($P < 0.05$), while in the treatment with *C. gloeosporioides*, a value of 164.68 mg EAA/100 g FW was obtained. However, they showed no significant differences compared to the control treatment at day one ($P > 0.5$), in which an antiradical activity of 174.45 mg EAA/100 g FW was recorded.

In contrast, all the treatments increased their antiradical activity on day three and kept on day 5, showing no significant differences between the treatments ($P > 0.05$) (Figure 4(a)).
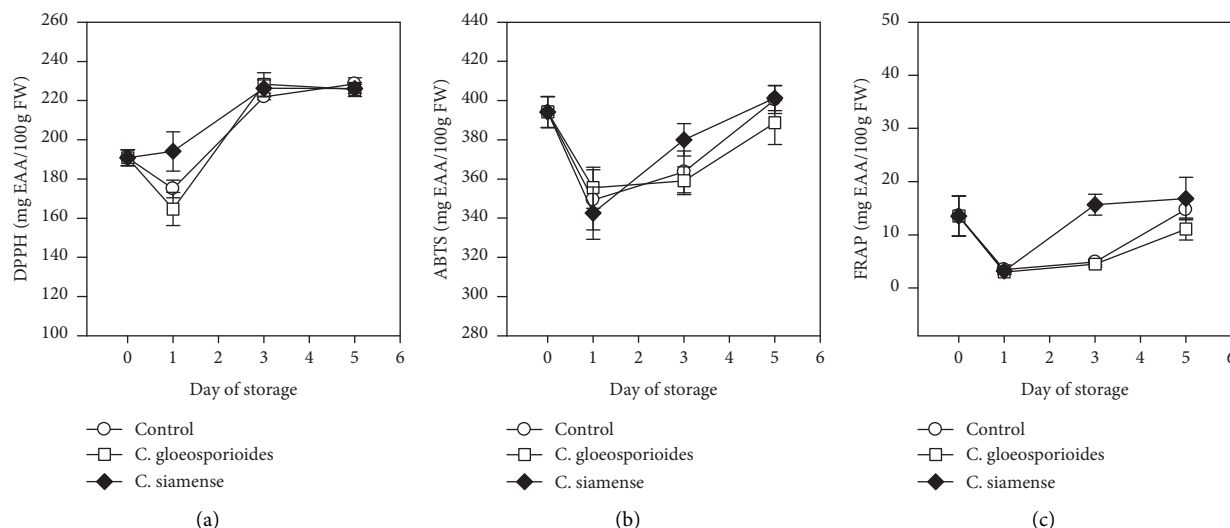
FIGURE 4: Antioxidant activity by the DPPH method● (a), antioxidant activity by the ABTS●+ (b), and antioxidant activity by the FRAP method (c) in soursop fruits with three different treatments. Control (*C*), *C. gloeosporioides*, and *C. siamense*.

The DPPH method can trap the DPPH radical. The increase in this specific activity is given by the rise in the concentration of phenolic compounds on the third and fifth day of storage in all treatments. This increase in antioxidant activity in the soursop fruits could be related to the rise of phenolic compounds because they can neutralize free radicals by the presence and action of hydroxyl groups of phenolic compounds [46].

Our results coincide with those reported by Balois-Morales et al. [47] who reported an increase in antiradical activity ranging from 86.86 to 67.35 mg EAA/100 g FW in soursop fruits after four days of storage at room temperature. Thus, we can conclude that the increase in antiradical activity at the third and fifth day occurs as part of the healthy metabolism of the soursop fruits.

The soursop fruits inoculated with both pathogens did not show significant differences ($P > 0.05$) in antioxidant activity evaluated by ABTS●+ concerning control at day 1. The fruits showed an increase in their antioxidant activity at day three of storage where their maximum activity was up to date five with a value of 388.70 ± 12 mg EAA/100 g FW for both pathogens and 371.34 ± 24.65 mg EAA/100 g FW for control (Figure 4(b)).

The increase of antioxidant activity on the ABTS●+ radical in the ripening period can be due to the high metabolic activity characteristic of maturation and the climacteric period, besides to the action of ascorbic acid, which has a high concentration in the days after the harvest [48].

The results obtained in this study agree with those reported by Balois-Morales et al. [47] who said the antioxidant activity of 77.07–86.28 mg EAA/100 g FW in soursop fruits since day 0 to after four days exposed to a temperature of 22°C.

The fruits inoculated with *C. siamense* showed a late response on day three of storage in antioxidant reducing activity evaluated by FRAP, since they increased their antioxidant activity (16.81 mg EAA/100 g FW) compared

to the control (4.84 mg EAA/100 g FW) (Figure 4(c)). The above is an essential mechanism of plant defense since plants trap iron as a strategy to reduce pathogen virulence; also, they may use the iron to increase local oxidative stress in defense responses against pathogens [49].

The changes in antioxidant activity (ABTS●+, DPPH●, and FRAP) are related to the high metabolic activity of the maturation process, besides the action of ascorbic acid, which has a high concentration days after harvest, together with the increase in phenolic compounds [48]. Soursop fruits showed antioxidant activity by this principle, coinciding with other investigations reported with the FRAP method in soursop fruits [50]. However, the pathogens may alter this behavior as observed in the present work with fruits treated with *C. siamense*.

The antioxidant activity DPPH● and FRAP of the soursop fruits inoculated with the pathogens allowed to see a marked difference concerning the control fruits. Liu et al. [51] showed that the high antioxidant capacity of soursop fruits is due to the high polyphenols content, which blocks oxidation and activates other antioxidants.

The increase of antioxidant activity may be related to the secondary responses of soursop fruits to defend against an infective process, which includes an elevation of the levels of constitutive defensive toxins, of pathogen receptors and structural reinforcement of cell walls in tissues. The process determines a substantial change in the metabolic profile of the active cells [52].

The marked responses in the fruits inoculated with *C. siamense* (early and late response) in comparison with those inoculated with *C. gloeosporioides* and the control fruits, whether in phytochemicals or antioxidant activity, might be related to the fact that the soursop presents higher resistance to the attack of *C. siamense,* so that its response mechanism is more sensitive.

At day 5, when fruits are senescent, there are no statistical differences among treatments ($P > 0.05$) in

TABLE 2: Identification of soluble phenols in soursop by UPLC-ESI-QTOF MS^E.

No.	Compound name	Retention time (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Adduct
1	p-Coumaric acid	5.18	$C_9H_8O_3$	164.05	164.05	−2.24	[M-H]$^-$
2	Ferulic acid	5.71	$C_{10}H_{10}O_4$	194.06	194.06	−2.24	[M-H]$^-$
3	Quercetin 3-O-glucoside	5.84	$C_{21}H_{20}O_{12}$	464.10	464.10	0.42	[M-H]$^-$
4	Kaempferol	5.89	$C_{15}H_{10}O_6$	286.05	286.25	−5.05	[M-H]$^-$
5	Kaempferol 3-O-rutinoside	6.22	$C_{27}H_{30}O_{15}$	594.16	594.16	−0.20	[M-H]$^-$
6	Quercetin	7.87	$C_{15}H_{10}O_7$	302.05	302.04	−2.03	[M-H]$^-$
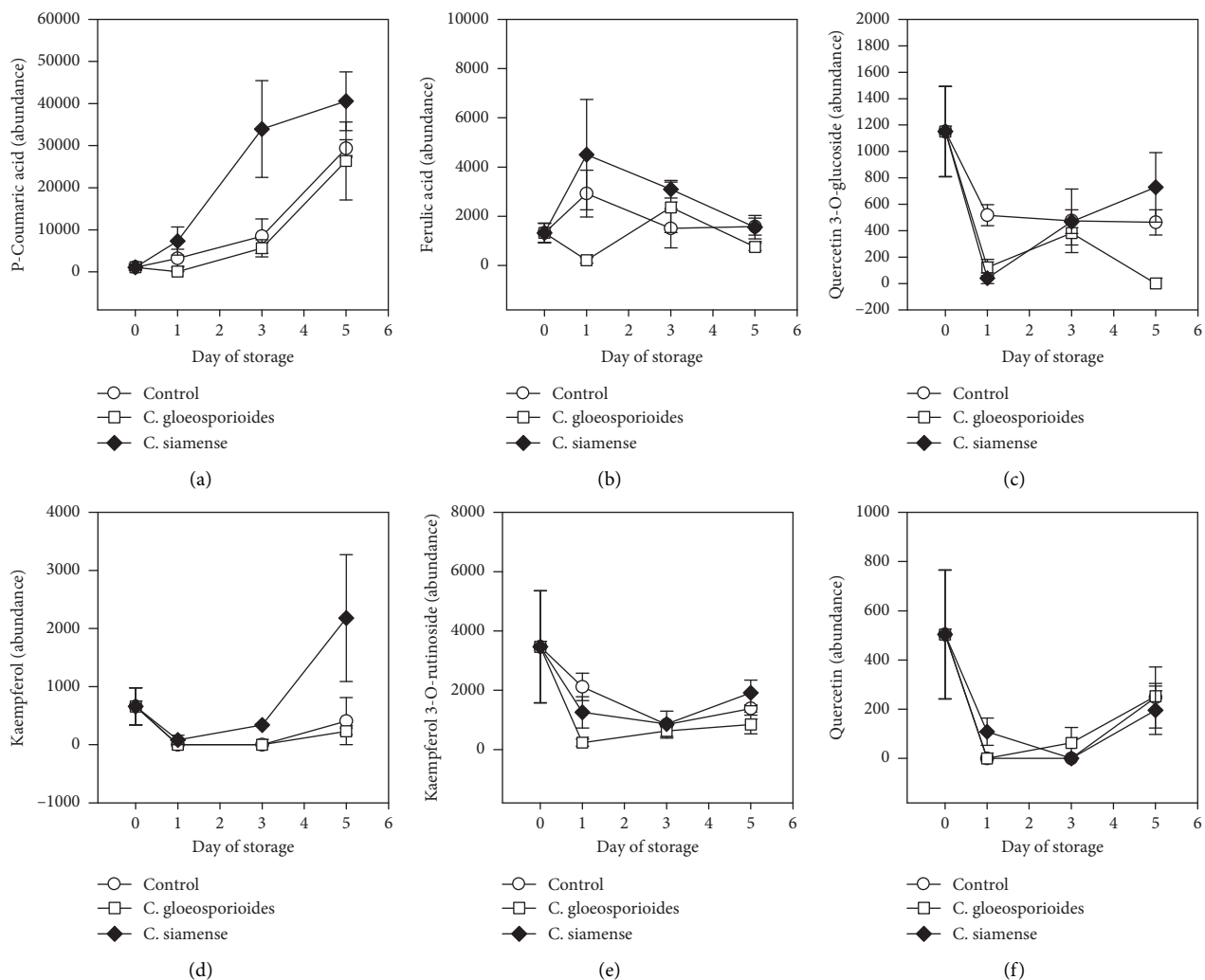
FIGURE 5: p-Coumaric acid (a), ferulic acid (b), quercetin 3-O-glucoside (c), kaempferol (d), kaempferol 3-O-rutinoside (e), and quercetin (f) abundance in soursop fruits with three different treatments during storage. Control (C), *C. gloeosporioides*, and *C. siamense*.

antioxidant activity (ABTS•+, DPPH•, and FRAP). Arauz [28] mentioned that the infection of climacteric fruits by *C. gloeosporioides* is an adaptation example of the pathogen to the host physiology, and the infection seems to be a case of coevolution, presenting an advantage, on the one hand, for the nutrition of the pathogen, and on the other hand, for the propagation of the fruit seeds.

3.2.3. Identification and Dynamics of the Soluble Phenolic Compounds. Table 2 shows the identification of the main soluble phenols found in soursop. Two phenolic acids (ferulic and p-coumaric acids) and four flavonoids, specifically flavonols (kaempferol, kaempferol 3-O-rutinoside, quercetin, and quercetin 3-O-glucoside), were found in the soursop samples. These phenolic compounds have been found previously in soursop [53, 54]. Besides, these compounds have been reported for their antifungal activity [55].

Figure 5 shows the dynamic of the soluble phenol compounds identified. We observed an early response on day one. The level of quercetin was increasing in fruits treated with *C. siamense*, while the concentration of kaempferol 3-O-rutinoside and ferulic acid decreases in the fruits treated with *C. gloeosporioides* and quercetin 3-O-glucoside for fruits treated by both fungi regarding control fruits ($P < 0.05$).

Besides the above, a late response on day three was recorded, increasing the levels of p-coumaric acid and kaempferol in fruits treated with *C. siamense* regarding control fruits ($P < 0.05$). However, the level of quercetin 3-O-glucoside decreased in fruits treated with *C. gloeosporioides* at day five ($P < 0.05$).

The induction of the quercetin production on day one indicates recognition of *C. siamense* by the fruits and the activation of its defense mechanism. Wilkinson et al. [56] mention that the first inducible defenses are faster and stronger against pathogen attack, which could be related to the low virulence of *C. siamense* against soursop fruit. Quercetin has been reported as a compound with antifungal activity [57], and it is produced as a strategy of plant defense against phytopathogenic fungi [58]. Likewise, quercetin can cause severe damage to fungi as *Penicillium expansum*, and this compound can increase defense proteins in the plants [57].

On the other hand, the metabolism changes on day three. Fruits change the response metabolites regarding day one, increasing the production of p-coumaric acid and kaempferol in fruits treated with *C. siamense*. These changes could be related either to the use of a more cost-efficient mechanism [56] or to an adaptation of fungi metabolism to plant defense, since the fungi can change from a biotrophic to a necrotrophic metabolism [59].

In another way, even though kaempferol 3-O-rutinoside, quercetin 3-O-glucoside, and ferulic acid have been reported with fungicide activity [58], these compounds decreased their levels regarding control on day one and quercetin 3-O-glucoside on day five in fruits treated with *C. gloeosporioides*. Likewise, quercetin 3-O-glucoside decreased in fruits treated with *C. siamense* on day one. The above could be due to the production of effectors by fungi. Van der Dous and Rep [60] mention that the fungi can secrete effectors, which are proteins that protect fungi from plant defense compounds or could suppress the host's immune system. So, these compounds can promote the colonization and proliferation of disease symptoms. The above could be related to a higher virulence of *C. gloeosporioides* and the appearance of more severe symptoms on fruits treated with this fungus.

4. Conclusion

Soursop fruits had a response to the attack of pathogens during ripening at the physicochemical and oxidative levels, which are associated with the production of compounds that, in high concentrations, inhibit the development of the pathogens. Both pathogens cause an early response in the fruit, increasing the concentration of saponins and decreasing the production of quercetin 3-O-glucoside. *C. siamense* decreased total soluble phenols and flavonoids and increased antiradical activity DPPH. *C. gloeosporioides* decreased the levels of kaempferol 3-O-rutoside and ferulic acid. On the other hand, both pathogens cause a late response in soursop fruits decreasing the concentration of saponins and increasing flavonoids and phytosterols. *C. siamense* increased total soluble phenols, p-coumaric acid, kaempferol, and antiradical activity FRAP. Also, *C. gloeosporioides* decreased the production of quercetin 3-O-glucoside. As well as, pathogens could produce compounds that inhibit the production of defense compounds by fruits, which could be related to an increase of disease severity. The soursop fruits showed higher sensitivity to the attack of *C. siamense*, which could be related to higher resistance to the attack of this pathogen compared to *C. gloeosporioides*.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

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