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Antimicrobial activity of caatinga biome ethanolic plant extracts against gram negative and positive bacteria

Atividade antimicrobiana de extratos etanólicos do bioma Caatinga contra bactérias gram-negativas e positivas

Maria da Conceição A. de Sá,* Rodolfo de M. Peixoto,** Cristina da C. Krewer,*** Jackson Roberto G. da Silva Almeida,**** Agueda C. de Vargas,***** Mateus M. da Costa*

Resumo

A busca por tratamentos fitoterápicos intensificou-se nas últimas décadas. O uso abusivo das drogas antimicrobianas, a seleção de bactérias resistentes e as condições de manejo inadequadas são temas de impacto na medicina veterinária. Tendo em vista o acima descrito, objetivou-se avaliar a atividade antibacteriana de extratos etanólicos de plantas do bioma caatinga, contra patógenos de interesse veterinário. Foram utilizados seis extratos etanólicos de *Amburana cearensis* A.C.Smith*,* a *Selaginella convoluta* Arn.(Spring*),* a *Hymenaea courbaril L.*, a *Neoglaziovia variegata* (Arruda) Mez*., Bromelia laciniosa* Mart. e *Encholirium spectabile* Mart. A atividade antibacteriana destes extratos foi testada contra bactérias gram-negativas e positivas. Determinou-se a concentração bactericida mínima (CBM) para cada extrato. Os ensaios foram realizados em triplicata. Para os extratos avaliados foram encontradas atividades antibacterianas nos gêneros estudados com exceção de *Proteus* spp., *Nocardia* spp., *Staphylococcus caprae e Streptococcus agalactiae.* A umburana (*A. cearensis*) e o caroá (*Neoglaziovia variegata)* mostraram os menores valores de CBM. Considerando o baixo custo da fitoterapia e a atividade das plantas do bioma caatinga, outros estudos acerca da atividade *in vivo* e da caracterização fitoquímica tornam-se necessários.

Palavras-chave: extratos etanólicos, testes de sensibilidade, patógenos, veterinária.

Abstract

The search for phytotherapeutic (medicinal plant) treatments has been intensified in recent decades. The abusive use of antimicrobial drugs, selection of resistant bacteria and inadequate handling conditions are issues that have an impact on veterinary medicine. With this in mind, the objective of this study was to evaluate the antibacterial activity of ethanolic extracts of caatinga biome plants against pathogens of veterinary interest. Six ethanolic extracts of plants existing in the Caatinga biome of the Pernambuco semi-arid region were used, namely: *Amburana cearensis* A.C.Smith*, Selaginella convoluta* Arn.(Spring*), Hymenaea courbaril L.*, *Neoglaziovia variegata* (Arruda) Mez*., Bromelia laciniosa* Mart. and *Encholirium spectabile* Mart. The antibacterial activity of these extracts was tested against gram negative and positive bacteria. The minimum bactericidal concentration (MBC) for each extract was determined. Tests were carried out in triplicate. Antibacterial activities in the genuses studied were found for the extracts evaluated, with the exception of *Proteus spp., Nocardia* spp., *Staphylococcus caprae* and *Streptococcus agalactiae. Amburana cearensis* and *Neoglaziovia variegata* exhibited the lowest MBC values. Considering the low cost of phytotherapy and the activity of the caatinga biome plants, other studies related to the activity *in vivo* and the phytochemical characterization become necessary.

Keywords: ethanolic extracts, sensitivity tests, pathogens, veterinary medicine.

Introduction

Resistance to antimicrobial agents in human and animal health is a serious problem which requires not only the study of new approaches for the treatment of bacterial infections but also research for the development of new pharmaceuticals (Lathers, 2002). Therefore, the search for antibacterial properties of plant extracts has been stimulated and intensified (Miguel & Miguel, 1999, Stein et al., 2005).

* Laboratório de Microbiologia e Imunologia Animal da Universidade Federal do Vale do São Francisco, 56300-990, Petrolina,PE, Brasil

** Instituto Federal de Educação, Ciência e Tecnologia do Sertão Pernambucano, Campus Floresta, 56400-000, Floresta, PE, Brasil

*** Departamento de Morfologia da Universidade Federal de Santa Maria, 97119-900, Santa Maria, RS, Brasil

**** Núcleo de Estudos e Pesquisas de Plantas Medicinais da Universidade Federal do Vale do São Francisco, 56304-205, Petrolina, PE, Brasil

***** Departamento de medicina veterinária preventiva da Universidade Federal de Santa Maria, 97119-900, Santa Maria, RS, Brasil

Autor para correspondência: Mateus M. da Costa. E-mail: mateus.costa@univasf.edu.br; Tel. +55 81 3986 3800 Fax: +55 81 3986 3801.

Phytotherapy (medicinal plant therapy) is a method empirically used by humanity from ancient times until now (Weckesser et al., 2007). It is estimated that substances derived from plants constitute approximately 25% of medically prescribed agents in industrialized countries (Rates, 2001). In recent years, the prescription of phytotherapeutic products has grown in medical and veterinary clinical practice (Ryan et al., 2001).

Within this perspective, it is expected Brazil to be in a privileged position considering its extensive and diversified flora, holding approximately one third of the world's plants (Yunes, 2001). The Caatinga biome is the main ecosystem in the Brazilian Northeast Region, extending over the domain of semi-arid climates in an area of 73,683,649 ha, 6.83% of national territory, occupying the states of Bahia, Ceará, Piauí, Pernambuco, Rio Grande do Norte, Paraíba, Sergipe, Alagoas, Maranhão and Minas Gerais (Brasil, 2008). It contains various plants species of phytotherapeutic interest. In this diversity of plants we find *Amburana cearensis* A.C.Smith*, Selaginella convoluta* Arn.(Spring*), Hymenaea courbaril L., Neoglaziovia variegata* (Arruda) Mez*., Bromélia laciniosa* Mart., and *Encholirium spectabile* Mart, plants with a history of use for diverse types of illnesses, with some being sold in public markets (Agra, et al., 2005). *Hymenaea courbaril* L.*,* belongs to the *Fabaceae* family and phytochemical studies have detected the presence of diterpenes in the resin exuded through the trunk and in bark extracts of this plant (Nogueira et al., 2001). Terpenes have various biological activities, such as protection against infections and insect attacks (Robberts et al., 1997). A study developed by Vera et al. (2006) showed the activity of plants from the Brazilian Northeast against isolated aerobic bacteria of patients with leishmaniasis. The objective of this study was to evaluate the antibacterial activity of ethanolic extracts of plants from the caatinga biome against pathogens of veterinary interest.

Material and methods

Location of the experiment

This study was developed from February 2007 to May 2008 in the Animal Microbiology and Immunology Laboratory of Universidade Federal do Vale do São Francisco.

Isolates used

The antibacterial activity of these extracts was tested against the following pathogens of veterinary interest: *Escherichia coli, Enterococcus faecalis, Klebsiella* spp*. , Salmonella* spp*., Staphylococcus aureus, Pseudomonas aeruginosa, Rhodococcus equi, Listeria* spp*., Corynebacterium* spp*., Aeromonas* spp*., Proteus* spp*., Yersinia enterocolitica, Staphylococcus epidermidis, Staphylococcus intermedius, Streptococcus agalactiae, Streptococcus suis, Nocardia* spp*., Vibrio* spp*., Micrococcus* spp, *Staphylococcus caprae* and *Edwardsiella tarda.*

Collection of plant material

The plant material of *Amburana cearensis* (umburana de cheiro or cumaru), *Encholirium spectabile* (macambira de fleche), *Hymenaea courbaril* (Jatobá), *Neoglaziovia variegata* (caroá), *Bromelia laciniosa* (macambira de porco) and *Selaginella convoluta*, whose common name is jericó collected in the municipality of Lagoa Grande, PE. The plant material was collected and identified by a specialist in the area. Herbarium specimens of the species were codified and placed in the Herbarium at the Universidade Federal do Vale do São Francisco (HVASF). Collection was made of the whole plant of *Selaginella convoluta*, *Encholirium spectabile*, *Bromélia laciniosa* and *Neoglaziovia variegata*, and the bark of the trunk of *Hymenaea courbaril* and *Amburana cearensis*.

Preparation of the crude

All material was processed at the laboratory of the Medicinal Plant Studies and Research Center …. It was then submitted to drying in a forced air circulation laboratory oven at a temperature of 40ºC for 72 hours and protected from light and moisture. After this period, the material was ground to powder and macerated for three days in a percolator with ethanol at 95%. The material underwent filtration under a vacuum system, the extract was then concentrated in a rotary evaporator under reduced pressure (60ºC) to approximately 1/5 of the original volume. The extract was then clarified through alcohol extractions for three days, with the final extract being conserved in an amber glass container under refrigeration.

In vitro *sensitivity test*

For determination of antibacterial activity, a microdilution protocol was used following descriptions of the M7-A4 document of the NCCLS (NCCLS, 1997). For this purpose, 0.01g of extract was diluted in 10 mL of ethanol. The stock solution was diluted in a serial to obtain the concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.90 µg/mL. In inoculum preparation, colonies maintained in Muller-Hinton Agar were used to obtain a bacterial suspension with turbidity equivalent to 0.5 turbidity on the MacFarland scale. From this suspension, 10 µL (1x 10^4 UFC) was inoculated in each well containing a dilution of the ethanolic extract. From the dilution where visible bacterial growth was not observed, an aliquot of 10µl was removed, seeding it on the MH Agar surface and incubating it for 24 h at 37ºC. Afterwards, the minimum bactericidal concentration (MBC) was determined as the smallest concentration of the ethanolic extract under study capable of causing the death of the inocula. Tests were performed in triplicate.

Results and discussion

The caatinga is the only exclusively Brazilian biome, but is often forgotten from the scientific point of view. Even under the supposition of little biodiversity, this is not justified in virtue of its broad flora and fauna composition (Leal et al., 2003). This diversity supports an as yet unexplored great biotechnological potential.

Members of the Enterobactericeae family are considered as important pathogens for animals, as well as a large public health problem. The resistance of these groups of microorganisms is very well known and is associated with the horizontal exchange of resistance plasmids (Sherley et al., 2004). In this study, the antibacterial activity of the extracts against *E. coli, Klebsiella* spp., *Salmonella* spp., *Y.*

enterecolitica and *Edwardsiella* spp. (Table 1) was observed. In relation to the bacterial groups *Proteus* spp*., Nocardia* spp*., S. caprae* and *S. agalactiae*, the activity of the extracts was the same as observed in the alcohol control, proving the absence of activity. For *E. coli*, it was observed that *Neoglaziovia variegata* and *Encholirium spectabile* presented good antimicrobial activity, with an average MBC of 83.33 μL and 125 μL respectively, with the latter also showing activity against *Corynebacterium* spp. (41.67 μL). Bravo et al. (1999) reported activity of *Amburana cearensis* against *E.coli* and *Shigella flexneri*. In our study, this activity was observed for all the enteric pathogens, although for *Edwardsiella* these values were the lowest (62.5 mL). In relation to *Salmonella* spp. lower activity was observed (104.2) for the *Selaginella convoluta* extract. For *Y. enterocolitica*, in addition to *Hymenaea courbaril* (62.50 μL), *Neoglaziovia variegata* and *Selaginella convoluta* also presented good antibacterial activity at 83.33 μL and 93.75 μL, respectively.

Among the main pathogens found in water are *Aeromonas* spp. *Vibrio* spp. and *Pseudomonas* spp. When the sensitivity of *Aeromonas* spp. and *Vibrio* spp. was analyzed, it was observed that, with the exception of *Neoglaziovia variegata* (62.5 μL and 31.25 μL, respectively) and *Amburana cearensis* (62.5 μL and 31.25 μL, respectively), the other extracts presented low activity (Table 1). Oliveira et al. (2007), upon analyzing phases of different plant extracts of the Southeast region of Brazil, showed the absence of activity for *Pseudomonas aeruginosa*. The resistance of *P. aeruginosa* to various antimicrobial compounds is widely recognized (Figueiredo et al., 2007).

In relation to the low percentage of sensitivity of the extracts against the isolates, in the Gram negative bacteria (Figure 1), the cell wall is internally and externally constituted by membranes separated by glycopeptide (Hirsh & Zee, 2003); this double protection cellular composition reduces the action of the antibacterial compounds. In addition, it may be observed that the origin of collection of these pathogens is the southern region of Brazil, where the bacteria have a high rate of resistance associated with excessive use of the antimicrobial agents (Costa et al., 2006).

Gram positive cocci are related to diverse infectious conditions in veterinary medicine, especially mastitis and pyodermititis, often having public health implications, especially *Staphylococcus aureus* (Fagundes & Oliveira, 2004). Considering the species *Staphylococcus* spp., the extract of *Hymenaea courbaril* showed the lowest MBC. The antibacterial activity of the plant extracts of the caatinga biome against *Streptococcus suis,* showed the lowest MBC for *Amburana cearensis*, *Bromélia laciniosa* and *Selaginella convoluta* (83.33 μL). In relation to *Micrococcus* spp. greatest sensitivity was observed to the ethanolic extract *Neoglaziovia variegata* (52.08 μL).

The Gram positive bacilli, especially those of the soil, are associated with the origin of resistance of diverse antimicrobial compounds. These are also important veterinary pathogens, principally in immunosuppressed hosts, where the treatment with antimicrobial drugs might not be successful and might cause its death. Therefore, the search for alternatives must be considered (D'Costa et al, 2006). For *Rhodococcus equi*, the extract with greatest antibacterial activity was *Neoglaziovia variegata* (93.75 μL). For *Listeria* spp., for its part, the greatest inhibition occurred for the extract of *Amburana cearensis* (125 μL). In contrast, for *Corynebacterium* spp., the greatest activity was observed for *Amburana cearensis* (41.67 μL) and *Encholirium spectabile* (41.67 μL).

The abusive use of antimicrobial drugs, the selection of resistant bacteria and inadequate management conditions are themes that have an impact on veterinary medicine (Mathew et al., 2007). There are diverse studies with plant extracts for determination of antihelmintic activity; nevertheless, there is a lack of studies for determination of antibacterial activity (Rochfort et al., 2008). In addition, when they are undertaken, there is no connection among the plants and object of study, as well as the methodologies evaluated. In our study, antibacterial activity was found in most of the extracts evaluated; nevertheless, in general, *Amburana cearensis* and *Neoglaziovia variegata* showed the lowest values of MBC. The extracts evaluated were not active for *Proteus* spp*., Nocardia* spp*., S. caprae* and *S. agalactiae.*

Conclusion

Considering the low cost of phytotherapy and the activity of the caatinga biome plants, other studies related to the activity *in vivo* and the phytochemical characterization become necessary.

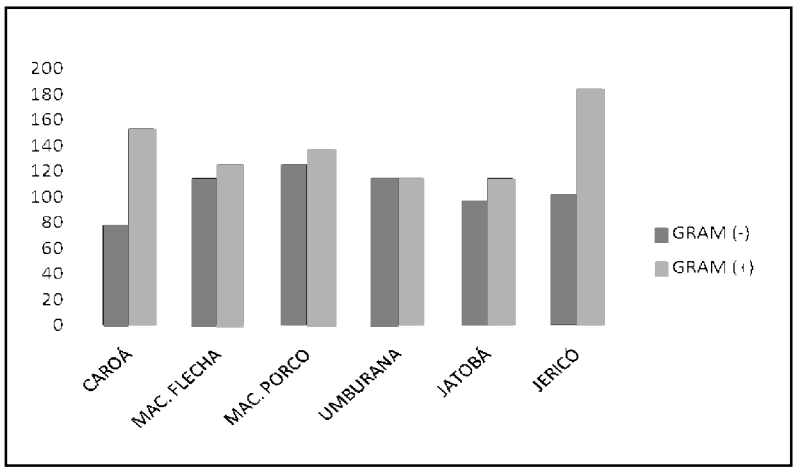

Figura 1: Means (μL) of inhibition of the ethanolic extracts of plants existing in the Caatinga biome of the Pernambuco semi-arid region against Gram (-) and Gram (+) bacteria.

Table 1: Susceptibility of the main pathogens of importance to Veterinary medicine in the face of plant extracts from the Caatinga Biome

	CAROÁ			MAC. DE FLECHA			MAC. PORCO			UMBURANA			JATOBÁ			JERICÓ		
	Mbc		Alcohol	Mbc		Alcohol	Mbc		Alcohol	Mbc		Alcohol	Mbc		Alcohol	Mbc		Alcohol
MICROORGANISM	Range	Mean	Mean	Range	Mean	Mean	Range	Mean	Mean	Range	Mean	Mean	Range	Mean	Mean	Range	Mean	Mean
Escherichia coli	62.5-125	83.33	354.17	0-125	125	354.17	0-125	125	354.17	125-250	166.7	354.17	62.5-125	104.2	354.17	62.5-250	145.8	354.17
Enterococcus faecalis	0-125	125	291.67	0-500	375	291.67	0-250	187.5	291.67	0-250	187.5	291.67	62.50-250	187.5	291.67	0-500	375	291.67
Klebsiella spp.	62.5-250	145.8	114.58	62.50-250	156.3	114.58	0-250	187.5	114.58	62.5-125	83.33	114.58	31.5-250	135.5	114.58	250-250	250	114.58
Salmonella spp.	62.5-250	145.8	250	125-250	166.7	250	125-500	250	250	62.5-250	145.8	250	62.5-250	145.8	250	62.5-125	104.2	250
Staphylococcus aureus	62.5-250	145.8	416.7	62.50-250	145.8	416.7	62.50-250	145.8	416.7	125-250	145.8	416.7	62.5-125	104.2	416.7	62.5-500	270.8	416.7
Pseudomonas aeruginosa	125-250	166.7	166.67	0-250	250	166.7	0-250	187.5	166.7	125-250	166.7	166.67	125-125	125	166.7	0-125	125	166.7
Rhodococcus equi	0-125	93.75	333.33	0-250	187.5	333.33	0-250	187.5	333.33	0-250	187.5	333.33	0-250	156.3	333.33	0-125	125	333.33
Listeria spp.	125-250	166.7	208.33	125-250	208.33	208.33	125-250	166.7	208.33	125-125	125	208.33	125.5	291.7	208.33	125-250	166.7	208.33
Corynebacterium spp.	250-500	333.33	437.5	31.25-62.50	41.67	437.5	31.25-62.50	52.08	437.5	31.25-62.50	41.67	437.5	62.5-62.5	62.5	437.5	31.25-62.50	41.67	437.5
Aeromonas spp.	0-62.50	62.5	125	0-125	125	125	0-500	500	125	0-62.50	62.5	125	0-62.50	62.5	125	0-250	250	125
Proteus spp.	0-250	250	250	0	0	250	0	0	250	0-500	281.3	250	0-500	281.3	250	62.5-500	270.8	250
Yersinia enterocolitica	62.5-125	83.33	104.2	62.5-125	104.2	104.2	62.5-250	145.8	104.2	62.5-125	104.2	104.2	62.5-62.50	62.5	104.2	31.25-125	93.75	104.2
Staphylococcus epidermidis	62.50-125	104.2	83.33	62.5-250	145.8	83.33	62.5-125	104.2	83.33	125-125	125	83.33	0-62.50	62.5	83.33	250-500	416.7	83.33
Staphylococcus intermedius	31.25-62.50	41.67	41.67	31.25-125	72.92	41.67	62.50-62.50	62.5	41.67	31.25-31.25	31.25	41.67	62.50-62.50	62.5	41.67	62.5-62.50	62.5	41.67
Streptococcus agalactiae	0-500	375	104.17	0-500	500	104.17	250-250	250	104.17	250-250	250	104.17	125-500	291.7	104.17	250-250	250	104.17
Streptococcus suis	125-250	166.7	104.17	125-125	125	104.17	62.50-125	83.33	104.17	62.5-125	83.33	104.17	125-125	125	104.17	62.50-125	83.33	104.17
Nocardia spp.	125-125	125	41.67	125-250	166.7	41.67	125-500	291.7	41.67	125-125	125	41.67	62.50-125	104.2	41.67	125-250	166.7	41.67
Vibrio spp.	31.25-31.25	31.25	62.5	31.25-62.50	52.08	62.5	31.25-125	72.92	62.5	31.25-31.25	31.25	62.5	15.62-125	67.71	62.5	15.62-125	57.29	62.5
Micrococcus spp.	31.25-62.50	52.08	83.33	62.5-125	104.2	83.33	62.5-125	104.2	83.33	125-125	125	83.33	62.50-125	104.2	83.33	62.50-125	83.33	83.33
Staphylococcus caprie	125-250	208.3	125	125-250	208.33	125	125-250	208.3	125	250-500	333.33	125	125-250	208.3	125	125-500	291.7	125
Edwadisiella spp.	62.50-62.50	62.5	104.17	125-250	166.7	104.17	62.5-125	104.2	104.17	62.50-62.50	62.5	104.17	62.50125	83.33	104.17	62.50-125	83.33	104.17

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