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BIOACTIVITY EVALUATION OF *EUGENIA PYRIFORMIS, PLINIA CAULIFLORA*, AND *HELICONIA ROSTRATA* AGAINST OXACILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* GENOTYPES

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ABSTRACT

In this study, the anti-Oxacillin Resistant *Staphylococcus aureus* (ORSA) activity of extracts from *Eugenia pyriformis* (leaf and stem**)**, *Plinia cauliflora* (rind fruit, leaf, and stem**)**, and *Heliconia rostrata* (flower, rhizome, leaf, and stem**)** was evaluated. *In vitro* screening of antibacterial activity of the extracts (extraction with ethanol:water [7:3, v/v]) was performed *using both agar* diffusion and *broth microdilution* assays against sixty isolates of ORSA from dental clinic (genotyped by using Multilocus Enzyme Electrophoresis method) and against *S. aureus* reference strain (ATCC 6538). To evaluate the toxicity of the extracts, the MTT reduction assay was performed. The *growth* of ORSA genotypes and *S. aureus* reference strain were *inhibited* by extracts from *E. pyriformis* and *P. cauliflora*. Moreover, the rind fruit from *P. cauliflora* showed the lowest Minimum Inhibitory Concentration value (6.25 mg/ml) against ORSA. The extracts were most active against *S. aureus* reference strain than on ORSA (p<0.05). All extracts from *H. rostrata* were inactive. *P. cauliflora* (rind fruit), *E. pyriformis* (leaf), and *H. rostrata* (leaf and rhizome) showed toxicity on cells from *Aedes albopictus*, wherein cytotoxic concentration values ranged from 1.3 to 19.0 mg/ml, whereas all other extracts showed no cytotoxicity at used concentrations. Thus, a potential anti-ORSA was demonstrated for the rind fruit from *P. cauliflora*.

INTRODUCTION

Currently, oxacillin-resistant *Staphylococcus aureus* (ORSA) began to appear on community accordingly assuming international importance as a cause of both hospital-and community-associated infections (HAI/CAI*)*, with outcome in diseases which account by high mortality rates all over the world (Zetola *et al.*, 2005; Klein *et al*; 2007). Although in Brazil health surveillance systems fails to report data on mortality rates of infections related to *S. aureus*, the underestimated data indicate a considerable contribution of this bacteria as a cause of death in hospitals (Teixeira *et al.*, 1995; Dos Santos Soares *et al.*, 2000; Moreira *et al.*, 2008; Sousa-Junior *et al.*, 2009; Scribel *et al.*, 2011). In the United States of America, data from health surveillance systems point out that hospital-acquired infections caused by *S. aureus* are a leading cause of illness, and the mortality rate in patients with multidrug-resistant *S. aureus* infections exceeds the combined deaths caused by HIV/AIDS and Tuberculosis (Klein *et al.*, 2007; Moreira *et al.*, 2008; Scribel *et al.*, 2011; Brown, 2013). The alarming spread of ORSA strains relates to specific clones, where in Brazil multiresistant clone BEC represents up to 80 % of the isolates (Teixeira *et al.*, 1995; Dos Santos Soares *et al.*, 2000; Sousa-Junior *et al.*, 2009; Feil et al., 2010). These clones are determined by using molecular typing, which is used in taxonomic and epidemiological studies, having a high applicability in the analysis of genetic diversity, being relevant to assess the

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degree of relationship between genetic strains of ORSA and to identify clones that may be associated with certain clinical patterns of infection and antimicrobial resistance (Mcdougal *et al.,* 2003; Aires and De Lencastre, 2004, Hanssen *et al.,* 2004; Alves *et al.,* 2006; Deurenberg *et al.,* 2007; Wyatt *et al.,* 2010). The use of medicinal plants in healthcare is essential throughout the world, especially among the poorest countries (Carmona and Pereira, 2013). Moreover, plants are also sources of bioactive compounds and screening of biodiversity can be considered a key step for the discovery of new antimicrobials, with unique mechanisms of action, in the race to overcome the underlying cross-resistance of microorganisms (Harvey, 1999; Ji *et al.,* 2009; Klančnik *et al.,* 2010). *Eugenia pyriformis* Cambess is belonging to the Myrtaceae Family. It occurs from southwest to southern Brazil, especially in the Atlantic Forest and Cerrado (Lorenzi, 2000; Delgado *et al.,* 2007; Fiúza *et al.,* 2008). Morphologically, *E. pyriformis* offers simple leaves that are glossy and subcoriaceous with solitary white flowers. It blooms from November to January, and its fruits are yellow, velvety, edible, maturing in January and February, being commonly used to make juice, vinegar or wine (Andrade *et al.,* 2000; Armstrong *et al.,* 2012; Scalon *et al.,* 2012). Studies have reported the use of *E. pyriformis* to treat gout and, moreover, antimicrobial (Stieven *et al.,* 2009), and antioxidant (Reynertson *et al.,* 2008) activities *in vitro* has been observed for this plant.

Plinia cauliflora Berg. (Myrtaceae) is a native tree found in Brazil (Lorenzi, 2000). It is widely distributed in the Atlantic, and its characteristics are the shrubs that grew up to 2.5 m, leaves with petioles from 1 to 1.2 mm, elliptic to oblong (Souza-Moreira *et al.,* 2010). This tree produces an edible fruit known as "Jabuticaba", which has a thick rind fruit and tougher. This traditional medicinal plant is used to treat hemoptysis, asthma, chronic inflammation of the tonsils and the peel of the fruit is commonly used in the treatment of diarrhea and skin irritations (Barros *et al.,* 2010; Souza-Moreira *et al.,* 2011). Chemical composition of *P. cauliflora* shows a predominance of ascorbic acid, tannins, glycosides, anthocyanins, and phenolic compounds (Reynertson *et al.,* 2006).

Heliconia rostrata Ruiz & Pav. (Heliconiaceae), also known as Hanging Lobster Claw or False Bird of Paradise, is cultivated in tropical areas, where it is found in Brazil throughout the Minas Gerais Cerrado. *H. rostrata* is herbaceous, with erect rhizomes and height ranging from 0.3 to 6 meters; the leaves have limbo, petiole and sheath. *H. rostrata* shows great importance due to its flower displays inflorescence, being used worldwide as an ornamental plant (Criley, 1988; Berry and Kress, 1991; Castro, 1995; Lorenzi, 2000; Marques *et al.,* 2004; Torres *et al.,* 2005). Although few studies have been published on this plant, recently have been demonstrated its anticoagulant (Estrada *et al*; 2009, Estrada *et al*; 2010) and antimicrobial activities (Abdullah *et al.,* 2012).
Given the need for new antimicrobials for fighting infections caused by multi-resistant *S. aureus*, in this study was evaluated the antimicrobial potential of extracts from *E. pyriformis*, *P. cauliflora*, and *H. rostrata* against ORSA strains (genotypes), and performed a screening of phytochemicals compounds and toxicity of these extracts.

MATERIALS AND METHODS

Ethics Statement

This study did not involve any endangered or protected species and no specific permits were required for the described studies. Botanical material from *Eugenia pyriformis* Cambess*, Plinia cauliflora Berg.*, and *Heliconia rostrata* were collected in particular area, with access permitted to researchers.

Plants and extracts

Samples of *Eugenia pyriformis* Cambess, *Plinia cauliflora* Berg., and *Heliconia rostrata* were obtained from southern Minas Gerais Cerrado (Chart 1) and identified by Prof. Dr. Marcelo Polo (Herbarium UALF, UNIFAL-MG). Several anatomical sites of these plants (20 % m/v) were macerated in alcohol 70° and kept for 168 h at dark and under daily agitation. Aliquots of theses alcoholic extracts were submitted to filtration and then they were placed in a rotary evaporator under reduced pressure (50–60 °C and 500mmHg). The final product was transferred to a reaction bottle 1 L (SCHOTT® DURAN®) and kept at 20 °C for 24 hours in order to evaluate the freezing of the final product and the efficacy of the solvent evaporation process. Then, aliquots (40 mL) of this final product was transferred into glass vials penicillin type (40 mL) and lyophilized and their dry mass was measured. The lyophilized final product was prepared into aqueous solvent (water type 1) at a concentration of 100 mg/mL, sterilized by filtration (Millipore Corporation, hydrophilic Durapore® PVDF, 0.22 µm, 47 mm, cat. # GVWP 047 00), and kept in sterile polypropylene tubes at 70 °C until use.

Bacteria

Sixty oxacillin-resistant *S. aureus* (ORSA) isolates were used. The bacteria were isolated, identified, and had their resistance profiles to antibiotics performed in a previous study (Silva *et al.,* 2014). Briefly, these isolates were passively harvested from a clinical environment (air) of a Dental School Clinic at the University José do Rosário Vellano (UNIFENAS), Alfenas city, Minas Gerais state, Brazil. Open plates containing MSA selective medium (mannitol salt phenol-red agar; Merck, Darmstadt, Germany) were placed for two hours during intense and periodical multi-activities (matutinal or evening) in the follows dental clinic environments: Room of Integrated Clinical I (Periodontics, Dentistry, Endodontics, Prosthodontics, Stomatology) at the Center for Rehabilitation of Cleft Lip-palate and Craniofacial Deformities; Room of Integrated Clinical II (Periodontics, Dentistry, Endodontics, Prosthodontics, Stomatology) and Dental Prosthesis samplings; Prophylaxis place; Purge Room; Sterilization Room; Teacher Room; Reception; Waiting Room; Social Assistance; Revelation room; Prosthesis room; Hallways; Hall of Pediatric Dentistry and the Center for Rehabilitation of Cleft Lip-palate and Craniofacial Deformities; Archives of Pediatric Dentistry; Waiting Room 1; and Waiting Room 2. These procedures were conducted for 6 months, twice per month, with intervals of ± 15 days between one harvest and another (from July to December, 2009). The plates were then incubated at 37 °C for 48 h. Colonies indicating mannitol fermentation by staphylococci were selected, and the characterization of *S. aureus* was performed by Gram staining; growth in a chromogenic medium (CHROMagar™ Staph aureus; Probac do Brasil Produtos Bacteriológicos Ltda., São Paulo, SP, Brazil); catalase and coagulase tests (Cefinase™ discs, Becton Dickinson & Company, USA); and screening test for resistance to oxacillin (CLSI M7-A6, 2003).

Genotyping

Multilocus enzyme electrophoresis–MLEE and genetic interpretation of the MLEE patterns of *S. aureus* isolates were accomplished using previously described methods (briefly in Chart 2) (Selander *et al.,* 1986; Boriollo *et al.,*2009; Boriollo *et al.,* 2010).

Antimicrobial activity

Agar diffusion assay

Screening of antibacterial activity of the extracts was evaluated by using agar diffusion assay according to the Clinical and Laboratory Standards Institute (CLSI M7-A6, 2003), with minor modifications as described previously (Silva *et al.,* 2014), against ORSA genotypes (n = 60 isolates or n = 60 strains) and *S. aureus* reference strain (ATCC 6538). Chlorhexidine solution (0.12 % m/v) and water type 1 were used as positive and negative controls, respectively. Diameter of the zones of growth inhibition were measured and reported in millimeters (mm).

Minimal inhibitory concentration (MIC)

All extracts from *E. pyriformis*, *P. cauliflora*, and *Heliconia rostrata* were tested against each ORSA genotype (n total = 60) and against *S. aureus* reference strain and MIC values were determined by broth microdilution, according to the CLSI (CLSI M7-A6, 2003). Bacteria were cultured overnight (approximately 18 hours at 35 °C) and then bacterial suspensions in *sodium chloride 0.9% (*w/v) were adjusted according to the McFarland Standard 0.5. Next, 100 µl of Mueller Hinton broth (MHB) was added per well on a microplate with 96 wells and, after that, were added 100 µl of the each extract. Serial dilutions were made, ranging from 50 to 0.78 mg/mL. Finally, 10 µl of microorganism (ORSA genotype or *S. aureus* reference strain) were added to each well. The reading was performed visually (CLSI M7-A6, 2003), whose presence of turbidity in the well after incubation for 24 hours at 35 °C was considered indicative of bacterial growth. MIC was considered as the lowest concentration of the extract in which no turbidity occurred in the well. The growth control was composed of 100 µl of MHB and 10 ml of inoculum. The control extract was composed of 100 µl of MHB and 100 µl of extract and the sterility control was only comprised of 100 µl of MHB. Chlorhexidine (0.12%) was used as positive control. These assays were performed in triplicate system.

Cytotoxicity screening by using MTT reduction assay

The cytotoxicity of the extracts was assessed by using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenytetrazolium bromide) reduction assay (Araújo *et al.,* 2008; Silva *et al.,* 2014). 1×10^4 cells (from *Aedes albopictus*) were seeded per well in 96-well tissue culture plates containing 0.1 mL of L-15 medium supplemented with 1% of fetal bovine serum and with decreasing dilutions from of the extracts (5 to 0.039 mg/mL). After incubation, 10 µL of MTT were added to the wells and incubated for 4 h at room temperature, in order to incorporate the MTT for the formation of the formazan crystals. Finally, 100 µL of DMSO were added per well for solubilization of the formazan and spectrophotometric analysis was performed at a wavelength of 570 nm. The percentage of cytotoxicity was calculated using the following formula: $\frac{AB}{A\times100}$, where A and B are values of optical densities of treated and controlled cells, respectively. Thus, the 50% cytotoxic (CC_{50}) and 90% cytotoxic (CC_{90}) concentrations were calculated and defined as the concentration of the plant extract that reduced the absorbance of treated cells in 50% and 90% respectively, when compared with those of the quality control.

Phytochemical constituents

The qualitative analyses of chemical substances of the extracts were determined by colorimetric and/or precipitator methods, according to previous studies (Costa, 1982; Silva *et al.,* 2014). These methodologies were selected to determine the presence of alkaloids, anthraquinones, flavonoids, tannins, and saponins.

Data Analysis

The agar diffusion assay was realized in triplicate and the results were statistically analyzed using Sisvar Software Version 5.3. With the aim of comparing the means, the ANOVA analysis followed by SCOTT-KNOTT (Scott and Knott , 1974) as a post test were performed, and a significant difference in the means were considered when $p < 0.05$ (α=0.05). Selectivity index (SI) was calculated as reported previously (Protopopova *et al.,* 2005; Silva *et al.,* 2014). The discriminatory power of MLEE method based on genetic interpretation of the electrophoretic patterns was set out by the numerical index of discrimination (D), according to the probability that two unrelated isolates sampled from the test population will be classified in different types (i.e., strains, genotypes, or ETs). This probability can be calculated using Simpson's index of diversity, which was developed for the description of species diversity within an ecological habitat (Simpson, 1949). This index might be derived from elementary probability theory (Armitage and Berry, 1987), and is given by the following equation:

$$D = 1 - \frac{1}{N(N-1)}\sum_{j=1}^{S} n_j(n_j - 1),$$

where N is the total number of isolates in the sample population, S is the total number of types (strains) described, and n_j is the number of isolates belonging to the j^{th} type (strain). This equation was derived as following. The probability that two isolates sampled consecutively will belong to that type (strain) is:

$$\frac{n_j(n_j - 1)}{N(N-1)}$$

These probabilities can be summed for all the described types (strains) to determine the probability that any two consecutively sampled isolates will be the same type (strain). This summation can be subtracted from 1 to obtain the equation above. This equation can be applied both to a direct comparison of the discriminating power of typing methods and to an analysis of the discriminating power of combined typing schemes. An index greater than 0.90 is desirable and the typing results can be interpreted with confidence (Hunter and Gaston, 1988; Hunter and Fraser, 1989).

RESULTS

Genetic interpretation of MLEE patterns for ORSA isolates

Interpretation of the electromorphs (isoenzyme bands) was performed following the commonly accepted rule for the deduction of the allelic composition of haploid organisms. The bands on the gels were numbered in order of decreasing mobility, and the corresponding alleles were numbered by using the same nomenclature. The lack of demonstrable for an enzyme activity was considered as a null allele at the corresponding locus. For each combination of alleles from all examined loci enzyme, it resulted in an electrophoretic type (Electrophoretic Type–ET), also called here a line, genotype, or clone (Selander *et al.,* 1986; Alfenas *et al.,* 1998; Boriollo *et al.,* 2009; Boriollo *et al.,* 2010). It was observed that the 60 ORSA isolates resulted in 57 different electrophoretic types (genotypes/strains). The allelic profiles for 60 samples are shown in Table 1 (bellow).

Antibacterial activity screening

Table 2 (below) shows inhibition zones and MIC values of the tested extracts against the 60 ORSA genotypes or *S. aureus* reference strain. Inhibition zone ranged from 8 to 23 mm and MIC values from 6.25 to 50 mg/ml. Through the agar diffusion assay, in general, the extracts were most active against *S. aureus* reference strain than against ORSA. The extract from the leaves of *P. cauliflora* showed inhibition zones significantly (p<0.05) higher than chlorhexidine (0.12 %). The extract from the stems of *P. cauliflora* against *S. aureus* reference strain and the extract from the rind fruit of *P. cauliflora* against ORSA genotypes showed the lowest MIC values (6.25 mg/ml in both cases).

Phytochemical screening

The chemical constituents present in the extracts are presented in Table 2. All extracts were negative for the presence of anthraquinones and almost all were positive for saponins, and only extracts from the rhizomes and leaves of *H. rostrata* showed absence of this group of compounds. Furthermore, there was variable presence of alkaloids, flavonoids, and tannins.

Cytotoxicity of extracts

The results of cytotoxicity assay performed on cell derived from *A. albopictus* are presented in Table 2, whose SI of the extracts are also shown. Extracts from the leaves and stems of *P. cauliflora*, stems and leaves of *E. pyriformis* showed no toxicity at the maximum concentrations used in the experiment (5 mg/ml). On the other hand, the cytotoxic concentration 50 % (CC_{50}) and 90 % (CC_{90}) for all other extracts ranged from 1.30 to 19 mg/ml.

DISCUSSION

Natural products have long been used as a complementary therapy and can be considered a viable source of new antimicrobial agents (Cowan, 1999; Butler, 2005; Ashafa and Afolayan, 2009; Ji *et al.,* 2009; Patwardhan and Vaydia, 2010). In our study, all extracts from *P. cauliflora* and *E. piryformes* were active against the 60 ORSA genotypes and *S. aureus* reference strain. As indicated in Table 2, in general, the extracts showed the lowest MIC values against *S. aureus* reference strain. However, the extract from the rind fruit of *P. cauliflora* had lower MIC against ORSA. MIC values for the extracts tested can be substantially influenced according to the extraction method used, here obtained with ethanol:water (7:3 v/v). Additionally, according to Gobbo-Neto and Lopes (2007), cultivation conditions of plants are also important, on influence of biotic and abiotic factors, it having effects on the production of bioactive metabolites and influencing hence the MIC values. Although were observed a wide interval of inhibition zone for each extract evaluated against 60 strains of ORSA, by agar diffusion assay (Table 2), this method may suffer interference in bioassays of plant extracts and hence these wide intervals cannot be because of differences displayed between strains of ORSA, as evidenced in Table 1.

Oliveira *et al*. (2011) reported the antimicrobial activity of extracts from the leaves of *P. cauliflora* against *Staphylococcus epidermidis*, *Escherichia coli*, *Lactobacillus acidophilus*, and *Candida albicans*, wherein the inhibition zones ranged from 11 to 15 mm and the MIC from 156 to 2500 mg/ml. Moreover, antiseptic formulations (topical cream and mouthwash) containing alcoholic extract from the leaves of this plant were developed and evaluated by agar diffusion, where showed activity against *S. epidirmidis*, *E. coli*, and *C. albicans*. Extracts from the fruit and leaves of *P. cauliflora* were evaluated by Souza-Moreira *et al*. (2010; 2011) against *Shigella sonnei* (clinical sample), *Enterococcus faecalis*, *E. coli*, and *Salmonella sp*. The extract from the leaves was active (MIC of 5 mg/ml), while the extract from the fruit was inactive. In our study, the MIC values found against ORSA genotypes for *P. cauliflora* were, in most of the cases, lower than those reported by Oliveira *et al*. (2011); Souza-Moreira *et al*. (2010; 2011). All extracts from *H. rostrata* were inactive against *S. aureus* reference strain and against the ORSA genotypes. Recently, Abdullah *et al*. (2012) showed that by agar diffusion, the methanol extracts from the flowers and stems of *H. rostrata* were active against *Bacillus subtilis* (ATCC 6633), with inhibition zone ranging from 8 to 11.5 mm, however, these extracts were inactive against *E. coli* (ATCC 25922). Regarding the profile of chemical compounds (Table 2), the extracts from *P. cauliflora* revealed a variation of chemical constituents according to the plant part. The leaves were positive for flavonoids, tannins, and saponins, extracts from the stems only were positive for tannins and saponins. Conversely, the rind fruit showed the presence of alkaloids and saponins, which may have influenced the low MIC against ORSA strains.

Table 1. Collection places and identification of Eugenia pyriformis Cambess, Plinia cauliflora Berg., and Heliconia rostrata used in this study

Family	Specie	Regional name	Collection place	Coordinates	Collection date	Voucher number
Myrtaceae	*Eugenia pyriformis* Cambess.	Uvaia,uvaieira, and uvalha	Alfenas city, MG state	21° 25' 44'' S 45° 56' 49'' W	January 2011	1459
Myrtaceae	*Plinia cauliflora* Berg.	Jabuticaba	Alfenas city, MG state	21° 25' 44'' S 45° 56' 49'' W	January 2011	1637
Heliconiaceae	*Heliconia rostrata*	Banana-de-jardim, Bananeira-do-brejo, falsa ave do paraíso, and bico-de-papagaio	Areado city, MG state	21° 24' 39.44'' S 46° 08' 53.81'' W	January 2011	1636

Table 2. Systems and solutions utilized for MLEE analysis of enzymes metabolic from S. aureus

Enzyme EC number	Name	Symbol	Compound for staining Substrate	Buffer	Salt	Coenzyme	Dye catalyser
1.1.1.1	Alcohol dehydrogenase	ADH	Ethanol (3 mL) Isopropanol (2 mL)	200mM Tris–HCl pH 8.0 (50 mL)[a]		NAD 1% (2 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.14	Sorbitol dehydrogenase	SDH	Sorbitol (250 mg)	50 mM Tris–HCl pH 8.0 (50 mL)[b]		NAD 1% (2 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.17	Mannitol-1-phosphate dehydrogenase	M1P	Mannitol-1-phosphate (5 mg)	200 mM Tris–HCl pH 8.0 (50 mL)[a]		NAD 1% (2 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.37	Malate dehydrogenase	MDH	2M Malic acid (6 mL)[e]	200 mM Tris–HCl pH 8.0 (40 mL)[a]		NAD 1% (2 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.47	Glucose dehydrogenase	GDH	D-glucose (500 mg)	200 mM Tris–HCl pH 8.0 (50 mL)[a]		NAD 1% (1 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.48	□-Galactose dehydrogenase	GLDH	Galactose (450 mg)	100 mM Tris HCl pH 8.4[c]		NAD 1% (1 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.1.1.49	Glucose-6-phosphate dehydrogenase	G6PDH	Glucose-6-phosphate disodium salt hydrate (100 mg)	200 mM Tris–HCl pH 8.0 (50 mL)[a]	100 mM $MgCl_2$ (1 mL)[f]	NADP 1% (1 mL)	PMS 1% (500 mL) MTT 1.25% (1 mL)
1.11.1.6	Catalase[g]	CAT					
3.1.1.1	α–Esterase	α-EST	α-Naphthyl acetate (1% solution in acetone: 15 mg/1.5mL)	Sodium phosphate pH 7.0 (40 mL)[d]			Fast Blue RR salt (25 mg)
3.1.1.1	β-Esterase	β-EST	β-Naphthyl acetate (1% solution in acetone: 15 mg/1.5mL)	Sodium phosphate pH 7.0 (40 mL)[d]			Fast Blue RR salt (25 mg)

In phytochemical analysis of *P. cauliflora* presented in previous studies (Reynertson *et al.,* 2006; Reynertson *et al.,* 2008), the extracts from the leaves and their polar fractions showed the presence of tannins, and high levels of total phenolics and flavonoids. The extracts from the leaves of *E. pyriformis* were positive for all classes of studied compounds, with exception of anthraquinones, the stem showed positive reaction for tannins and saponins. For *H. rostrata*, phytochemical screening evidenced flavonoids and tannins in the leaf extract; tannins and saponins in the stem extract; saponins in the flower extract; and flavonoids in the rhizome extract. Thus, the complexity of plant extracts and the crucial effect of synergism are likely to be relevant for the antimicrobial action.

Additionally, *in vitro* antimicrobial activity of flavonoids, tannins, alkaloids, and saponins have been demonstrated. There is an extensive literature providing evidence gathered for the role of saponins in antimicrobial activity. This metabolite is considered a membrane disruptor which may, in part, be associated with its action, which leads to subsequent microbial death (Scalbert, 1991; Bruneton, 1999; Cowan, 1999; Schenkel *et al.,* 2001). However, based on combinations of pleiotropic molecules, the biological activity may also be the result of a combination of various compounds present in the extracts (Williamson, 2001), including the result of the action of reactive chemical species (oxidants) produced by plants (Brynildsen *et al.,* 2013), in which microbicidal effect has been reported for plant defense against pathogens such as bacteria and fungi (Resende *et al.,* 2003).

Table 3. Allelic profiles of 57 electrophoretic types of oxacillin-resistant S. aureus from dental clinical environments

Code of isolates	ET (strain/genotype)	Number of isolates	Alleles of 39 enzymatic loci																																						
			Adh-		Sdh-			M1p-				Mdh-						Gdh-					Gldh-				G6pdh-				Cat-		α-Est-					β-Est-			
			1	2	1	2	3	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	1	2	3	4	5	1	2	3	4
----------	Reference strain*	----------	-	*a*	*a*	-	-	-	*a*	*a*	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
I.A4.1b	ET1	1	*e*	-	-	*b*	-	-	*a*	*a*	-	-	-	-	*b*	*b*	-	-	-	*c*	*c*	-	-	*a*	*b*	-	-	-	*a*	-	*c*	-	-	*a*	-	*c*	-	*e*	-	-	-
II.A8.1d	ET3	1	*e*	-	-	-	-	-	*a*	*a*	-	-	-	*a*	*c*	-	-	-	*a*	*a*	*a*	-	-	*a*	*b*	-	-	*a*	-	-	*a*	-	*f*	*b*	-	-	-	-	-	-	-
II.A7.5a	ET6	1	*e*	-	*a*	*b*	*a*	-	-	*a*	*a*	-	-	-	*b*	-	-	-	*a*	*a*	*a*	*a*	-	*b*	*a*	*a*	-	*a*	-	-	*a*	-	*d*	*a*	-	-	-	*e*	-	-	-
III.A4.4a	ET7	1	*e*	*a*	*b*	-	-	-	-	-	-	-	-	-	*c*	*c*	-	-	*a*	*a*	*a*	*a*	-	*a*	*b*	-	-	*c*	-	*a*	*a*	-	-	*c*	-	*b*	-	*c*	-	-	-
IV.A2.14a	ET9	1	*e*	-	*b*	-	-	-	*a*	*a*	-	-	-	-	-	*c*	-	-	*a*	*a*	-	-	-	*a*	*b*	-	*c*	-	-	*a*	*c*	-	*e*	-	-	-	-	-	*b*	-	-
IV.B4.2b	ET10	1	*e*	-	*a*	*b*	-	-	-	-	-	-	-	-	*b*	*c*	-	-	*a*	*b*	*a*	-	-	*a*	*b*	-	-	*c*	-	-	*c*	*b*	*e*	-	-	-	-	-	*a*	-	-
VIII.A9.6a	ET23	1	-	*a*	*a*	*a*	-	-	-	*a*	-	-	*b*	-	-	-	-	-	-	*c*	-	*a*	-	-	*a*	-	-	*b*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
VI.A1.8a	ET24	1	-	*a*	-	*a*	-	-	-	*a*	-	-	*b*	-	-	-	-	-	-	*c*	-	*a*	-	-	*a*	-	-	*b*	-	-	*c*	-	*d*	-	-	-	-	-	*b*	-	-
VI.A2.14a	ET25	1	*a*	-	*a*	*a*	-	-	-	*a*	-	-	*b*	-	-	-	-	-	-	*c*	-	*a*	-	-	*a*	-	-	*b*	-	-	*c*	-	-	*c*	-	*c*	-	-	*b*	-	-
V.A13.7f	ET29	1	-	*a*	*a*	*a*	-	-	-	*a*	-	-	*d*	-	-	-	-	-	-	*c*	-	*a*	-	*a*	*a*	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	*b*	-	-
VII.B3.1b	ET35	1	-	*a*	-	*a*	-	-	-	*a*	-	-	*d*	-	-	-	-	-	-	*c*	-	*a*	-	-	*a*	-	-	*b*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
V.A13.2e	ET36	1	-	*a*	-	*a*	*b*	-	-	-	-	-	*d*	-	-	-	-	-	-	*c*	-	*a*	-	-	*b*	-	-	*d*	-	-	*c*	-	-	*b*	-	*b*	-	-	*a*	-	-
VI.A11.1a	ET38	1	-	*a*	-	*a*	-	-	-	-	-	-	*b*	*b*	-	-	-	-	-	*c*	-	*a*	-	-	-	-	-	*d*	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-
VIII.A13.6g	ET44	1	-	*a*	*a*	*a*	-	-	*a*	-	-	-	-	-	*a*	-	*a*	-	-	*c*	-	-	-	*a*	*a*	-	-	*d*	-	-	*c*	-	*g*	-	-	*b*	*a*	-	-	-	-
X.A1.9a	ET47	1	-	*a*	*a*	*a*	-	-	*a*	*a*	-	-	-	-	*a*	-	*a*	-	-	-	*b*	-	-	*c*	-	-	-	*d*	-	-	*c*	-	-	-	-	-	-	*e*	-	*a*	*a*
XI.A2.4b	ET55	1	-	-	*a*	*a*	-	-	*a*	*a*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	-	*d*	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-
X.A10.1c	ET56	1	*b*	*b*	*a*	*a*	-	-	*a*	*a*	-	-	-	-	-	-	-	*a*	*b*	*d*	-	-	-	-	-	-	*d*	*d*	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-
IX.A4.6b	ET58	1	*c*	-	*a*	*a*	-	-	*a*	*a*	-	-	-	-	*a*	-	-	-	-	*a*	*b*	-	-	*a*	-	-	-	-	-	-	*c*	-	-	-	-	-	-	*d*	-	-	-
X.A13.10f	ET59	1	-	-	*a*	*a*	-	-	*a*	*a*	-	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	*c*	-	*c*	-	-	-	-	-	-	-	-
XI.A4.6a	ET61	1	*e*	-	*a*	*a*	-	-	*a*	*a*	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	*b*	-	-	-	*a*	-	*c*	-	-	-	-	-	-	-	*a*	-	-
IX.A1.10a	ET62	1	*d*	*a*	*a*	*a*	-	-	*a*	*a*	-	-	-	-	-	*a*	*a*	-	-	*d*	*a*	-	-	*b*	*b*	-	-	*d*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
VIII.A7.2a	ET89	1	*f*	*a*	*a*	-	-	*b*	*a*	-	-	*c*	*c*	-	-	-	-	-	*b*	-	-	-	*b*	*a*	-	-	-	-	*a*	*b*	*c*	-	-	*b*	-	-	-	-	*a*	-	-
XIII.A1.1a	ET91	1	-	-	-	-	-	-	-	*a*	-	*c*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	*b*	-
XIII.A12.1c	ET94	1	-	-	-	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	-	*c*	-	*d*	-	-	-	-	*b*	-	-	-
XII.A9.7b	ET104	1	-	-	-	-	-	-	-	*a*	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	*d*	-	-	*c*	-	*d*	-	-	-	-	-	-	-	-
XII.A13.2b	ET106	1	-	-	-	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*d*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
II.A7.4b	ET111	1	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	-	-	-
XI.A7.1a	ET116	1	-	-	-	-	-	-	-	*a*	-	-	*d*	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*d*	-	-	-	-	*b*	-	-	-
XVIII.A4.5a	ET125	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*b*	-	-	-	-	-	*a*	-	-
XXII.A4.6a	ET126	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	-	-	-	-	-	*a*	-	-
XXI.B5.2a	ET126	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	-	-	-	-	-	*a*	-	-
XIX.A2.1b	ET130	1	*g*	*a*	*a*	-	-	*b*	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*b*	-	-	-	-	*c*	-	-	-
XXII.A2.13b	ET131	1	-	*a*	*a*	-	-	*b*	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*b*	-	-	-	-	*c*	-	-	-
XX.A13.1c	ET134	1	-	*a*	*a*	-	-	*b*	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*a*	-	-	-	-	*c*	-	-	-

ET: electrophoretic type (bacterial strain/genotype); Adh: alcohol dehydrogenase; Sdh: sorbitol dehydrogenase; M1p: mannitol-1-phosphate dehydrogenase; Mdh: malate dehydrogenase; Gdh: glucose dehydrogenase; Gldh: D-galactose dehydrogenase; G6pdh: glucose-6-phosphate dehydrogenase; Cat: catalase; α-Est: α-esterase; β-Est: β-esterase. The letters from *a* to *g* correspond to different alleles/haplotypes. [-] null allele. *ATCC® 25923

……………. *Continued*

Code of isolates	ET (strain genotype)	Number of isolates	Alleles of 39 enzymatic loci																																						
			Adh-		Sdh-			M1p-				Mdh-						Gdh-					Gldh-				G6pdh-				Cat-		α-Est-					β-Est-			
			1	2	1	2	3	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	1	2	3	4	5	1	2	3	4
XIX.B1.2c	ET138	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*c*	-	-	-	*c*	-	-	-
XVIII.A1.10d	ET142	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	*c*	-	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
XIX.A13.2a	ET143	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
XXII.A3.1a	ET143	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
XV.A5.4a	ET143	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	-	*a*	-	-
XX.A12.1a	ET144	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	*a*	-	-	-
XVIII.A2.14b	ET147	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	*c*	-	-	*a*	*a*	*b*	-	*a*	-	-
XX.A13.1a	ET149	1	-	*a*	*a*	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	-	*b*	-	-	-	*c*	*a*	-	-
XXII.A2.10a	ET152	1	-	*a*	*a*	-	-	-	*a*	-	-	*b*	-	-	-	-	-	-	-	*c*	-	-	-	*a*	-	-	-	-	*a*	-	*c*	-	*f*	-	-	-	-	*c*	-	-	-
XV.A9.8b	ET163	1	-	-	-	-	-	-	-	-	-	*b*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XIV.A13.10e	ET169	1	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XV.A2.11a	ET174	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	*d*	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XVII.A1.14a	ET179	1	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XVI.A1.2a	ET180	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XIV.A2.12a	ET181	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	-	-	-	-	-	-	-	-	-	*a*	-	*c*	-	-	-	-	-	-	-	-	-	-
XVI.A2.10d	ET184	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
XVI.A1.4b	ET185	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	*a*	-	-	-	-	-	-	-	-
XVII.A9.4a	ET187	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-
IV.A13.9b	ET192	1	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	-	-	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-	-	-	-
V.A3.1a	ET193	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	-	-	-	-	*a*	-	*b*	-	-	-	-	-	-	-	-	-	-
VII.B1.20a	ET194	1	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	-	*c*	-	-	-	-	-	-	-	*c*	-	-	*b*	-	-	*b*	-	*b*	-	-	-	-	-
VII.A13.5c	ET195	1	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-	-	-	*b*	-	*a*	*b*	-	-	-	-	-	-	-	*a*	-	-
IX.A1.3a	ET196	1	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-	-	-	-
XIV.A1.18d	ET201	1	-	-	-	-	-	-	-	-	-	-	-	-	*c*	-	-	-	-	*c*	-	-	-	-	-	-	-	*c*	-	-	*b*	-	-	-	-	-	-	-	-	-	-
XVII.A13.2c	ET205	1	-	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	-	-	*c*	-	-	-	-	-	-	-	-	*a*	-	*b*	-	-	-	-	-	-	-	-	-	-
XXII.A3.1b	ET211	1	-	-	-	-	-	-	-	-	-	-	-	-	*a*	*a*	-	-	-	-	-	-	-	-	-	-	-	-	*a*	-	*b*	*a*	-	-	-	-	-	-	-	-	-

ET: electrophoretic type (bacterial strain/genotype); Adh: alcohol dehydrogenase; Sdh: sorbitol dehydrogenase; M1p: mannitol-1-phosphate dehydrogenase; Mdh: malate dehydrogenase; Gdh: glucose dehydrogenase; Gldh: D-galactose dehydrogenase; G6pdh: glucose-6-phosphate dehydrogenase; Cat: catalase; α-Est: α-esterase; β-Est: β-esterase. The letters from *a* to *g* correspond to different alleles/haplotypes. [-] null allele. *ATCC® 25923; Discriminatory power equal to 0.99688.

Table 4. Interval and mean1 of the growth inhibition zone (IZ)2, MIC values, assessment of toxicity, and qualitative results from the phytochemical analysis of the crude hydroalcoholic extracts from Eugenia pyriformis Cambess., Plinia cauliflora Berg., and Heliconia rostrata

Specie	Crude hydroalcoholic extract	Interval and mean [3] of the IZ (mm)		MIC (mg/ml)		Assessment of toxicity		Selectivity Index (SI)		Active constituents				
		ORSA [4]	*S. aureus* ATCC 6538	ORSA	*S. aureus* ATCC 6538	CC_{50} (mg/ml)	CC_{90} (mg/ml)	ORSA	*S. aureus* ATCC 6538	Al	An	Fl	Ta	Sa
Eugenia	Leaf	10-16 (13) [d]	11-13 (12) [c]	50.00	25.00	9.56	19.00	0.4	0.77	+	-	+	+	+
pyriformis	Stem	9-16 (12) [c]	10-12 (11) [b]	50.00	25.00	NT	NT	NA	NA	-	-	-	+	+
Plinia	Rind fruit	9-16 (12) [c]	15-16 (15) [e]	6.25	25.00	1.30	2.55	0.41	0.1	+	-	-	-	+
cauliflora	Leaf	6-17 (13) [d]	15-18 (17) [f]	50.00	50.00	NT	NT	NA	NA	-	-	+	+	+
	Stem	5-15 (10) [b]	12-15 (13) [d]	50.00	6.25	NT	NT	NA	NA	-	-	-	+	+
Heliconia	Stem	0 (0) [a]	0 (0) [a]	N	N	NT	NT	NA	NA	-	-	-	+	+
rostrata	Flower	0 (0) [a]	0 (0) [a]	N	N	NT	NT	NA	NA	-	-	-	-	+
	Leaf	0 (0) [a]	0 (0) [a]	N	N	1.40	2.60	NA	NA	-	-	+	+	-
	Rhizome	0 (0) [a]	0 (0) [a]	N	N	2.15	6.90	NA	NA	-	-	+	-	-
Controls	Chlorexidine 0.12	10-26 (15) [e]	15 -18 (16) [e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Distilled water	0 (0) [a]	0 (0) [a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[1] Between parenthesis; [2] Tests by agar diffusion with extracts at a concentration of 100 mg/ml; [3] means followed with different letters are significantly different from each other according to Scott & Knott test ($p<0.05$); [4] n = 60 samples / 57 strains; MIC: Minimum inhibitory concentration; CC_{50}: 50% cytotoxic concentration; CC_{90}: 90% cytotoxic concentration; SI = CC_{90} / MIC_{100}; NT = Not toxic at the concentrations used; NA = Not applicable; N = absence of inhibition at the maximal concentration used in the test (50 mg/ml); Al: Alkaloids; An: Anthraquinones; Fl: Flavonoids; Ta: Tannins; Sa: Saponins; +: present; -: absent.

Concerning the evaluated extracts, as for their cytotoxicity, the extract from the rind fruit of *P. cauliflora* showed the same MIC value than the extract from the stems of this plant, however, the stems extract showed a more desirable characteristic, since they do not showed toxicity at 5 mg/ml. The presence of alkaloids in the extract from the rind fruit can at least in part explain this. Although antimicrobial activity of alkaloids has been demonstrated, the cytotoxic effect of this compound on cells has also been reported (Gonçalves and Lara, 2009; Silva *et al.,* 2014).

The delineation of strains by multilocus enzyme electrophoresis (MLEE) has allowed the evaluation of genetic diversity and population structure (Selander and Whittam, 1983; Rattazzi *et al.,* 1983), as high-supplied discriminatory power, as well as reproducibility (Boerlin, 1997; Soll, 2000; Van Belkum *et al.,* 2001; Boriollo *et al.,* 2009; Boriollo *et al.,* 2010). Metabolic isoenzymes are useful auxiliary tools in taxonomy, systematic genetic, and epidemiological characterization of bacteria and yeasts of medical interest (Selander and Whittam, 1983; Rattazzi *et al.,* 1983; Selander *et al.,* 1986; Bert *et al.,* 1997; Boerlin, 1997; Soll, 2000; Van Belkum *et al.,* 2001; Napimoga *et al.,* 2004; Boriollo *et al.,* 2009; Boriollo *et al.,* 2010). As regards the activity of the extracts against ORSA genotypes, a significant difference was found between the 60 ORSA samples evaluated, in which only the electrophoretic types (ETs) 126 and 143 had more than one sample with this same allelic profile (Table 1). Therefore, we deduce that different electrophoretic types (genotypes) did not affect the MIC values for the evaluated extracts (Table 2), whereas all 60 samples showed the same MIC values against each one of the evaluated extracts, and when the extract did not inhibit the growth of all ORSA genotypes (*e.g.,* extracts from *H. rostrata*) it occurred equally well for all 60 ORSA genotypes. *S. aureus* has been considered the most frequent cause of infections in HAI and *CAI as well,* and its multi-resistance to antimicrobials has led to high rates of morbidity and mortality associated (Bernardo *et al.,* 2005; Fowler *et al.,* 2007; Boucher and Corey, 2008; Jappel *et al.,* 2008; Gelatti *et al.,* 2009; Xie *et al.,* 2011; Tan *et al.,* 2012).

Infections caused by *S. aureus* are especially difficult to treat because of multi-resistance to antimicrobial agents, mainly to methicillin (Aires and De Lencastre, 2004; Klein *et al.,* 2007; Boucher and Corey, 2008). Plant extracts, widely applied in traditional and modern medicine. Currently, the association between extract and antibiotic has been thoroughly evaluated against multi-resistant pathogens, including *S. aureus* (Lee and Gould, 2002; Hemaiswaryaa *et al.,* 2008; Diarra *et al.,* 2013; Müller *et al.,* 2013).Therefore, because of a possible synergy between extracts plant and antibiotics, this will be an effective therapeutic option in the future. Through the results obtained, it can be concluded that all extracts from *E. pyriformis* and *P. cauliflora* were active against both *S. aureus* reference strain and ORSA genotypes. On the other hand, all extracts from *H. rostrata* were inactive. The extract from the rind fruit of *P. cauliflora* evidenced the best anti-ORSA activity. These findings demonstrate that rind fruit from *P. cauliflora* shows potential to further studies. Notwithstanding, our study should be emphasized as complementary to other researches, either these aiming isolate compounds with antimicrobial action through a bioassay-guided approach or assessing a possible synergism between extract and antibiotic. It may provide opportunities in running by novel antimicrobials and providing therapeutic alternatives for treating infections by antimicrobial-resistant bacteria, such as ORSA infections.

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Author Disclosure Satatement

The authors declare that they have no competing interests.

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