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Research Article

BACTERIAL INFECTION IN PRESSURE INJURY: EVALUATING IN VITRO ANTIMICROBIAL ACTIVITY OF NATURAL EXTRACTS

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ABSTRACT

Introduction: Pressure injury are susceptible to infection due to sterile loss of the innate barrier function of the skin and dermal appendages, facilitating the development of microbial communities. **Objective:** The aim of this study was evaluating *in vitro* antimicrobial activity of natural products against bacteria associated by pressure injury. **Methods:** The test was conducted with crude extracts of leaves and crude extracts of stem bark of vegetal species *Caesalpiniaferrea* and *Caesalpiniapluviosa* against of strains *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), through in agar-well diffusion assay and determination of Minimal Inhibitory Concentrations (MIC). **Results:** The results showed that crude extract of leaves of *Caesalpiniaferrea* and *Caesalpiniapluviosa* were the most active with the diameter of inhibition zones statistically significant to all tested microorganisms, when compared with standard drug, beside that presented the best results of MIC. Conclusion: The result obtained potentiates the species *Caesalpineaferrea* and *Caesalpineapluviosa*as promising in the treatment of bacterial infections in pressure injury.

Keywords: Pressure injury. Antimicrobial activity.Caesalpinaceae

INTRODUCTION

Pressure injury (PrIs) and their treatment represent one of the most challenging clinical problems faced by patients, who generally is elderly, neurologically impaired, chronically hospitalized, or have chronic spinal cord injury [1]. The characteristic processes of pressure lesion, as poor blood circulation, complications from osteomyelitis, necrosis of muscle and soft tissue, ulceration of the skin or erosion into neighboring vital structures can devastate patients' health and quality of life affected [2, 3].

However, of according Singh et al. [4], the main problem of PrIs is bacterial infection due to exudative material, serous, crusted or hemorrhagic present on the surface of the pressure lesion which favors the growth the species bacterial as *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Proteus mirabilis*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*, that according Dai et al. [5] are species prevalent in pressure injury.

Bacterial contamination of chronic ulcers especially those associated PrIs is a universal and inevitable occurrence because of the evolutionary advantage of microorganisms to cut risks to their survival, in result, the treatment of pathology is done with antibiotic therapy [6]. According Lee et al. [7] the antibiotic therapy has been greatly important cornerstones of clinical medicine since the second half of the 20th century, however, the last decade of the 20th century and the first decade of the 21th century have witnessed the emergence and spread of antibiotic resistance in pathogenic bacteria around the World, and the consequent failure of antibiotic therapy.

In the context, currently, due the bacterial resistance, the search by antimicrobial agents of vegetal origin has increased substantially, because in your therapeutic arsenal there is compounds with high probability have potential antimicrobial and be an alternative of new antimicrobial agents, once these substances have a chemical composition which generally minimizes the development of resistance by the pathogens [6, 7].

Particularly to this study they were chosen two species of Leguminosae family, because your use in folk medicine to treatment of infections of skin and record to the presence of alkaloids, flavonoids, terpenoids and carotenoids, and other natural compounds that are directly related your biological activities [8], as study of Awouafack et al. [9] which recorded susceptibility of species *S. aureus*, *E. faecalis* and *E. coli* to crude extracts of twigs of *Eriosemarobustum,* supporting the biological activities to species this vegetal family.

The present study aimed investigate *in vitro* the antimicrobial activity of crude extracts from leaves and stem bark of species *Caesalpiniaferrea*and *Caesalpiniapluviosa* on growth of bacteria associated the infection of pressure injury.

MATERIAL AND METHOD

Biological Samples

Antimicrobial activity was carried out against strains of *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the Tecpar Laboratory®.

Plant material

The leaves and stem bark of *Caesalpiniaferrea* and *Caesalpiniapluviosa* were dried at room temperature and ground in a grinder. 3 kg were extracted, separately, with ethanol at a concentration of 70% (v/v) getting the crude extracts of leaves of *Caesalpiniaferrea* (CELCF) and crude extracts of leaves of *Caesalpiniapluviosa* (CELCP) and 4.5 kg were extracted, separately, with ethanol at a concentration of 70% (v/v) getting the crude extracts of stem bark of *Caesalpiniaferrea* (CESBCF) and crude extracts of leaves of *Caesalpiniapluviosa* (CBCP). An evaporation of the ethanol was performed using a rotary evaporator. Before used in antimicrobial activity bioassays, the extracts were dissolved in Dimethyl Sulfoxide 0.01% (DMSO 0.01%). A dilution was carried in double series from the maximum concentration of 500 µL/mL to 7.8 µL/mL.

Antimicrobial assays

Bacterial strains of standardized culture grown on Muller-Hinton nutrient agar at 37 °C for 24 h after time were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland scale. Then were, inoculated in Petri dishes with agar medium; four well of 6 mm diameter, were made in each plate

with sterile Pasteur pipettes and in each well was added 20 µl of each test material. DMSO 0.01% was used as negative control experiment and disc of ciprofloxacin (20 µg/mL) and gentamicin (20 µg/ml) were used as positive controls. The agar plates were incubated at 37 °C for 24 h. After this time then the plates were observed for the presence of inhibition of microbial growth that was indicated by clear zone around the wells. This zone around the wells was measured and each experiment together with control was replicated three times.

For the determination of the MIC (Minimal inhibitory concentrations), the-broth micro-dilution method was used as per NCCLS [10]. Strains *of S.aureus*, *S. epidermidis*, *E.coli* and *P. aeruginosa*were previously normalized to a concentration based on the standard of the 0.5 McFarland scale (1.5 x 10^8 CFU/mL) and inoculated in culture medium Mueller-Hinton for bacterial growth. In 96-well micro-plates were added a volume of 5 µL of bacterial inoculum in each well for each microorganism tested and 100 µL of the tested sample. A dilution of crude extract was carried in doble series from the maximum concentration of 500 µL/ml to 7.8 µg/ml. The negative control was the DMSO 0.01% and to positive control were used 20 µg/ml of gentamicin and ciprofloxacin. The 96-well micro-plates were placed in a bacteriological incubator at 37 °C for 24 h. After that time were added in the 96-well micro-plates 30 µL of resazurin reagent to indicate the presence of uninhibited bacterial growth (a pink/purple color) or inhibition (blue color) of growth in each well. Each experiment together with the control was replicated three times, and then the lowest concentration of each botanical as well as antimicrobial standard that inhibited the microbial growth after incubation was recorded [11].

Statistical analyses

Statistical evaluation of the results was performed using the BioEstat 5.3 and analysis of variance (ANOVA) was applied.

RESULTS AND DISCUSSION

In generally studies have highlighted that the bacterial infection delays the fibroblast development and inflammatory responses affecting the process of healing of pressure injury [12]. Singh and collaborators [4], evaluating exudate of Pressure injury in Spinal Injury Patients, recorded infection by *E. coli* in 38.88% and *Staphylococcus aureus* in 38.09% of patients. The study of Salcido and Lorenzo [13], showed that most common organisms isolated from PrIs were *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Corynebacterium.

Because of high bacterial infection in PrIs, the intervention recommended normally is antibiotic therapy, which enhances the development of bacterial resistance, since this is an inherent feature of microorganisms [8].

This is study it sought investigate the antimicrobial activity of crude extracts vegetal with alternative to treatment of bacterial infection, because these substances have chemical compositions which generally minimizes the development of resistance by the pathogens [7]. This perspective the crude extract of species *Caesalpiniaferrea* and *Caesalpiniapluviosa* showed promising results.

Data in table 1 indicated that the crude extract of leaves and crude extract of stem bark *C. ferrea*and *C.pluviosa*showed considerable antimicrobial activity against to all tested microorganisms. However, the crude extract of leaves the two species showed the best results with the diameter of inhibition zones statistically significant to all tested microorganisms, when compared with standard drug.

A few study with natural products have recorded susceptibility of different bacterial strains occurring in pressure lesion to crude extracts and organic fractions, as the study of Adhan [14] that investigated the antimicrobial activity of extracts of species vegetal *MenthaPiperita*, *MenthaLongifolia* and *OcimumBasilicum* against different bacterial species occurring in pressure lesion, as *Staphylococcus aureus*, *Klebsiellapneumoniae* and *Escherichia coli*, and recorded that combinations of *M. piperita,M. longifolia* and *O. basilicum* show synergistic effects against of bacteria; and study of Pattanayak et al. [15] that recorded susceptibility of *Escherichia coli* to extracts of leaves of *Mikaniascandens* and susceptibility of *Staphylococcus aureus* to methanolic extract of *M. scandens* in *in vitro* study of antimicrobial activity in wound.

The crude extracts of leaves *C. ferrea* and *C. pluviosa* from the results obtained in this study, inhibited of significant form the growth of *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*, with inhibition zone means between 20.5, 22.0, 21.5 and 34.0 mm, respectively, to crude extracts of leaves *C. ferrea*; and with inhibition zone means between 21.0, 30.5, 21.0 and 33.5 mm, respectively, to crude extracts of leaves *C. pluviosa*. These dates resemble to dates Noori [16] that recorded susceptibility of species *S. aureus* and *Klebsiella* sp. to extracts natural honey.

Table 1 :*In vitro* antimicrobial activity of crude extracts of leaves of *Ceaesalpineaferrea* (CELCF) and *Caesalpineapluviosa* (CELCP) and crude extracts of steam bark of *Ceaesalpineaferrea*(CESBCF) and *Caesalpineapluviosa* (CESBCP) against bacteria occurring Pressure lesion using in agar-well diffusion assays.

Treatment	Inhibition zone means (mm±SE)			
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***
CELCF				
500 µg/mL	20.5±1.0[b]	22.0±1.0[b]	21.5±1.0[b]	34.0±0.5[a]
250 µg/mL		21.0±0.5[b]		22.0±1.0[c]
125 µg/mL	20.0±1.0[b]	18.0±1.0[c]	21.0±0.5[b]	20.5±0.5[c]
62.5 µg/mL		11.0±0.5[e]		NA
31.2 µg/mL	18.5±0.5[c]	5.0±1.0[f]	19.0±0.5[c]	NA
15.6 µg/mL		NA		NA
7.8 µg/mL	NA	NA	16.5±1.0[d]	NA
	NA			
	NA		11.5±0.5[e]	
	NA			
			NA	
			NA	
CELCP				
500 µg/mL	21.0±0.5[b]	30.5±0.5[a]	21.0±0.5[b]	33.5±0.5[a]
250 µg/mL		21.0±1.0[b]		30.0±1.0[a]
125 µg/mL	20.5±1.0[b]	22.0±0.5[b]	21.0±0.5[b]	28.0±0.5[b]
62.5 µg/mL		NA		22.0±1.0[c]
31.2 µg/mL	20.0±0.5[b]	NA	17.0±1.0[d]	17.5±0.5[d]
15.6 µg/mL		NA		NA
7.8 µg/mL	15.0±1.0[d]	NA	15.5±0.5[d]	NA
	NA		8.0±1.0[f]	
	NA		5.0±0.5[g]	
	NA		NA	
CESBCF				
500 µg/mL	21.0±0.5[b]	18.0±1.0[c]	21.5±0.5[b]	28±1.0[b]
250 µg/mL		15.0±0.5[d]		NA
125 µg/mL	18.5±0.5[c]	NA	15.0±1.0[d]	NA
62.5 µg/mL		NA		NA
31.2 µg/mL	16.0±1.0[d]	NA	12.5±0.5[e]	NA
15.6 µg/mL		NA		NA
7.8 µg/mL	NA	NA	8.0±1.0[f]	NA
	NA		6.5±0.5[g]	
	NA		2.0±1.0[h]	
	NA		NA	
CESBCP				
500 µg/mL	21.5±0.5[b]	20.5±1.0[b]	15.0±1.0[d]	36.0±1.0[a]
250 µg/mL		20.5±0.5[b]		34.5±0.5[a]
125 µg/mL	20.0±1.0[b]	15.5±1.0[d]	11.5±0.5[e]	28.0±1.0[b]
62,5 µg/mL		11.0±0.5[e]		27.0±0.5[b]
31,2 µg/mL	18.0±0.5[c]	NA	NA	NA
15,6 µg/mL		NA	NA	NA
7,8 µg/mL	14.0±1.0[e]	NA	NA	NA
			NA	
	10.5±0.5[f]		NA	
	NA			
	NA			

Getamicin	32.0±0.5[a]	31.0±0.5[a]	33.0±0.5[a]	35.0±0.5[a]
Ciprofloxacin	21.0±0.5[b]	21.0±0.5[b] NA	22.0±0.5[b]	21.0±0.5[c] NA
DMSO 0.01%	NA		NA	

NA = no active

In the same column, means followed by the same letters do not differ statistically itself (p ≤0.05).

The figure 1 shows the Minimum Inhibitory Concentrations (MIC) values. The crude extracts of leaves *C. ferrea* and *C. pluviosa* showed the best results of MIC to all bacterial strains tested, with inhibiting the growth in concentrations between 250 – 125 µg/mL. These dates resemble to dates of Cooper et al. [17], that in assays in vitro with compounds isolated of honey against bacterial strains isolated of wounds, recorded the minimums values de MIC to these compounds. According Wagner and Ulrich-Merzenich [18], the mixture of bioactive constituents in plant extracts are responsible for biological activities the many vegetal species.

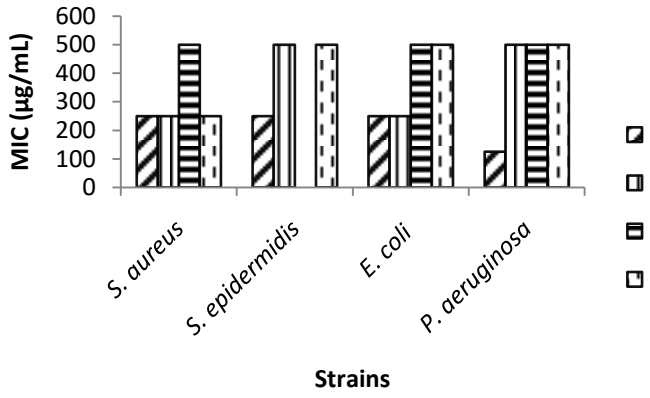

Fig. 1: MIC of crude extract of leaves (CELCF) and crude extract of stram (CESBCF) of *Caesalpineaferrea* and crude extract of leaves (CELCP) and crude extract of stram (CESBCP) of *Caesalpineapluviosa* using the microtitre assay.

There are few studies of antimicrobial activity of natural products against bacteria occurring in pressure lesion, most are with honey use, but the natural products it is a promising source of new chemical compounds for drug discovery [19, 20, 21], particularly in this study, the results obtained potentiates the species *Caesalpineaferrea*and*Caesalpineapluviosa* as promising in the treatment of bacterial infections in pressure lesion.

CONCLUSION

The crude extracts of leaves *Caesalpineaferrea*and*Caesalpineapluviosa* showed antimicrobial activity significant against the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*, associated to pressure lesion, making them promising in the treatment of bacterial infections in pressure injury.

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