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Original Article

Screening and isolation of antibacterial proteinaceous compounds from flower tissues: Alternatives for treatment of healthcare-associated infections

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ABSTRACT

Healthcare-associated infection represents a frequent cause of mortality that increases hospital costs. Due to increasing microbial resistance to antibiotics, it is necessary to search for alternative therapies. Consequently, novel alternatives for the control of resistant microorganisms have been studied. Among them, plant antimicrobial protein presents enormous potential, with flowers being a new source of antimicrobial molecules. In this work, the antimicrobial activity of protein-rich fractions from flower tissues from 18 different species was evaluated against several human pathogenic bacteria. The results showed that protein-rich fractions of 12 species were able to control bacterial development. Due its broad inhibition spectrum and high antibacterial activity, the protein-rich fraction of *Hibiscus rosa-sinensis* was subjected to DEAE-Sepharose chromatography, yielding a retained fraction and a non-retained fraction. The retained fraction inhibits 29.5% of *Klebsiella pneumoniae* growth, and the non-retained fraction showed 31.5% of growth inhibition against the same bacteria. The protein profile of the chromatography fractions was analyzed by using SDS-PAGE, revealing the presence of two major protein bands in the retained fraction, of 20 and 15 kDa. The results indicate that medicinal plants have the biotechnological potential to increase knowledge about antimicrobial protein structure and action mechanisms, assisting in the rational design of antimicrobial compounds for the development of new antibiotic drugs.

Keywords healthcare-associated infections, human pathogenic bacteria, flower protein, antimicrobial

INTRODUCTION

Healthcare-associated infections (HAIs) are a major cause of mortality in the nosocomial environment, endangering lives and increasing hospital costs throughout the world. The incidence of HAIs is variable depending on the country. In the U.S., data show spends between 5.7 and 6.8 billion dollars per year on HAIs (Pittet et al., 2008; WHO, 2011).

The pathogens commonly involved in the etiology of HAIs are bacteria, fungi and viruses; however, bacteria represent the main causal agent (Hughes et al., 2008). The constant and gradual selective pressure caused by indiscriminate use of antibiotics promotes remarkable and rapid microbial resistance. Over the past few decades, an alarming increase in infections caused by antibiotic-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase-producing (ESBL-producing) Escherichia coli and *Klebsiella spp*., among others, has been observed, particularly in intensive care units. Additionally, the emergence of resistant strains has become a major nosocomial problem, and indeed was the main health problem of the last century, whereas the development of effective new therapeutic agents is proving to be increasingly complicated (Hughes et al., 2008; Petrosillo et al., 2010; Pittet et al., 2008).

In order to contribute to the development of new alternatives to control multi-resistant pathogens, different strategies, such as bioprospecting and characterization of new molecules from a wide variety of plants, have been studied. Medicinal plants that are widely used in ethnopharmacology to control disorders and combat infections present vast biotechnological potential, thanks to the presence of multiple bioactive compounds, including antimicrobial proteins/peptides (Benko-Iseppon and Crovella, 2010; Choi et al., 2009; Pascual et al., 2001; Pelegrini et al., 2011; Silva et al., 2011). There is an enormous diversity of antimicrobial proteins, constitutively produced by the host plant or induced by pathological situations, including viral, fungal and bacterial infections, or related situations, including nematodes, insect pests and herbivorous animals (Antoniw et al., 1980; Castro et al., 2005; Iriti et al., 2007; Liu et al., 2006; Tavares et al., 2008). In recent years, screening and isolation of defense plant proteins/peptides with antimicrobial activity has become a new biotechnological tool in the search for new antibiotics (Guaní-Guerra et al., 2010; Lima et al., 2011; Mandal et al., 2011; Maria-Neto et al., 2011;

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Ribeiro et al, 2010; Silva et al., 2011; Wong et al., 2012).

Antimicrobial proteins from all plant tissues have been widely studied after being isolated from roots, stems, bulbs, leaves, fruits, seeds and flowers; of these, seeds have been used most, due to their higher protein content, compared to other plant parts (Cândido et al., 2011; Lay et al., 2003a,b; Mandal et al., 2012; Maria-Neto et al., 2011; Ribeiro et al., 2010; Sartori et al., 2003). However, flowers may also represent an important source for boosting our antimicrobial arsenal, since this is a structure of extreme value for the species' survival, responsible for reproduction, and presumed to have great potential in the production of antimicrobial compounds (de Beer and Vivier, 2011; Lay et al., 2003a,b; Moreira et al., 2011; Sartori et al., 2003; Tavares et al., 2008).

In summary, this study aims to expand the knowledge about the antimicrobial potential of proteinaceous extracts from flowers of medicinal and/or ornamental plants from eighteen species that occurs in Brazil. All of them have been traditionally used in ethnomedicine being generally associated with secondary metabolites or essential oils (Favarin et al., 2013; Samy et al., 2013).

Among the medicinal plants selected in this study, some of them are native and/or endemic to Brazil and in most cases are not reported active protein compounds responsible for these activities, such as *Hancornia speciosa* (*Apocynaceae*), where latex, leaves and bark extracts have been used to decrease gastrointestinal diseases, inflammations, tuberculosis and fungal infections, being also applied onto diabetes and hypertension treatment. (Endringer et al., 2010; Marinho et al., 2011; Pereira et al., 2012; Silva et al., 2011). Similarly, bark and stem extracts from *Hymenaea stigonocarpa* (*Fabaceae*) have been traditionally used also to treat gastric disorders, ulcers, diarrhea and inflammation (Rodrigues et al., 2012).

Moreover, *Myracrodruon urundeuva* (*Sapindales*) also show different medicinal properties from leaves, seeds, stem and bark, which can present anti-diarrheal, anti-ulcer, intestinal wound healing and larvicidal activities (Ferreira et al., 2011). Furthermore, extracts from fruits, stem, bark and leaves from the species *Spondias tuberosa* (*Anacardiaceae*) may present antioxidant, antibacterial and antiviral properties (Silva et al., 2012; Araújo et al., 2012). Lastly, bark and leaves extracts from *Handroanthus serratifolius* (*Bignoniaceae*) are traditionally used as anti-inflammatory, due the presence of a phenolic substance known as lapachol, which can present strong antimicrobial, anti-allergic, wound healing and antitumor activities (Morais et al., 2005).

The others medicinal plants included in this study are not native, but are widely spread throughout the tropical regions and are easily found in South America, including Brazil, also being known by their medicinal properties including *Verbenaceae* family. In this group, extracts from aerial parts (leaves and flowers) of *Lippia rotundifolia* also can be used to control tuberculosis (Leitão et al., 2006). Otherwise, some species are primarily known as ornamental plants, being worldwide distributed due aesthetic purpose. Studies reveals that they may have several medicinal properties and its leaves, flowers, stems and bark extracts are used by folk medicine, for the treatment of microbial infections and inflammation, but also utilized to reduce constipation, arthritis and the other pathologies. Leaves from small shrub-like *Catharanthus roseus* (*Apocynaceae*) have been normally used to control infectious diseases and common cold, which are related to their antimicrobial activity (Guimarães et al., 2012; Rahmatullah et al., 2012; Shakir et al., 2013). Moreover, the tree *Delonix regia* (*Fabaceae*) has been frequently used for ornamental purposes in many countries from tropical region where leaves, flowers, stems and bark extracts are used by folk medicine mainly for the treatment of microbial infections and inflammation, but also utilized to reduce constipation, arthritis, hemiplegia, leucorrhoea and rheumatism (Shabir et al., 2011). Similarly, the small shrub *Dianthus caryophyllus* (*Caryophyllaceae*), cultivated on large scale in South America for ornamentation, have their flower buds traditionally used in the treatment of wounds, gastro-intestinal disorders and throat or gum infections (Mohammed and Al-Bayati, 2009)

Lastly, the species *Hibiscus rosa-sinensis* (*Malvaceae*), as well known as medicinal and ornamental are widely cultivated in tropical regions. Extracts from roots, leaves and floral tissues have been used initially for aphrodisiac or rejuvenator issues, but posteriorly, applied for fertility and infections control. The antimicrobial activity of proteins from *H. rosa-sinensis* flowers was also evidenced in previous studies, showing in vitro inhibition of human pathogenic bacteria (Ruban and Gajalakshmi, 2012; Vasudeva and Sharma, 2008).

Other species of ornamental plants found in Brazil without records of biological activities tested were also here exploited such as *Bougainvillea glabra* (*Nyctaginaceae*), *Grevillea banksii* (*Proteaceae*), *Rosa alba* (*Rosaceae*), *Tibouchina granulosa* (*Melastomataceae*) and *Zantedeschia aethiopica* (*Araceae*).

In resume, experiments here reported were performed using the protein-rich fractions of floral tissues (petals, bracts, inflorescences, spathes, spadix and entire flowers) from different medicinal and ornamental flowering plants, representing numerous botanical families in order to analyze antimicrobial potential toward healthcare-associated bacterial infections. Protein-rich fractions that showed the highest antimicrobial activity were subsequently used for further isolation studies and functional characterization.

MATERIAL AND METHODS

Biological material and protein extraction

Floral tissues were collected from native Brazilian species and ornamental species acquired in greenhouses, totaling 18 different species and twenty different samples (pink and white variants of *Catharanthus roseus* flowers; spathe and spadix of *Zantedeschia aethiopica*). Bracts from *Bougainvillea glabra* (*Nyctaginaceae*); petals from white-flowering *Catharanthus roseus* (*Apocynaceae*), red-flowering *C. roseus* (*Apocynaceae*), *Delonix regia* (*Fabacea*), *Dianthus caryophyllus* (*Caryophyllaceae*), *Hancornia speciosa* (*Apocynaceae*), *Handroanthus serratifolius* (*Bignoniaceae*), *Hibiscus rosa-sinensis Linn*. (*Malvaceae*), *Hymenaea stigonocarpa* (*Fabaceae*), *Myracrodruon urundeuva* (*Sapindales*), *Rosa alba* (*Rosaceae*), *Spondias tuberosa* (*Anacardiaceae*); inflorescences from *Grevillea banksii* (*Proteaceae*), *Lippia rotundifolia* (*Verbenaceae*), *Lippia rubella* (*Verbenaceae*), *Lippia salvifolia* (*Verbenaceae*), *Lippia sidoides* (*Verbenaceae*); entire flowers from *Tibouchina granulosa* (*Melastomataceae*); spadix and spathes from *Zantedeschia aethiopica* (*Araceae*) were macerated with liquid nitrogen and mixed with an extraction solution containing NaCl 0.6 M and HCl 0.1% (1:3 m/v), remaining in overnight agitation. The extracts were centrifuged at 8,000 g for 30 min, at 4℃, and the supernatant subjected to ammonium sulfate precipitation (0 - 100%). After precipitation, the samples were centrifuged again at 8,000 g for 30 min, at 4℃, and the pellet was dialyzed (3.0 kDa cut off) against distilled water during 24 h. After new centrifugation at 8,000 g for 20 min, at 4℃, the supernatant was lyophilized and these protein-rich fractions were finally used for bioassay.

Protein quantification was performed by fluorimetic method according to the manufacturer's instructions (Quant-iT™ Protein Assay Kit, Invitrogen).

Antibacterial activity bioassays

The antibacterial activities of protein-rich fractions and isolated proteins from floral samples were determined by microdilution method (CLSI, 2009). A final concentration of 250 µg/ml from protein-rich fractions from all flower tissues was prepared in sterile 96-well microplates. Subsequent to initial tests, selected samples of 100 µg/ml from DEAE-Sepharose chromatography fractions of H. rosa-sinensis protein-rich fraction were submitted to the same treatment for bacterial growth inhibition bioassays. The wells were filled with 100 µl of Luria-Bertani liquid medium (LB) for antibacterial approach; final bacterial inoculate was 105 CFU/ml and incubated aerobically at 37℃ for 6 h. Sterile distilled water was used as negative control and chloramphenicol 40 µg/ml as positive control. Bacterial growth evolution was monitored by measuring the optical density at 595 nm, every 30 min, to determine the bacterial growth phases. The experiment was performed in triplicate. To perform the bioassays Gram-negative bacteria (Escherichia coli ATCC-8739, *Klebsiella pneumoniae* ATCC-13883, Proteus mirabilis ATCC-43071, *Salmonella typhimurium* ATCC-14028 and *Shigella flexneri* ATCC-12022) and Gram-positive bacteria (*Streptococcus pyogenes* ATCC-19615 and *Staphylococcus aureus* ATCC-25923) were used, the major human pathogens that cause nosocomial infections.

Protein isolation of *Hibiscus rosa-sinensis L.*

After the initial antibacterial bioassays, the H. *rosa-sinensis L.* sample was elected as the main focus of this work, given its antimicrobial potential and high availability of floral tissue. After dialyses, 10 mg of the protein-rich fraction was applied on a DEAE-Sepharose (GE Healthcare) column, with a weak anion exchange resin capable of binding to negatively charged molecules, generating a non-retained fraction (NRF) and a retained fraction (RF) (Fig. 1). The column was previously equilibrated with triethanolamine buffer 20 mM, pH 7.5, through a flow of about 1 ml/min. The non-retained fraction was eluted with the same buffer, and the retained fraction was eluted with the triethanolamine buffer 20 mM, pH 7.5 containing NaCl 1.0 M. Chromatography was monitored by spectrophotometry at 280 nm. Finally, the chromatographic fractions were dialyzed against distilled water (3.0 kDa cut off) and lyophilized to prepare for subsequent analyses.

Molecular mass analyses by SDS-PAGE

Molecular mass profiles from *H. rosa-sinensis* protein-rich fraction and chromatography fractions were obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 12%, conducted according to established procedures by Laemmli and co-workers (1970). The gel was further silver nitrate stained (Blum et al., 1987). To determine the apparent molecular mass a molecular mass marker was used, with range between 10 kDa and 200 kDa (FERMENTAS-Unstained Protein Molecular Weight Marker #SM0431).

RESULTS AND DISCUSSION

In recent decades, a significant increase in the resistance of pathogenic bacteria to conventional antibiotics has been observed. Members of the family Enterobacteriaceae, such as *E.coli*, *Klebsiella sp*. and *Proteus sp*., are now a major cause of healthcare-associated infections, especially in developing countries, with higher mortality observed in immunocompromised patients, premature infants and elderly, obese, diabetic, and cancerous patients, or those with other serious illnesses and have undergone medical and surgical treatments (Apostolopoulou et al., 2004; Pelegrini et al., 2008; Ruef, 2005).

In this study we evaluated the antibacterial activity of floral protein-rich extracts from native Brazilian and ornamental species. The extracts were subjected to bioassays using healthcare-associated infection-related bacteria. Among the 20 samples from 18 flowering plants species studied, only 13 samples presented some bacterial growth inhibition activity. Protein extracts from seven species (*D. caryophyllus*, *H. serratifolius*, *L. rubella*, *L. salvifolia*, *L. sidoides*, *R. alba* and spadix of *Z. aethiopica*) were unable to inhibit the growth of any tested pathogens (data not shown).

Among the active samples, seven presented remarkable biological activity, showing high or complete bacterial growth inhibitory activity (> 93 - 100%) against most of the microorganisms tested. The human pathogenic bacteria E. coli was inhibited by the extracts from *T. granulosa* (96%), *Z. aethiopica* (96.3%), *H. rosa-sinensis* (99.3%), *H. speciosa* (100%), *H. stigonocarpa* (100%), *M. urundeuva* (100%) and *S. tuberosa* (100%). Nevertheless, higher deleterious activity was obtained with *H. rosa-sinensis* extracts, which in addition to inhibiting *E. coli* growth, was also able to decrease efficiently the development of *K. pneumoniae* (98.2%), *P. mirabilis* (98.3%) and *S. pyogenes* (93.3%), with the floral proteic extract showing most potential for bacterial control. Similar results were showed by Ruban and Gajalakshmi (2012), who demonstrated the antibacterial potential of aqueous extracts of *H. rosa-sinensis* flowers against *S. aureus*, *Streptococcus sp*., *Bacillus subtilis*, *E. coli*, *Salmonella sp*. and *Pseudomonas aeruginosa*, using disc diffusion and agar-well methods. Using an alternative strategy, Arullappan and co-workers (2009) showed the antimicrobial activity of different extracts of *H. rosa-sinensis* flowers against the human pathogen MSRA. They tested organic solvents such as petroleum ether, ethyl acetate and methanol to extract the antibacterial compounds and used the disc diffusion method for the test. Best results were demonstrated with petroleum ether extract against MSRA. In contrast with our results with aqueous extracts, no antibacterial activity was detected against *E. coli* and *K. pneumoniae* with the organic extracts.

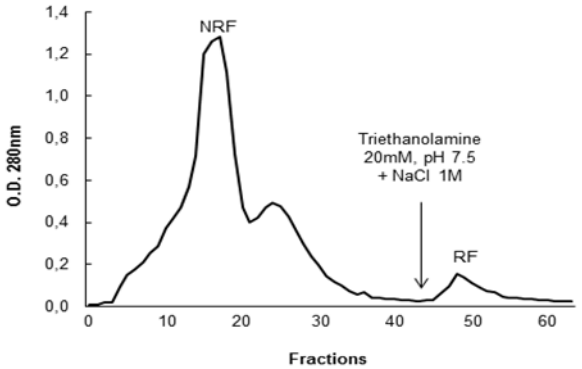

Fig. 1. Anionic exchange chromatography profile of DEAE-Sepharose from *H. rosa-sinensis* proteins. NRF corresponds to non-retained fraction and RF corresponds to retained fraction, which was eluted with triethanolamine buffer pH 7.5, containing 1M NaCl (black arrow)

Due to the higher bacterial growth inhibitory activity against all pathogens tested, added to the cosmopolitan distribution of these ornamental plants, which bloom

throughout the year, *H. rosa-sinensis* was chosen for subsequent protein purification. Thus, protein-rich extracts from *H. rosa-sinensis L*. petals were applied on ion exchange chromatography, using a DEAE-Sepharose (GE Healthcare) column, with a weak anion exchange resin capable of binding to negatively charged molecules, generating a non-retained fraction (NRF) and a retained fraction (RF) (Fig. 1).

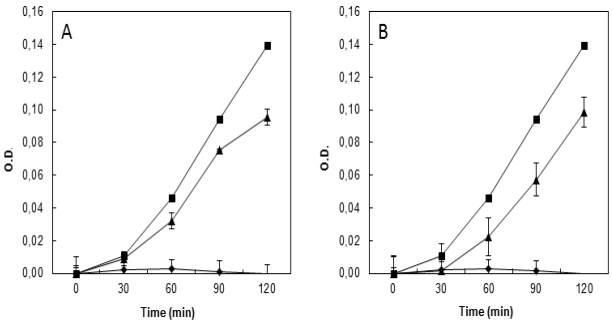

Fig. 2. Antibacterial activity of DEAE-Sepharose chromatographic fractions against *Klebsiella pneumoniae*. (A) indicates the non-retained fraction (NRF) and (B) corresponds the retained fraction (505 nm). The line with rhombs indicates the negative control (distilled water); the line with squares indicates the positive control (chloramphenicol 40 µg/ml); the line with triangles indicates the sample (100 µg/ml from each chromatographic fraction). Vertical bars represent the standard deviation.

The fractions NRF and RF were dialyzed, lyophilized and then new antibacterial tests were carried out against *K. pneumoniae*, with concentration of 100 µg/ml. The NRF showed 31.7% and RF showed 29.5% of bacterial growth inhibition activity (Fig. 2). This fact demonstrates the possibility of two or more proteins with bacterial growth inhibitory activity in the same extract, a characteristic which can be explained by the complex plant defense system, capable of producing an antimicrobial arsenal in response to infections, including a variety of antimicrobial proteins, such as the expression of various R genes and the production of NBS-LRR or PR proteins (Iriti et al., 2007; Liu et al., 2006; Pelegrini et al., 2008). Nevertheless, interestingly, initially purified proteins presented a substantial reduction in antibacterial activity after each purification step, decreased approximately from 95% of activity in the protein-rich fraction to around 30% of activity in chromatographic fractions. Several hypotheses could be drawn, but the main possibility leads us to believe that NRF and RF fractions have proteins with antimicrobial activity which could act synergistically against bacterial cells, by controlling development. Supporting this theory is the remarkable reduction noted in inhibitory activity when these proteins were separated, a phenomenon also observed in previous works with plant antimicrobial proteins and peptides (Ribeiro et al., 2010; Thevissen et al., 1999). However, it is also plausible that the decrease in antimicrobial activity may be related to protein sample processing during storage and purification steps (Table 1 and Fig. 2).

After activity confirmation, the chromatographic fractions were analyzed by SDS-PAGE in order to determine the apparent molecular mass. The protein profiles showed a wide range of molecules with molecular masses lower than 10 kDa to over 100 kDa in NRF, while only two bands were visualized in the RF: one band with apparent mass below 20 kDa and another band with apparent weight below 15 kDa, revealed by silver nitrate staining (Fig. 3). Among all the antimicrobial proteins/peptides isolated from plants, only a few have been isolated from flowers, with representatives in the following families: defensins (Lay et al, 2003a,b; Van der Weerden et al., 2008), snakins (Segura et al., 1999), hevein-type peptides (Koo et al., 1998; Van Damme et al., 1999), lipid transfer proteins (Garcia-Olmedo et al., 1995), myrosinase-binding proteins (Rask et al., 2000), pathogenesis-related proteins (Lotan et al., 1989; Sels et al., 2008), nucleotide binding site and leucine-rich repeat proteins (Moreira et al., 2011).

Analyzing the molecular masses observed in RF, the lower band, between 10 kDa and 15 kDa, may possibly represent classes of antimicrobial proteins, like: Lipid-transfer proteins (LTP), similar to anther-specific sugar beet bvLTP-1 (Beta vulgaris Lipid Transfer Protein 1) with 12 kDa, belonging to a longer group of LTPs. This class has basic proteins with four disulfide bonds and high molecular chains, capable of binding fatty acids and transferring phospholipids between membranes, also reported to be involved in antimicrobial activity and plant defense due to a typical hydrophobic tunnel-like cavity that may be inserted into the microbial membrane, forming a pore and leading to cell death (Capella et al., 2001; Ferreira et al., 2007; Lin et al., 2005; Matsuhira et al., 2007; Tavares et al., 2008). Another possibility is that proteins from the lower band are members of the Myrosinase Binding Proteins (MBP). Actually, two similar forms from *Arabidopsis thaliana* flowers (MBP1 and MBP2) have been described, each of 14 kDa. MBPs have two disulfide bonds and a cationic charge, and are reported to be involved in several plant physiological aspects, including defense against herbivorous predators and pathogens thorough a myrosinase-glucosinolate system, producing metabolites that are very effective against microbes, among other functions (Capella et al., 2001; Tavares et al., 2008).

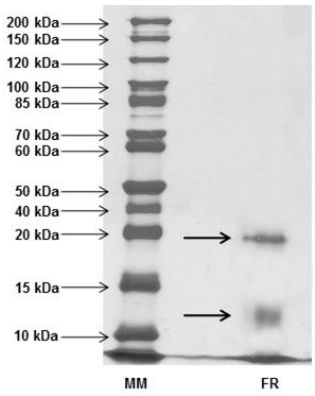

Fig. 3. Proteic molecular mass profile analyses from DEAE-Sepharose chromatographic fractions of *H. rosa-sinensis* by SDS-PAGE (12%). Gel was silver stained. MM corresponds to molecular weight marker (10 kDa to 200 kDa) and RF corresponds to retained fraction.

Considering the molecular weight of the RF higher band, less than 20 kDa, due the high molecular mass, it may represent a member of the Pathogenesis Related Proteins (PRP) defense proteins super-family, which has a large variety of molecular sizes and different functions, currently comprising 17 families. One group in which this protein could be classified is the PR-10 family, similar to Parsley "PR-1" with molecular mass up to 19 kDa. This group is formed by Ribosome-Inactivating Proteins (RIP) with RNA N-glycosidase activity that paralyzes protein synthesis from pathogens (Ferreira et al., 2007; Matsuhira et al., 2007). However, an important observation to be discussed at this point is the fact that among the 17 existing classes of PRP, most of them present antifungal activity, which although not tested in this study, permits us to consider a possible classification of the lower protein into, for example, the PR-1 family (with molecular masses about 15 - 17 kDa and homology to the superfamily of cysteine-rich proteins), among

Table 1. Antibacterial activity of floral protein extracts against bacteria pathogenic human

Species	Rich Protein Fraction Antibacterial Activity [% of Inhibition]					
	E. coli	*K. pneumoniae*	*P. mirabilis*	*S. aureus*	*S. pyogenes*	*S. flexneri*
Bougainvillea glabra	4.3 ± 0.000	-	-	ND	-	-
Catharanthus roseus red	50.3 ± 0.004	ND	-	17.2 ± 0.003	-	-
Catharanthus roseus white	45.0 ± 0.100	-	-	-	-	-
Delonix regia	35.0 ± 0.080	30.5 ± 0.010	-	-	-	-
Grevillea banksii	5.0 ± 0.100	-	-	-	-	-
Hancornia speciosa	100.0 ± 0.000	-	-	-	-	-
Hibiscus rosa-sinensis	99.3 ± 1.500	98.2 ± 1.300	98.3 ± 1.800	-	93.3 ± 0.500	-
Hymenaea stigonocarpa	100.0 ± 0.000	-	-	-	-	-
Lippia rotundifolia	12.6 ± 0.500	49.6 ± 0.600	36.6 ± 0.400	-	44.3 ± 0.600	-
Myracrodruon urundeuva	100.0 ± 0.000	-	-	-	-	-
Spondias tuberosa	100.0 ± 0.000	-	-	-	-	-
Tibouchina granulosa	96.0 ± 0.001	29.7 ± 0.001	-	4.9 ± 0.001	ND	31.1 ± 0.001
Zantedeschia aethiopica	96.3 ± 0.000	-	-	ND	-	-

Extracts not tested against the microorganism. ND corresponds to not detectable. The results are expressed as mean ± standard deviation.

a few other cases (Ferreira et al., 2007).

On the other hand, no inferences could be drawn about the NRF, with so many different proteins, as it was impossible to attribute the biological activity of the fraction to any observed band, which may contain molecules of any antimicrobial protein class.

Floral protein extracts studied in this research demonstrate the great potential of these species as a source of active molecules in the treatment of bacterial diseases. Although, few antimicrobial proteins or peptides have so far been isolated from flowers compared with other plant tissues, there are promising precedents in the case of NaD1, a defensin isolated from *Nicotiana alata* flowers or PhD1 and PhD2, defensins isolated from *Petunia hybrida* petals. All of these show antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum* (Lay et al., 2003). Some AMPs described in flowers were originally purified from other parts of the plant and then located later in other tissues, through analysis of total RNA by Northern blot, as is the case of snakin-1 and -2 from *Solanum tuberosum* (Berrocal-Lobo et al., 2002; Segura et al., 1999). Others have not yet been properly purified: through the cDNA library, Northern and Southern blots, sequences have been analyzed, cloned and, by heterologous expression, purified and tested, as is the case of Pth-St1, defensin *Solanum tuberosum* (Kovalskaya and Hammond, 2009). However, several descriptions in the literature analyze sequence similarities and have found AMPs, without antimicrobial activity tests, as is the case of pathogenesis-related proteins from *Nicotiana tabacum* (Lotan et al., 1989) and MBP1 and MBP2, myrosinase-binding proteins from *Arabdopsis thaliana* (Capella et al., 2001).

Until now, there is no record of publications that describe proteins or peptides extracted from flowers that have been found to be active against healthcare-associated infection pathogens, especially bacteria. It is clear that floral tissues have been under-explored in the area of defense mechanisms of reproductive structures, which may be due not only to very low amount of proteins found in this tissue so far, but also to the presence of a variety of pigments and secondary compounds in flowers. The results shown here provide new and important information regarding the purification of proteins with antimicrobial activity, present in native and ornamental flowers, opening new prospects for other studies. Given the great need for implementation of new molecules for pathogenic bacteria control, the authors suggest that these proteins have biotechnological potential for developing new drugs against Gram-negative and Gram-positive bacteria. So, further studies are needed to isolate and identify these possible antimicrobial proteic compounds from the different species here studied and totally purify the antibacterial proteins from *H. rosa-sinensis* for its molecular characterization and correct biochemical classification.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article, as well as the authors has no conflicting financial interests.

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