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Antioxidant, antimicrobial and anti-quorum sensing activities of *Rubus rosaefolius* phenolic extract

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ABSTRACT

Phenolic compounds are plant secondary metabolites that present many biological effects including antioxidant and antimicrobial activities. Studies have shown that these compounds can also inhibit quorum sensing bacterial communication. The aim of this study was to characterize the centesimal composition, mineral and phenolic content, to determine the antioxidant and antimicrobial effect as well as the inhibition of quorum sensing by the phenolic extract obtained from wild strawberry (*Rubus rosaefolius*). Centesimal composition and minerals were in the range expected for fruits of the *Rubus* generum, even though Fe and Zn presented higher levels. The phenolic content was 5902.89 mg GAE/L, also approaching the levels found for fruits of *Rubus* sp. The antioxidant activity determined through the ABTS method was 162.4 ± 5.6 and 120.8 ± 1.5 μM Trolox/g of fruit in the DPPH assay, indicating an elevated potential for ABTS and medium potential for DPPH method. The phenolic extract was able to inhibit all the evaluated bacteria presenting MICs in the range of 491.90–1475.74 mg GAE/L. In sub-MIC concentrations, the phenolic extract inhibited all the phenotypes typically regulated by quorum sensing in bacteria, including violacein production, swarming motility and biofilm formation. The phenolic extract of *R. rosaefolius* presented antioxidant, antimicrobial and anti-quorum sensing activities which are in agreement with previous studies linking phenolic compounds to these properties.

1. Introduction

The health benefits of fruits and vegetables are well established and they have been shown to provide essential components for the basic functions of many living organisms (Sreeramulu and Raghunath, 2010). Among these components are vitamins and phenolic compounds. Those are secondary metabolites implicated in the defense against phytopathogens, radiation and antioxidant activities observed in fruits and vegetables (Tiveron et al., 2012; Roginsky and Lissi, 2005). Due to the presence of antioxidant compounds, fruits and vegetables exhibit protective functions, reducing the risks of non-transmissible chronic diseases such as cancer and cardiovascular diseases (Jonville et al., 2011; Faller and Fialho, 2010; Scalbert et al., 2005).

The phenolic compounds, besides having antioxidant capacity, are also capable of inhibiting the growth of microorganisms according to their concentration (Martins et al., 2015; Pinho et al., 2014). These characteristics justify the growing interest of the food industry for natural products containing these compounds.

It is commonly believed that food deterioration, at least in part, is regulated by a mechanism of cell-to-cell communication known as quorum sensing (Bai and Rai, 2011). This system is important for defining bacterial activity since it regulates important cellular functions such as sporulation, biofilm formation, bacteriocin production, conjugation, competence, virulence gene expression, pigment and bioluminescence production, among others (Hammer and Bassler, 2003; Smith et al., 2004). By using quorum sensing inhibitors, mainly of natural origin, virulence factors could also be inhibited in pathogenic microorganisms besides affecting food deterioration phenotypes (Kalia, 2013).

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The species *Rubus rosaefolius* is original from the Himalaia, Western Asia and Australia, belonging to the group of raspberry and blackberry (Souza et al., 1995). The round thin-skinned fruit from *R. rosaefolius*, popularly known as "amora-do-mato" (wild strawberry) in Brazil has agreeable colors, which varies from purple to dark red as ripe, appreciable aroma and flavor. The fruit is rich in anthocyanins, mainly perlagonidins and cyanidins, which have been related to its biological and therapeutic properties (Bowen-Forbes et al., 2010; Campbell et al., 2015; Santoni et al., 2015). Even though studies concerning the phenolic content, antioxidant, antimicrobial and anti-quorum sensing activities are scarce, results obtained with raspberry and blackberry, which belong to the genus *Rubus*, provide an indication for these activities. Therefore, the aim of this study was to evaluate the aforementioned activities of the phenolic extract obtained from the fruits of *R. rosaefolius*.

2. Material and methods

2.1. Phenolic extract preparation

Fruits of *R. rosaefolius* 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., 28763).

Fruits were washed and sanitized with sodium hypochloride at 50 mg/L for 15 min. The seeds were manually removed and the pulp was homogenized and kept frozen at −20 °C until use. Phenolic compounds were extracted as previously described (Bertoldi, 2009). 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 of the extracts was determined by the Folin–Ciocalteu assay (Shahidi and Naczk, 1995) and expressed as mg of galic acid equivalent per L (mg GAE/L).

2.2. Antioxidant activity (ABTS and DPPH)

The antioxidant activity of the phenolic extract from *R. rosaefolius* was determined by using the ABTS+ radical, according to the methodology described by Rufino et al. (2007). For the standardization of the results, trolox was used as an external standard and the results expressed as antioxidant activity in μM Trolox equivalent per gram of fresh pulp.

The antioxidant capacity was also determined according to the DPPH method described by Brand-Williams et al. (1995). Trolox was also used as an external standard in this assay and the results expressed as μM Trolox equivalent per gram of fresh pulp.

2.3. Mineral content determination

The content of potassium, calcium, iron, copper, zinc and chloride of the extract was analyzed by Total Reflection X-ray Fluorescence (TXRF). 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 discsand dried. The results were obtained by integration of the signal on a 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 fruit.

2.4. Centesimal composition

This experiment was performed with the pulp of the fruit by quantifying the content of moisture, ash, total lipids, total soluble solutes, proteins, carbohydrates and fibers according to the AOAC (1995), AOAC (2003), AOAC (2005) methodologies and IAL Institute (2005). The carbohydrate content was calculated by subtracting the protein, total lipids, moisture and ash from 100%.

2.5. Antimicrobial activity

The antimicrobial activity of the phenolic extract, in different concentrations, 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 fluorescens* 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 described by Wiegand et al. (2008). All quorum sensing assays were performed in sub-MIC concentrations with no measurable interference on the bacterial growth, as determined by the enumeration of colony forming units per mL (CFU/mL) in LB agar.

2.6. 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 and 30 °C in Luria-Bertani (LB) broth, according to Pinto et al. (2007).

2.6.1. Quorum sensing inhibition in C. violaceum

Initially, an agar diffusion assay was performed according to the method described by Tan et al. (2012), with modifications. 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.

2.6.2. Quantification of violacein production in C. violaceum

The assay was performed as described by Tan et al. (2012), with modifications. *C. violaceum* was cultivated at 30 °C and the assay was performed in test tubes. The positive control for quorum sensing inhibition used was ((Z-)-4-Bromo-5(bromomethylene-2(5h)-furanone)—C30 (Sigma–Aldrich), at concentration of 39.4 μM. The percentage inhibition of violacein production was calculated by using the following formula:

$$\%\ \text{inhibition of violacein production} = (\text{OD of control at } 585\ \text{nm} - \text{OD of treatment at } 585\ \text{nm} / \text{OD of control at } 585\ \text{nm}) \times 100$$

Table 1
Centesimal composition of *R. rosaefolius* pulp (g/100 g of pulp).

Proteins	0.66 ± 0.001
Lipids	0.02 ± 0.01
Ash	0.57 ± 0.12
Moisture	80.22 ± 0.15
Carbohydrates	18.53 ± 0.07[a]
Fibers	4.97 ± 1.08
Total soluble solutes (°Brix)	10.4 ± 0.5

Numbers represent the mean values of triplicates ± standard deviation.
[a] Value obtained by the difference (100%—protein, lipid, moisture and ash content).

2.6.3. Swarming motility assay

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

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

This experiment was performed according to the protocol described by Conway et al. (2002).

2.7. Statistical analysis

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).

3. Results and discussion

3.1. Centesimal composition

The centesimal composition of the *R. rosaefolius* pulp is shown on Table 1.

R. rosaefolius pulp presented a high content of moisture, carbohydrates and total soluble solutes, while presenting a low protein, ash and lipid content. These are common characteristics of most commercially consumed fruits such as apples, oranges, mangoes and others (Silveira et al., 2011). A study performed by Hirsch et al. (2012) with three cultivars of *Rubus* ssp. showed very similar results for most of the parameters evaluated in the present study; however, their lipid content was considerably higher (0.15 g/100 g). We believe that this difference could be accounted for by the different methodologies since Hirsch et al. used cold extraction with reagents that present different properties from those described by AOAC (1995). Guedes et al. (2014) evaluated the chemical composition of *R. rosaefolius* and found a moisture content similar to the present study.

3.2. Trace elements

Total reflection X-ray fluorescence spectroscopy (TXRF) is a non-destructive, rapid and multielemental method that provides all the results in a single measurement of the sample. For this reason, TXRF was employed to quantify trace elements in the phenolic extract. Trace element quantities found in the pulp of *R. rosaefolius* were determined for potassium, calcium, iron, copper, zinc and chlorine (Table 2).

The most abundant element present was potassium, followed by chloride, iron and zinc. These results are of great nutritional importance, since according to the recommendations from the World Health Organization (2003), the daily intake of iron and zinc would be 45 mg and 11 mg, respectively. Thus, the consumption of *R. rosaefolius* fruits could contribute to reach the recommended nutrient intake.

Table 2
Spectroscopy results determined by Total Reflection X-ray Fluorescence TXRF of trace elements found in *R. rosaefolius*, in mg/g in the pulp of fruit.

Metals	Fruit pulp (mg/g)
Potassium (K)	0.965
Calcium (Ca)	0.185
Iron (Fe)	0.155
Copper (Cu)	NF
Zinc (Zn)	0.039
Chlorine (Cl)	0.563

NF—not found.

To the best of our knowledge there are no reports describing the use of TXRF to evaluate the trace element composition for this fruit. However, Kinupp and Barros (2008) evaluated the macro and micronutrients by inductively coupled plasma optical emission spectrometry (ICP-OES), for 69 native species from the south of Brazil, including *R. rosaefolius.* The authors found higher contents for calcium and potassium than the present study. For iron, the content was lower (0.0053 mg/g) in their study and for zinc, the values were similar. These differences could be due to many factors including genetic properties of the species, climate conditions, soil composition and the degree of maturation upon harvest (Martínez-Ballesta et al., 2010).

Despite the importance of consuming minerals for a healthy human diet, there is little information on the presence of these constituents in fruits, even for those highly consumed (Martínez-Ballesta et al., 2010; Kinupp and Barros, 2008). The trace elements are widely distributed in nature and are important for the healthy functioning of the human body, participating in enzymatic reactions, bone formation, coagulation, cicatrization and oxygen transport (Martínez-Ballesta et al., 2010; Kinupp and Barros, 2008). Besides, the organism antioxidant defense and the control of free radical production are influenced by many minerals (Barbosa et al., 2010; Koury et al., 2007; Mafra et al., 1999). Thus, the presence of these micronutrients in fruits in significant levels is of great importance for quenching radicals and contributing to the protective action of our diet.

3.3. Phenolic composition

The phenolic content found in the extract was 5902.89 ± 7.42 mg GAE/L (the phenolic extract had an equivalent of 3300 g of pulp/L). This amount is equal to 177.26 mg GAE/100 g of pulp which falls within the range found for other fruits from the *Rubus* generum (Zielinski et al., 2015; Ferreira et al., 2010).

Rufino et al. (2010) suggested a classification scheme where the phenolic content of fruits could be grouped as low if the concentration was <100 mg GAE/100 g of fruit, medium for the range 100–500 mg GAE/100 g and high for >500 mg GAE/100 g. Based on this classification, *R. rosaefolius* would be classified as having a medium content of phenolic compounds. It is important to note that fruits from the same species could be classified in different categories since the phenolic content can vary according to maturation, cultivation practices, location, harvest conditions and storage (Soares, 2002). The methods used to extract the phenolic compounds can also interfere in the quantification since some other reducing compounds from non-phenolic origin can also be extracted. Contrasting with many published studies in which the extracts were obtained by maceration with solvents, followed by filtration (Bowen-Forbes et al., 2010; Ferreira et al., 2010; Zielinski et al., 2015; Sariburun et al., 2010), in the current study several interfering components were eliminated by the solid phase extraction, including reducing sugars, soluble proteins and acids which

Table 3
Antioxidant activity of phenolic extracts from the pulp of *R. rosaefolius* determined by the ABTS and DPPH methods.

Antioxidant activity	
ABTS (μM Trolox/g of fruit)	DPPH (μM Trolox/g of fruit)
162.4 ± 5.6[a]	120.8 ± 1.5[a]

[a] Values represent the mean of triplicates ± standard deviation.

minimize the possibility for overestimation of the phenolic content and the antioxidant activity of fruits (Rijke et al., 2006; Dias et al., 2010).

There is no recommended daily intake for the consumption of phenolic compounds, despite its important role in the human diet. It is estimated that the average intake by Brazilians is 48,3 mg/day (Faller and Fialho, 2010). Since fruits and vegetables are the major sources of these compounds, and based on the fact that the WHO recommends consumption of 400 g of fruits and vegetables daily (about 5 portions), the Brazilian population does not consume adequate amounts (Brasil, 2010).

3.4. Antioxidant activity

Table 3 presents the results for the antioxidant activity of the phenolic extract of *R. rosaefolius*. No studies reporting the antioxidant capacity of *R. rosaefolius* were found. However, there have been reports on other fruits from the generum *Rubus*. For instance, Sariburun et al. (2010) evaluated the methanolic extract of raspberry fruits (*Rubus idaeus* L.) and blackberry fruits (*Rubus fruticosus* L.) and found values ranging from 64.14 to 153.70 μM Trolox/g of fruit for the DPPH assay and from 64.36 ± 1 to 117.07 ± 0.94 μM Trolox/g of fruit for the ABTS method. On the other hand, Souza et al. (2014) found that the antioxidant activity of *R. idaeus* fruits were 6.27 ± 0.02 μM Trolox/g by the ABTS methods, while Çekiç and Özgen (2010) found values ranging from 8.9 μM Trolox/g to 21.5 μM Trolox/g in the same assay. Our study revealed that *R. rosaefolius* presented a good antioxidant activity as revealed by both methods and the comparison with the literature.

In the present work, a higher antioxidant activity was observed with the ABTS assay, which could be related to the characteristics of this method since ABTS can be a better measure for hydrophilic and lipophilic compounds and those with high pigmentation (Floegel et al., 2011). It is worth mentioning that most of the values reported in the literature are from extracts dissolved in organic solvents, which differ from the current study that focused on the phenolic extracts which are likely to contain anthocyanins like cyanidin-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-(2g-glucosylrutinoside), cyanidin-3-sophoroside, cyanidin, peonidin, malvidin, pelargonidin, and delphinidin (Rijke et al., 2006; Bowen-Forbes et al., 2010; Guedes et al., 2014). Therefore, the results described for extracts in the literature are less specific and represent the antioxidant capacity of both phenolic and non-phenolic compounds.

Table 5
Minimal inhibitory concentration (MIC) of phenolic extract from *R. rosaefolius* (values expressed as mg of GAE/L of extract and equivalent extract dilution).

Bacteria	Extract concentration in mg GAE/L	Equivalent extract dilution
A. hydrophila	491.90	1:14
B. cereus	491.90	1:14
C. violaceum	491.90	1:14
E. coli	737.87	1:8
H. alvei	491.90	1:14
L. monocytogenes	1475.74	1:4
P. aeroginosas	491.90	1:12
P. fluorescens	491.90	1:14
S. aureus	421.63	1:14
S. marcescens	737.87	1:8
Salmonella spp.	737.87	1:8

3.5. Antimicrobial activity

The results for the plate diffusion assay with the phenolic extract obtained from *R. rosaefolius,* at different concentrations, are shown in Table 4.

As observed in Table 4, the phenolic extract inhibited all the evaluated bacteria, at least at two concentrations. The most sensitive bacterium was *P. fluorescens* which was inhibited in all tested concentrations and presented the greatest inhibition zone (14.2 ± 0.11 mm). As it is often difficult to compare studies with plant extracts, Alves et al. (2000) suggested a classification scheme where extracts with halos <9 mm were classified as inactive, from 9–12 mm, partially active, from 13–18 mm active, and >18 mm very active. If we follow this classification scheme, the present extracts were partially to active against the tested bacteria.

Once again, no studies were found for the antimicrobial activity of *R. rosaefolius* fruits. However, Zeidan et al. (2013) studied the antimicrobial activity of the ethanolic and methanolic extracts from leaves and fruits of *R. sanguineus,* which is a related species, against strains of pathogenic *E.coli*, *P. aeruginosa*, *S. aureus*, *B. cereus* and *Candida albicans,* by the plate diffusion assay. They found that all the organisms showed some degree of sensitivity to the leaves and the fruits (halos varied from 7 ± 0.5 mm to 22 ± 0.5 mm).

Complementing the inhibitory potential of the phenolic extract of *R. rosaefolius,* the results for the MIC are presented on Table 5.

Studies determining the MIC of any type of extract prepared with *R. rosaefolius* have not been reported. However, Mauro et al. (2002) evaluated the antimicrobial acitvity of the aqueous extract obtained from the root, stem and leaves of *R. sanguineus* which inhibited *S. aureus*, *P. aeruginosa, E. coli* and *C. albicans.* Azevedo,

Table 4
Growth inhibition by different concentrations of phenolic extract from *R. rosaefolius*, measured as inhibition zone around the wells (mm), by the plate diffusion assay.

Bacteria	Concentration (mg GAE/L)				
	5902.8	2951.4	1475.0	983.8	737.8
A. hydrophila	10.5 ± 0.10	10.2 ± 0.05	–	–	–
B. cereus	11.5 ± 0.10	9.8 ± 0.05	–	–	–
E. coli	11.0 ± 0.14	11.0 ± 0.02	–	–	–
H. alvei	13.2 ± 0.15	8.2 ± 0.05	5.5 ± 0.01	–	–
L. monocytogenes	11.0 ± 0.04	12.0 ± 0.02	–	–	–
P. aeroginosas	13.0 ± 0.16	12.5 ± 0.02	–	–	–
P. fluorescens	14.2 ± 0.11	11.8 ± 0.05	9.2 ± 0.05	9.2 ± 0.05	7.2 ± 0.05
S. aureus	12.0 ± 0.00	9.0 ± 0.15	–	–	–
Salmonella spp.	13.0 ± 0.04	12.0 ± 0.15	10.0 ± 0.05	–	–

Inhibition zones around the wells were subtracted from the diameter of each well and expressed in mm.

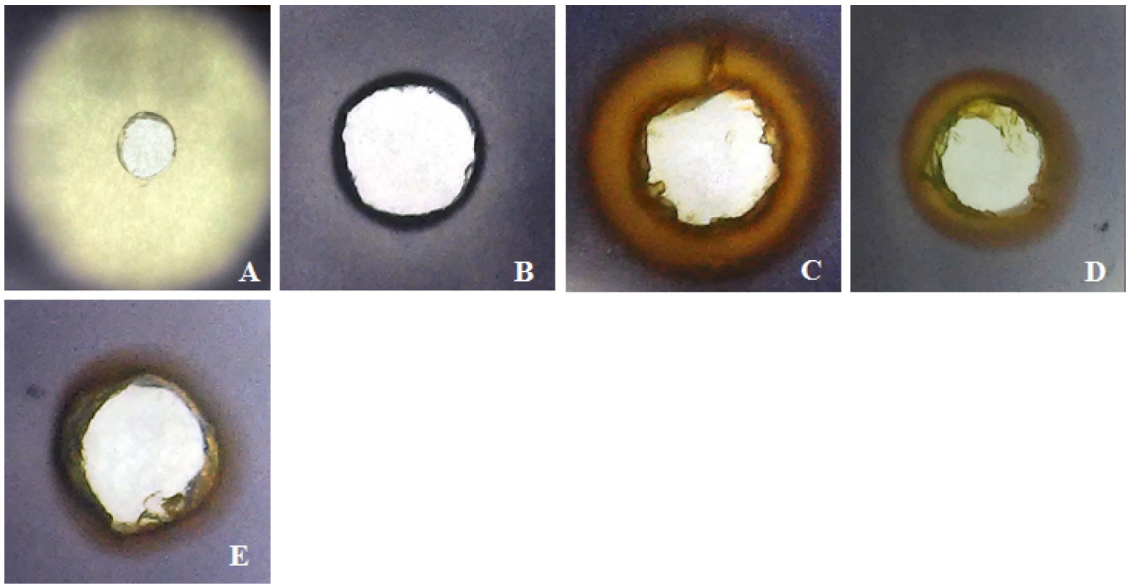

Fig. 1. Plate diffusion assay for the quorum sensing inhibition in *C. violaceum* by different concentrations of the phenolic extract from *R. rosaefolius*. (A) Kanamycin (100 μg/mL)—control for growth inhibition, (B) sterile distilled water—negative control, (C) 5902.8 mg GAE/L, (D) 2951.4 mg GAE/L, (E) 1475.7 mg GAE/L of phenolic extract. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

(2011) studied the antimicrobial activity of fruits of *R. fruticosus* finding inhibition against *S. enterica* serovar Enteritidis.

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

Fig. 1 presents the results for the quorum sensing inhibition assay in *C. violaceum* using different concentrations of the phenolic extract. The inhibition can be seen by the formation of a turbid halo, on a clear background around the well, on a purple plate, resulting from the inhibition of violacein production.

The phenolic extract inhibited the production of violacein, as can be observed in Fig. 1(C) which shows no color production, but bacterial growth can be observed by the turbidity of the halo. Contrasting with this result, the antibiotic Kanamycin inhibited bacterial growth, since a clear, transparent halo was formed around the well.

Zhang et al. (2014) studied phenolic compounds from *Rosa rugosa* extracts and also encountered colorless, but turbid halos indicating inhibition of quorum sensing. Other plant extracts have also shown similar results in this assay, as observed for garlic, geranium, lavender, rosemary and eucalyptus (Bodini et al., 2009; Del Monte et al., 2015; Khan et al., 2009; Szabó et al., 2010).

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

The phenolic extract was effective at inhibiting violacein production at all tested concentrations as shown in Fig. 2 ($p < 0.05$). The number of viable cells counted after 24 h of incubation did not differ between treatments and the control (absence of extract) indicating that there was inhibition only against violacein production (quorum sensing inhibition) and not against growth of the strain (Fig. 2).

The results highlight that violacein inhibition by the phenolic extracts, especially at 118.60 mg GAE/L, inhibited 88.6%, a value higher than the positive control for this experiment (furanone), which inhibited 68.6%. The extract at a concentration of 78.70 mg GAE/L did not differ statistically from furanone ($p > 0.05$), while at

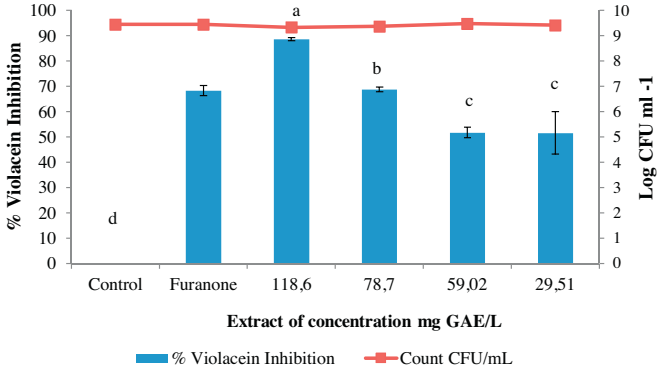

Fig. 2. Percentage inhibition of violacein production by the phenolic extract of *R. rosaefolius* at different concentrations and evaluation of microbial growth (Log CFU mL-1) after 24 h incubation in the presence of the extract. The control consisted of LB medium added of 200 μL of sterile distilled water and furanone C-30 (quorum sensing inhibition control) at 39.4 μM. Averages followed by the same letters do not differ statistically ($p < 0.05$).

the other concentrations, inhibition was observed, but at a level lower than from furanone.

No studies were found on the effect of *R. rosaefolius* fruits or any other parts of the plant on the production of violacein or on any other quorum sensing controlled phenotype. Likewise, studies showing the quorum sensing inhibitory effect of fruit extracts are scarce. However, studies have been performed with plants in general, including medicinal plants, with the aim of finding new antipathogenic and therapeutic compounds (Al-Hussaini and Mahasneh, 2009; Adonizio et al., 2006; Kalia, 2013; Del Monte et al., 2015). It is estimated that about 10% of the terrestrial flora presents some kind of activity that could be explored, but only 1% of this biodiversity has been evaluated (Al-Hussaini and Mahasneh, 2009).

3.8. *Effect of* R. rosaefolius *phenolic extract on the swarming motility of* S. marcescens *and* A. hydrophila

Swarming motility was evaluated in two important strains which are commonly present in refrigerated products (Pinto et al.,

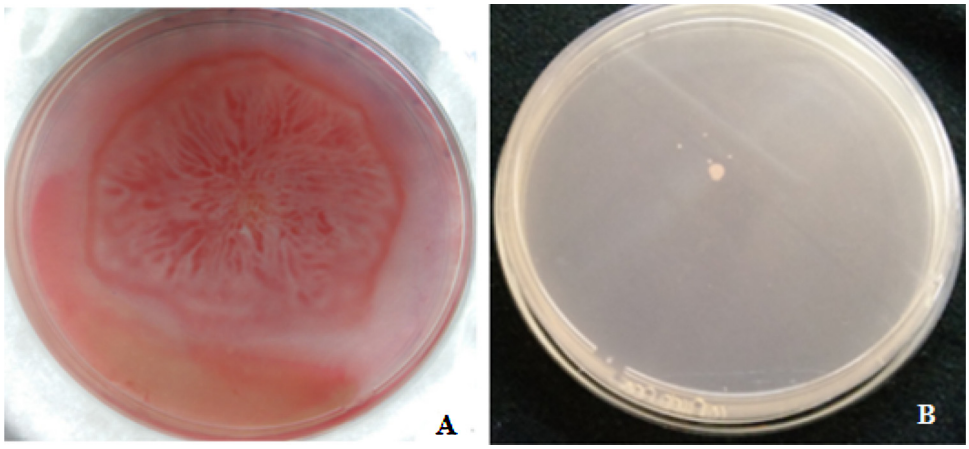

Fig. 3. Swarming motility against *S. marcescens*. (A) Control, *S. marcescens* in LB agar (B) LB agar added of phenolic extract of *R. rosaefolius* in the concentration of 236.11 mg GAE/L. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

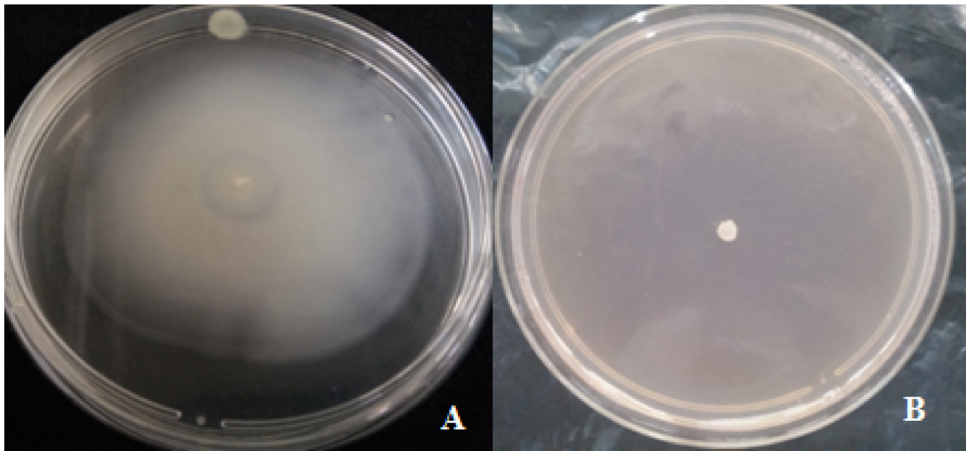

Fig. 4. Swarming motility against *A. hydrophila* IOC/FDA110-36. (A) Control, *A. hydrophila* in LB agar (B) LB agar added of phenolic extract of *R. rosaefolius* in the concentration of 236.11 mg GAE/L.

2007). The results presented in Figs. 3 and 4 show that the phenolic extract was able to inhibit swarming motility in both bacteria as well as prodigiosin production in *S. marcencens* (red pigment), both of which are regulated by *quorum sensing* (Packiavath et al., 2014)

This work is the first to provide results on the effect of *R. rosaefolius* phenolic extract on swarming motility in bacteria. Bakkiyaraj et al. (2012) have also evaluated the swarming motility inhibition against *S. marcescens* with bioactive compounds extracted from coral reefs. They observed that upon exposure to the compounds, there was inhibition on motility as well as on prodigiosin production, as observed in the present study. In another study, Packiavathy et al. (2012) found that eugenol, a secondary metabolite obtained from some plants, presented inhibitory activity against swarming motility on *Proteus mirabilis, P. aeruginosa* PAO1 and *S. marcescens.* The quorum sensing inhibition exhibited by the phenolic extract is likely to be related to their structural similarity with known autoinducer molecules (Bakkiyaraj et al., 2012; Packiavathy et al., 2012; Wang et al., 2006).

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

As observed in Fig. 5, all concentrations of the extract that were tested presented biofilm inhibitory capability against the tested strains, when compared to the control ($p < 0.05$), which was set to 100%. The AHL signaling molecules have already been reported as having an essential role on biofilm formation (Lazar, 2011; Tarver, 2009; Geske et al., 2005). As stated by Kalia (2013) the quorum sensing inhibitory effect observed in the present study could be related to the interference on the autoinducer molecule, either on its production or degradation or even by competing to the binding site on the quorum sensing receptor proteins. More studies are required in order to identify the mode of action of the phenolic compounds as well as to determine which of these compounds or how many of those actually present activity.

Natural compounds like penicillic acid, patulin, ajoene from garlic and *p*-coumaric acid have already been shown to interact with bacterial quorum sensing, sometimes stimulating or inhibiting the system (Lazar, 2011; Priha et al., 2014). Lately, berries have been studied for the presence of anti-quorum sensing compounds since they present a good concentration of phenolics (Priha et al., 2014). For instance, a study has shown that an exudate from cranberry containing many identified phenolic compounds was tested against strains of *Vibrio harveyi* inhibiting bioluminescence up to 57% (Feldman et al., 2009). Interestingly, these same phenolic compounds can be found in fruits of the generum *Rubus*, especially cyanidin-3-glucoside, which is commonly found in *R. rosaefolius* (Bowen-Forbes et al., 2010; Lee et al., 2012).

4. Conclusions

The phenolic extract from *R. rosaefolius* displayed great bioactive potential due to the presence of phenolic compounds that demonstrated antioxidant, antimicrobial and anti-quorum sens-

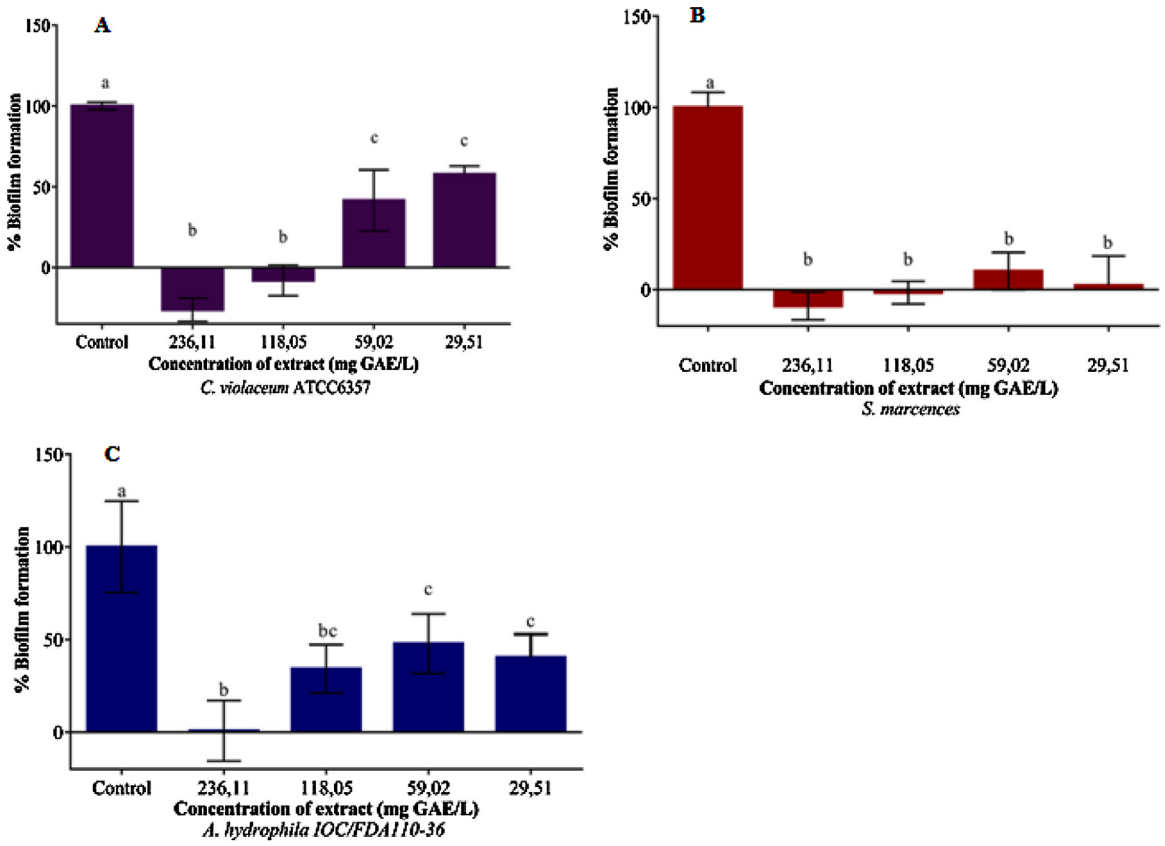

Fig. 5. Effect of the *R. rosaefolius* phenolic extract on biofilm formation against different bacteria. (A) *C. violaceum*; (B) *S. marcescens*; (C) *A. hydrophila*. Averages followed by the same letter do not differ statistically ($p \geq 0.05$).

ing activities. With respect to its mineral composition, iron and zinc have been found in the fruit and those can positively influence metabolic reactions related to the antioxidant action, which can further be improved by phenolic compounds. The consumption of this fruit is encouraged since it presents good antioxidant activity and trace elements which contribute to the reduction of the risk of non-transmissible chronic diseases. Berries like *R. rosaefolius* can be a good source of new bioactive compounds with quorum sensing inhibitory potential which could be further explored as a means to produce antivirulence drugs and new additives for the food industry.

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