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Microbial control and quorum sensing inhibition by phenolic compounds of acerola *(Malpighia emarginata)*

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Abstract

Recently, phenolic compounds have been tested for their ability to inhibit bacterial communication known as quorum sensing. The objective of this study was to characterize acerola pulp *(Malpighia emarginata)* regarding its centesimal composition and mineral content as determined by Total Reflection X-Ray Fluorescence as well as to determine the total phenolic content and the antioxidant, antimicrobial and anti-quorum sensing activities of the phenolic extract obtained from the pulp. The centesimal and mineral content presented values in good agreement with those shown for fruits of the *Malpighia* generum. The total phenolic content for the fruit was 5848.74 ± 4.18 mg GAE/L. The antioxidant activity determined by the ABTS method was 127.18 ± 6.6 µM Trolox/g of fruit and 112.02 ± 0.03 µM Trolox/g of fruit in the DPPH assay, expressive values as compared to other fruits. The phenolic extract inhibited all the evaluated bacteria with minimal inhibitory concentrations ranging from 487.39 mg GAE /L to 1462.18 mg GAE /L of extract. Interestingly, in sub-MIC concentrations the phenolic extract of acerola did not interfere with bacterial growth, but inhibited quorum sensing controlled phenotypes such as violacein production in *Chromobacterium violaceum*, swarming motility in *Aeromonas hydrophila* and more importantly, biofilm formation in these bacteria and also in *Serratia marcencens*. The phenolic extract from acerola presents important bioactive properties including the newly described ability to inhibit quorum sensing in bacteria which can be further explored as a means to control bacterial activities in foods.

Introduction

Phenolic compounds have attracted much attention in recent years due to their bioactive properties such as potent antioxidant activity (Ignat *et al*., 2011). The antioxidant activity of these compounds has been attributed to their ability to scavenge free radicals (alkoxyl, superoxide, hydroxyl radical, nitric oxide, peroxynitrite oxidant); interact with enzymes, transcription factors (NF-kB), receptors (e.g. estrogen receptors) which contribute to the reduction of oxidative stress common to cardiovascular diseases, neurodegenerative diseases, cancer amongst other diseases (Fraga *et al*., 2010).

Phenolic compounds can be structurally characterized by the presence of one or more aromatic rings bearing at least one free hydroxyl group. They are secondary metabolites found in plants and provide protection against ultraviolet radiation and some pathogens (Manach *et al.*, 2004). The principal sources of phenolic compounds are fruits (Oliveira *et al*., 2009). Some fruits can afford between 200-300 mg of phenolic compounds per 100 g of fresh fruit (Scalbert and Williamson, 2000).

Not only do phenolic compounds possess antioxidant capacity, they can also inhibit the growth of microorganisms. This property is of great interest to the food industry, and has potential applications as a natural alternative for food conservation and could alleviate growing concerns of consumers about the risks caused by artificial food preservatives (Papadopoulo *et al.*, 2005).

Quorum sensing has been associated with food spoilage and thus has generated a lot of attention within the scientific community (Pinto *et al*., 2007; Bai and Rai, 2011; Skandamis and Nychas, 2012). Quorum sensing is a form of transcriptional regulation in bacteria that consists of a signal molecule (which accumulates at high population density), a signal synthase and a signal receptor which most often

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functions by activating target genes in response to the activating signal (Pinto *et al*., 2012). Therefore, quorum sensing is very important for bacterial activity given that it controls diverse cellular functions such as sporulation, biofilm formation, siderophore production, swarming motility, competence, conjugation, virulence factor expression, among others (Bai and Rai, 2011). For this reason, the inhibition of this signaling mechanism could be exploited as an alternative mechanism for improving food safety and conservation as well as extending the shelf life of products (Bai and Rai, 2011; Skandamis and Nychas, 2012).

Acerola *(Malpighia emarginata)* is a fruit that has been highlighted for its relatively large quantities of vitamin C (Freitas *et al.*, 2006). In addition to vitamin C, acerola contains many phenolic compounds beneficial to human health (Oliveira *et al.*, 2012). The antioxidant capacity of acerola has been reported in the literature and acerola extracts have also demonstrated antigenotoxicity (Nunes *et al*., 2013). The objective of this study was to evaluate the antioxidant, antimicrobial and anti-quorum sensing activities of the phenolic extract of acerola.

Material and Methods

Extract preparation

Fruits of *Malpighia emarginata* were collected in the city of Ouro Preto – MG, herborized and a voucher specimen deposited in the Professor José Badini Herbarium at the Federal University of Ouro Preto (Oliveira, B.D, 28569). Fresh fruits were washed and sanitized with sodium hypochloride (50 mg/L) for 15 min at room temperature. Next, the seeds were manually removed and the pulp was homogenized using a blender for 20 seconds at room temperature and kept frozen at -20°C until further use. Phenolic compounds were extracted by solid phase extraction (SPE) as previously described (Noratto *et al*., 2010). The term phenolic extract used frequently within this report has no relation to the solvent system used to obtain the extract. Phenol was not used as an extraction solvent and instead the term phenolic extract is used to describe an extract rich in phenolic compounds.

The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu assay (Shahidi and Nacsk, 1995) and expressed as mg of gallic acid equivalent per L (mg GAE/L). Non-phenolic reducing compounds including sugars, organic acids (particularly ascorbic acid) amongst others were eliminated by SPE in order to improve the specificity of the Folin-Ciocalteu assay, as suggested by Sánchez-Rangel *et al.* (2013).

Centesimal composition

This experiment was performed with the pulp of the fruit by quantifying the content of proteins, lipids, ash, moisture, carbohydrates, fibers, and total soluble solutes as recently reported (Oliveira *et al.*, 2016). The carbohydrate content was calculated by subtracting the sum of the percentages of protein, total lipids, moisture and ash from 100%.

Trace content determination

The content of potassium, calcium, iron, copper, zinc and chloride of the extract was analyzed by Total Reflection X-Ray Fluorescence (TXRF) as in Oliveira *et al.* (2016). The analysis was performed on a S2-PICOFOX instrument (Bruker AXS, Berlin, Germany) with a molybdenum anode operating at 50 kV and 750 mA. A solution of the extract in distilled water was made and 10 µl of the solution was transferred on to quartz discs and dried. The results were obtained by integration of the signal on Bruker Spectra software (version 6.1.5.0) with quantification being performed by means of comparing with internal standards of known concentration. The minerals were expressed as mg/g of pulp fruit.

Antioxidant activity (ABTS and DPPH)

The antioxidant activity (AA) of the phenolic extract from acerola *(Malpighia emarginata)* was determined by using the $ABTS^{+}$ radical method, according to the methodology described by Rufino *et al.* (2007) and by DPPH free radical method, as described by Brand-Williams *et al.* (1995). For the standardization of the results, trolox was used as an external standard for both assays and the results were expressed as antioxidant activity in µM Trolox equivalent per gram of fresh pulp.

Antimicrobial activity

The antimicrobial activity of the phenolic extract was evaluated by plate diffusion assay and by determining the minimal inhibitory concentration (MIC). The tests were performed against Gram negative bacteria *Escherichia coli* ATCC10536, *Aeromona hydrophila* IOC/FDA110, *Pseudomonas aeruginosa* ATCC15442, *Pseudomonas fluorescense* NCTC10038, *Salmonella* spp. (laboratory stock), *Serratia marcescens* UFOP-001 (laboratory stock), *Hafnia alvei* ATCC11604, and the Gram positives *Staphylococcus aureus* ATCC6538P, *Listeria monocytogene* ATCC7644 and *Bacillus cereus* ATCC 11778, all of great relevance in food microbiology. The tests were also performed against

Chromobacterium violaceum ATCC6357 which was used in the quorum sensing bioassays. For the plate diffusion assay, the procedures described by Salawu *et al.* (2011) were followed, while MIC was performed as previously reported by Wiegand *et al*. (2008). All quorum sensing assays were performed in sub-MIC concentrations with no measurable interference on the bacterial growth.

Anti-quorum sensing activity

The quorum sensing inhibitory effect of the phenolic extract was evaluated in *C. violaceum* ATCC6357, *A. hydrophila* IOC/FDA110-36 and *S. marcescens* UFOP-001. The strains were cultured at 28°C and 30°C in Luria-Bertani (LB) broth, according to Ravn *et al.* (2001).

Quorum sensing inhibition in C. violaceum

Agar diffusion assay was performed according to Oliveira *et al*. (2016). The test was performed with *C. violaceum* ATCC6357 at 30°C. To each well, 20 µl of phenolic extract in different concentrations were added. Distilled sterilized water and kanamycin (100 µg/ml - Sigma Aldrich) were used as controls. The quorum sensing inhibitory activity in *C. violaceum* was verified by the formation of a turbid halo, indicated by bacterial growth, with no pigment production around the well on a purple background on the plate.

Quantification of violacein production in C. violaceum

The assay was performed as described by Oliveira *et al.* (2016). The percentage inhibition of violacein production was calculated by using the formula:

% inhibition of violacein production =((a-b)/a)x 100

Where "a" is the OD of control at 585 nm e "b" the OD of treatment at 585 nm.

Swarming motility assay

The swarming motility assay was performed as described by Huber *et al.* (2003) with *A. hydrophila* and *S. marcescens.*

Biofilm formation in C. violaceum, S. marcescens *and* A. hydrophila

This experiment was performed according to the protocol described by Conway *et al.* (2002) in which the biofilms were stained with crystal violet and read on microtiter plates.

Statistical analysis

Table 1. Centesimal composition (g/100g fresh pulp) and trace element content of acerola pulp (mg/g fresh pulp)

	Acerola
Protein	0.94
Lipids	0.01
Ash	0.13
Moisture	95.63
Carbohydrates	3.29*
Fibres	2.11
Soluble solutes (ºBrix)	4.4
Potassium (K)	0.845
Calcium (Ca)	0.390
Iron (Fe)	0.186
Copper (Cu)	0.012
Zinc (Zn)	0.039
Chlorine (Cl)	NF

NF not found

* Value obtained by the difference (100%—protein, lipid, moisture and ash content)

Assays were performed in triplicate and the results expressed as average with standard deviation. The significance ($p<0.05$) was obtained by the ANOVA test and Tukey test by using the GraphPad Prism version 5.00 software for Windows (San Diego, Califórnia, USA).

Results and Discussion

Centesimal composition

The centesimal composition and mineral content of acerola are shown in Table 1.

Vendramini and Trugo (2004) evaluated the centesimal composition of acerola at different stages of maturation and found protein and moisture contents similar to the results described in the present study. However, the ash content was higher and the amount of soluble solids was lower. Although most of the data has shown similarities to the those reported in the literature, slight differences in the results are influenced by factors such as harvest time, maturation, variety, climate and soil conditions, exposure time to the sun, the location of fruit on the plant and post-harvest handling (Amira *et al*., 2011). The the high proportion of fibers encountered among the carbohydrates in this study is also noteworthy.

Trace elements

Table 1 shows the results for the trace elements found in the pulp of acerola.

The element potassium was present in greatest proportion, followed by calcium, iron, zinc and copper, respectively. No other assays determining the quantities of metals by TXRF in acerola were encountered in the literature. Brunini *et al.* (2004) evaluated the metal content in acerola from different regions and found the following values: 0,9 to 2,9 mg/g of Ca; 0,23 to 0,46 mg/g of Fe; 0,009 to 0,029 mg/g of Cu and 0,019 top 0,089 mg/g of Zn; amounts that approximate those found in this study. On the other hand, with respect to the quantity of K, Brunini *et al.* (2004) found between 13,9 to 22,9 mg/g, which is significantly higher than our results. Acerola may be considered a good source of iron; however, the contents of other minerals are very low and contributing little to attain the recommended daily quota. The study from Brunini *et al*. (2004) confirms that regionality can strongly dictate the composition of the fruit, and explains the large variations in the mineral content from different regions.

Phenolic composition

The content of phenolic compounds found in the phenolic extract of acerola was 5848.74 ± 4.18 mg GAE/L. This is equivalent to 224.95 mg of GAE per 100 g of fresh pulp. Higher values obtained from the aqueous extract of acerola were reported by Vieira *et al.* (2009) (835.25 ± 32.44 mg GAE/100g of pulp) and Vissotto *et al.* (2013), respectively (658 ± 179 mg GAE/100g acerola pulp).

In general, large variations in the content of phytochemicals between the fruit and its value added products (frozen pulp, juice) is associated to intrinsic (specie, cultivar and maturity) and extrinsic (production, storage, transport) characteristics as well as different extraction methods or solvents used for phenolic compounds extraction. However, considering high levels of ascorbic acid in acerola (506 ± 54 mg GAE/100g pulp), as reported by Vissotto et al. (2013), differences in total phenolic content (TPC) of this fruit may be likely related to the lack of specificity of Folin- Ciocalteu method for predicting phenolic compounds content since non-phenolic reducing compounds including ascorbic acid are detected by Folin- Ciocalteu reagent.

Various phenolic compounds from acerola have been identified by chromatographic and spectroscopic techniques, including anthocyanins (cyaniding-3-α-O-rhamnoside, pelargoidin-3-α-O-rhamnoside,malvidin-3,5-diglucoside), flavan-3-ols (epigallocatechin gallate, epicatechin), rutin (quercetin-3-O-rutinoside), clorogenic acid and quercitrin (quercitrin-3-α-O-rhamnoside (Hanamura *et al*., 2005; Mezadri *et al*., 2008; Santini and Huyke, 1956).

Antioxidant activity (AA)

In the present study, the antioxidant activity of the phenolic extract of acerola determined by the DPPH free radical method was 112.02 µmol trolox/ g fresh fruit, a higher value than that (53.2 µmol trolox/ g fresh matter) found by Kuskoski *et al.* (2006), using the same method. This large variation probably is not associated to a lower TPC from the sample analyzed (580mg GAE/100g frozen pulp of acerola) as compared to ours (224.95 mg GAE/100 g of fresh pulp) but most likely is due to the presence of ascorbic acid in the extract that was evaluated by Kuskoski *et al*. (2006). Additionally, according to Lima *et al.* (2003), some antioxidant phenolic compounds present in acerola, particularly anthocyanins, degrade during frozen storage, underestimating TPC as compared to fresh fruit. Rufino *et al.* (2010) studied 18 non-traditional Brazilian tropical fruits and found camu-camu (1176 mg GAE/100 g fresh fruit) and acerola (1063 mg GAE/100 g fresh fruit) to be the richest in phenolic compounds. Amongst the fruits tested, the greatest AA were found for puçá-preto (EC50 = 414 g/g DPPH), camu-camu (EC50 = 478 g/g DPPH) and acerola (EC50 = 670 g/g DPPH), as determined by DPPH method. The study highlighted an association between AA and TPC from fruits and, in the case of acerola and camu-camu, between AA and vitamin C contents, probably related to the lack of specificity of Folin-Ciocalteu method for quantification of phenolic compounds (Rufino *et al.* 2010; Vissotto *et al.*, 2013). Therefore, differences in AA values reported in the literature for a specific fruit are related to large variations in the content of reducing compounds extracted from fruit or from fruit pulp, which depends on extraction method or solvents used for extraction of these compounds, besides intrinsic (maturity, cultivar) and extrinsic factors (storage, processing conditions) (Lima *et al*., 2005). These differences make direct comparisons between antioxidant activity of different fruits very difficult and it is also a problem for ranking their antioxidant potential.

Even though many studies show high correlation among antioxidant activity and content of phytochemicals, antioxidant capacity values also vary according to the selected method because method specificities differ, including the chemistry underlying the assays, wavelengths that are used to monitor the reaction as well as the types of molecules

Table 2. Minimal inhibitory concentration of phenolic extract of acerola pulp expressed as mg of GAE/L for the respective extract dilution

Bacteria	Extract concentration in mg GAE/L	Equivalent extract dilution
L. monocytogenes	1462.18	1:4
E. coli	1462.18	1:4
S. aureus	487.39	1:12
P. aeroginosas	731.00	1:8
Salmonella spp.	1462.18	1:4
A. hydrophila	1462.18	1:4
B. cereus	1462.18	1:4
H. alvei	1462.18	1:4
P. fluorescens	1462.18	1:4
C. violaceum	487.39	1:12
S. marcescens	974.79	1:6

detected (Prior *et al.*,2005; Thaipong *et al.,* 2006).

In our study, antioxidant activity of acerola, determined by the $ABTS^{+}$ radical method, was 127.18 µmol trolox/g fresh fruit. In the work conducted by Rufino *et al.* (2010), AA of fruits, also evaluated by the same method, ranged from 6.3 to 153 µmol trolox/g fresh matter and were organized in order of increasing antioxidant capacity as: umbu < yellow mombin < carnauba < cashew apple < mangaba < açaí < uvaia < java plum < gurguri < jabuticaba < puçá-coroa-de-frade < murta < jussara < acerola < puçá-preto < camu-camu. In that study, acerola presented a lower value (96.6 µmol trolox/g fresh matter) as compared to our finding, which suggests that phenolic compounds, which were concentrated by solid phase extraction in our study, may present a higher contribution to the antioxidant activity of the fruit as compared to non-phenolic reducing compounds (ascorbic acid), which were not removed by Rufino *et al*. (2010). Therefore, antioxidant activity from acerola was expressive as compared to other fruits and has a contribution from both phenolic and non-phenolic compounds.

Antimicrobial activity

In the microbial inhibition test by the plate diffusion assay, no bacterial sensitivity to the acerola phenolic extract was observed, even in the higher concentrations that were tested. This may be related to the characteristics of the method which may not be compatible with the compounds present in the extract, e.g., charge and molecular weight characteristics of the molecules that could hamper their diffusion in a solid medium. Another possibility would be related to the phenolic composition of the extract which has no strong inhibitory effect against the evaluated bacteria. In fact, Meléndez and Capriles (2006) evaluated the antimicrobial activity of 172 species of plants through the plate diffusion assay and found no sensitivity of cultures of *S. aureus* and *E. coli* to many extracts, including that prepared from acerola *(M. ermaginata)*, indicating that this fruit presents low potential for inhibiting bacterial growth.

Table 2 presents the MICs for the phenolic extract of acerola against different types of bacteria. Despite the negative results in the plate diffusion assay, all the tested bacteria presented some sensitivity to the phenolic extract of acerola (Table 2), even though the concentrations were relatively high as compared to the concentrated phenolic extract (dilutions ranging from 1:4 to 1:16). In a similar fashion, Paz *et al.* (2015) observed sensitivity of *P. aeruginosa*, *L. monocytogenes, E. coli* and *Salmonella* spp. to an extract of acerola. However in their study, the extract was prepared with a mixture of ethanol and water with no purification step performed for phenolic compounds. In contrast, Delva and Goorich-Schneider, (2013) found inhibition by the flavonoid extract of acerola to *S. aureus*. Motohashi *et al*. (2004) also found similar results in ethyl-benzene extracts, finding antimicrobial activity against *S. epidermidis*. We have recently shown that *Rubus rosaefolius* phenolic extract prepared in a similar fashion to the present study also presented antimicrobial activity (Oliveira *et al.*, 2016), indicating that phenolic compounds are indeed responsible for the observed inhibition.

Differences related to MIC determinations and other antimicrobial tests can be attributed to many factors including the strains of microorganisms evaluated, origin of the plant, time of harvest as well as the method used for extract preparation (Ostrosky

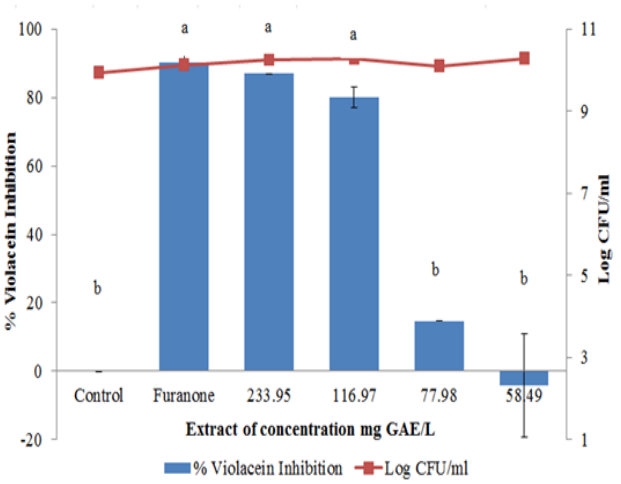

Figure 1. Inhibition of violacein production by the phenolic extract of acerola at different concentrations and evaluation of microbial growth (Log CFU/mL) after 24 h incubation in the presence of the extract. The control consisted of LB medium added of 200 µl of sterile distilled water. Averages followed by the same letters do not differ statistically ($p<0.05$)

et al., 2008). Another important factor is the diversity of compounds present in the plant since it is unlikely that a single compound would be responsible for the antimicrobial activity. In fact, synergy among different compounds has been shown (Shami *et al.*, 2013).

Quorum sensing inhibition in C. violaceum *(plate diffusion assay)*

No inhibition was observed in this experiment, which would be perceived by the formation of a cloudy halo around the well, on a purple background, resulting from the inhibition of the production of the violacein pigment. Despite the fact that this test indicated no interference with quorum sensing system of *C. violaceum*, it is not a definitive result since it is possible that the methodology is not compatible with the molecules present in the extract. Unlike the extracts, the well containing kanamycin showed a translucent halo, indicating inhibitory effect on bacterial growth by the antibiotic.

The lack of inhibition by the phenolic extract from acerola contrasts with previous studies using similar extracts obtained from plants which have shown inhibition in this assay as observed with extracts from fruits of *R. rosaefolius* (Oliveira *et al.*, 2016) and *Eugenia brasiliensis* (Rodrigues *et al.*, 2016), *Rosa rugosa* tea (Zhang *et al.*, 2014), and other fruits and spices (Musthafa et al., 2010).

Quantification of violacein production in the presence of R. rosaefolius *phenolic extracts*

Despite the lack of response in the plate diffusion assay, the phenolic extract of acerola at three different concentrations displayed significant inhibition on the production of violacein when compared to the control ($p<0.05$) (Figure 1). Additionally, the CFU/ml counts in the presence of the extract in different concentrations did not show any significant differences to the control indicating that at those concentrations, the extract preferentially exhibited quorum sensing inhibiting properties (Figure 1).

Inhibition of violacein production was observed up until 77.98 mg GAE/L of extract. The higher concentrations inhibited violacein production with values approaching those of furanone which is a well-known quorum sensing inhibitor (Galloway *et al.*, 2011). The results show for the first time the inhibitory potential of acerola against the quorum sensing system of *C. violaceum*. In general, studies on fruits with inhibitory activity of quorum sensing are rarely found. Acerola, which until then had not tested positive for inhibition of violacein production by the diffusion method, has presented itself as a potential inhibitor of quorum sensing.

These findings are in agreement with studies carried out by other researchers who found inhibitory activity of the quorum-sensing system for extracts and natural compounds obtained from various plants. Chenia (2013) evaluated the inhibition of violacein production by kigelia fruit extracts *(Kigelia africana)* dissolved in hexane, dichloromethane, ethyl acetate and methanol and reported satisfactory results in which >90% inhibition was observed. Olivero-Verbel *et al.* (2014) studied the inhibitory capacity of essential oils extracted from *Lippia alba* on quorum sensing, and found inhibition of violacein production. We have also found that phenolic extract obtained from *R. rosaefolius* strongly inhibited violacein production (Oliveira *et al.*, 2016).

Effect of acerola (Malphigia emarginata) *phenolic extract on the swarming motility of* S. marcescens *and* A. hydrophila

The phenolic extract of acerola did not inhibit the swarming motility of *S. marcescens*. It also did not inhibit prodigiosin pigment production as previously observed by us with the phenolic extract from *R. rosaefolius* (Oliveira *et al.*, 2016). In contrast to the result obtained with *S. marcescens*, the acerola extract was capable of inhibiting swarming motility of *A. hydrophila* IOC/FDA110-36 (Figure 2).

A study by Borges *et al.* (2012) revealed inhibition capacity of two types of phenolic compounds, ferulic

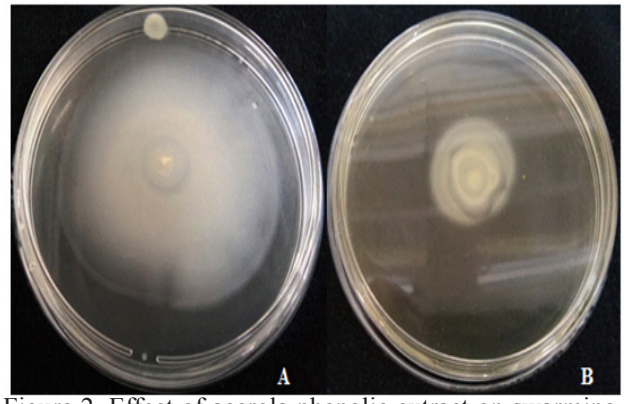

Figure 2. Effect of acerola phenolic extract on swarming motility of *A. hydrophila* IOC/FDA110-36. A) Control, LB agar B) LB agar added of phenolic extract of acerola in the concentration of 233.94 mg GAE/L

acid and gallic acid, against motility of *S. aureus, P. aeroginosas, L. monocytogenes* and *E. coli*. Their results demonstrated a strong potential of these phenolic compounds for inhibiting swarming motility. The secondary metabolite Eugenol isolated from the cumin extract was investigated by Packiavathy *et al.* (2012) and demonstrated inhibition against swarming of *Proteus mirabilis, P. aeruginosa* PAO1 and *S. marcescens*. Our results and those from other studies indicate that phenolic compounds at sub-inhibitory concentrations can affect bacterial motility. The mechanism of inhibition is still not understood, but it is likely related to quorum sensing regulation of swarming (Daniels *et al*., 2004).

Biofilm formation on C. violaceum *ATCC6357,* A. hidrophila *IOC/FDA110-36,* C. violaceum *ATCC6357 and* S. marcescens

The effect of the phenolic extract of acerola at different concentrations on biofilm formation for different strains is shown in Figure 3. All concentrations tested displayed inhibition of biofilm formation, for all species, when compared to control ($p < 0.05$). It has already been well reported that N-Acyl homoserine lactones (AHLs) play an essential role in the formation of biofilms (Geske *et al.*, 2005; Tarver, 2009; Lazar, 2011). It is possible that the inhibitory potential of acerola phenolic extract is due to the synthesis of AHLs or to their detection by the receptor protein which is a LuxR homologue (Pinto *et al.*, 2012; Kalia, 2013). Another factor which may be responsible for the reported results could be destabilization of the cell membrane caused by the phenolic compounds present in sub-MIC concentrations (Burt *et al.*, 2014). Furthermore, biofilm formation is a phenotype that depends on multiple factors, including quorum sensing signaling (Ponce-Rossi *et al.,* 2016). Additional studies are required in order to evaluate the mechanisms of

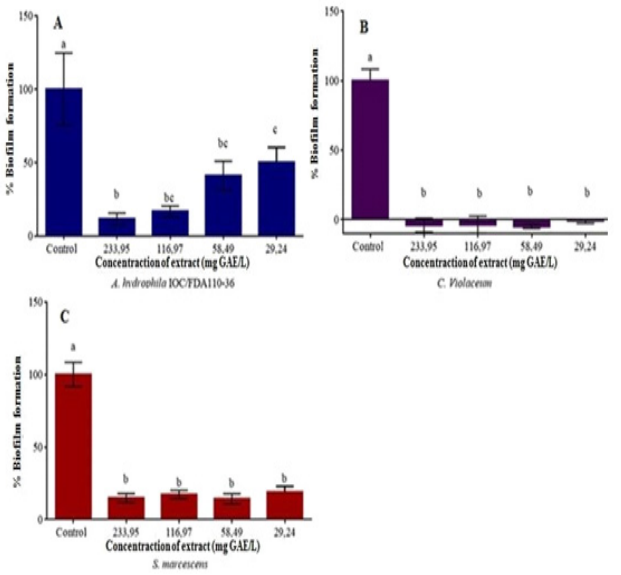

Figure 3. Effect of the acerola phenolic extract on biofilm formation of different bacteria. A) *A. hydrophila* IOC/FDA110-36; B) *C. violaceum* ATCC6357; C) *S. marcescens* UFOP-001. Averages followed by the same letter do not differ statistically ($p \geq 0.05$)

biofilm inhibition observed in this and many other studies. However, it is likely that quorum sensing inhibition played an essential role in blocking biofilm formation in this study.

Similar works found inhibition of biofilm formation by natural compounds. The methanol extract of *Capparis spinosa* (capers) inhibited formation of biofilm biomass in *E. coli, P. aeruginosa* PAO1, *P. mirabilis* and *S. marcescens* (Abraham *et al*., 2011). It has been shown that, naringenin, kaempferol, quercetin, petunidin, malvidin, cyanidin, apigenin and catechin are examples of phenolic compounds that have displayed anti-quorum sensing actvity (Vandeputte *et al*., 2010; Vikram *et al.*, 2010; Gopu *et al*., 2015). An exudate containing cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, peonidin-3-galactoside, 3-peonidin-glucoside, peonidin-3-arabinoside and proanthocyanidins was tested against phenotypes of various *Vibrio harveyi* strains, and inhibited the bioluminescence emission by up to 57% (Feldman *et al*., 2009). Most of these compounds are common to extracts of acerola and it can therefore be inferred that the activity of the extract is due these compounds (Vendramini and Trugo, 2004; Hanamura *et al*., 2005; Mezadri *et al*., 2008). However, the exact nature of the phenolic compounds from the present study that were responsible for the observed phenotypes remains to be determined. Another important question that remains is the contribution of each individual phenolic compound on any observed phenotype and

how different phenolic compounds can be used to inhibit not only quorum sensing but also microbial growth.

Conclusion

Phenolic compounds rich acerola extract showed antimicrobial and antioxidant activities and, at sub-inhibitory concentrations, inhibited quorum sensing regulated phenotypes, a bioactive property highlighted for the first time for these compounds from acerola. Besides the expressive levels of antioxidants, including polyphenolics and ascorbic acid, acerola contributes to providing macro and micronutrients, as revealed in this study. Future studies aimed at identifying the direct contribution of individual phenolics at the molecular events involved in quorum sensing inhibition are needed in order to provide the food industry with an additional option in the control of quality and safety of food products.

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