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MEDICINAL PLANTS FROM BRAZILIAN CAATINGA: ANTIBIOFILM AND ANTIBACTERIAL ACTIVITIES AGAINST *Pseudomonas aeruginosa*[1]

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ABSTRACT - The Caatinga biome covers a vast area in northeastern Brazil and presents a high level of biodiversity. It is known that about 400 plant species are used by semi-arid local communities for medical purposes. Based on ethnopharmacological reports, this study aims to screen 24 species from Caatinga regarding the ability to prevent biofilm formation and to inhibit the growth of *Pseudomonas* a*eruginosa* - a major opportunistic human pathogen and an important causative agent of morbidity and mortality. The effects of aqueous extracts, at 0.4 and 4.0 mg mL^{-1}, on biofilm formation and on growth of *P. aeruginosa* ATCC 27853 were studied using the crystal violet assay and the OD_{600} absorbance, respectively. The most active extracts were analyzed by thin-layer chromatography and high performance liquid chromatography. Our investigation pointed extracts of four species with potential application for the control of *P. aeruginosa*: *Anadenanthera colubrina* (Vell.) Brenan, *Commiphora leptophloeos* (Mart.) J.B. Gillett, *Myracrodruoun urundeuva* Allemão, whose antibiofilm effects (89%, 56% and 79% inhibition of biofilm, respectively) were associated with complete inhibition of bacterial growth, and *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson, which were able avoid 68% of biofilm formation and inhibited 30% bacterial growth. The qualitative phytochemical analyses reveal the complexity of the samples as well as the presence of compounds with high molecular weight.

Keywords: Biofilm. *Anadenanthera colubrina*. *Commiphora leptophloeos. Myracrodruoun urundeuva. Pityrocarpa moniliformis*. Traditional medicine.

PLANTAS MEDICINAIS DA CAATINGA BRASILEIRA: ATIVIDADES ANTIBIOFILME E ANTIBACTERIANA CONTRA *Pseudomonas aeruginosa*[1]

RESUMO - O bioma Caatinga abrange uma vasta área no nordeste do Brasil e apresenta uma expressiva biodiversidade. Sabe-se que aproximadamente 400 espécies de plantas são utilizadas por comunidades locais para fins medicinais. Com base em relatos etnofarmacológicos, este estudo tem por objetivo rastrear 24 espécies de plantas da Caatinga quanto à capacidade de impedir a formação de biofilme e de inibir o crescimento de *Pseudomonas aeruginosa* - importante patógeno oportunista humano e agente causador de morbidade e mortalidade. Os efeitos dos extratos aquosos, nas concentrações de 0,4 e 4,0 mg mL^{-1}, sobre a formação de biofilme e o crescimento de *P. aeruginosa* ATCC 27853 foram avaliados através do ensaio de cristal violeta e da densidade óptica a 600 nm, respectivamente. Os extratos mais ativos foram analisados por cromatografia em camada delgada e cromatografia líquida de alta eficiência. Nossa investigação indicou extratos de quatro espécies com potencial aplicação para o controle de *P. aeruginosa*: *Anadenanthera colubrina* (Vell.) Brenan, *Commiphora leptophloeos* (Mart.) J.B. Gillett, *Myracrodruoun urundeuva* Allemão, cujo efeitos antibiofilme (89%, 56% e 79% de inibição de biofilme, respectivamente) foram associados com a completa inibição do crescimento bacteriano, e, *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson, a qual foi capaz de evitar 68% da formação de biofilme e inibiu em 30% o crescimento bacteriano. As análises qualitativas fitoquímicas indicam heterogeneidade entre as amostras, bem como a presença de compostos com elevado peso molecular.

Palavras-chave: Biofilme. *Anadenanthera colubrina*. *Commiphora leptophloeos. Myracrodruoun urundeuva*. *Pityrocarpa moniliformis.* Medicina tradicional.

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INTRODUCTION

The folk medicine has a great contribution to human health care long before modern medicine began. Nowadays, the use of medicinal plants as a source of remedies remains common to the medical traditions of many cultures and there is a strong evidence of integration between traditional and modern methods of health care. Understanding the practices of the past which are maintained by the traditional knowledge passed generation by generation, it provides insight into and may lead to improvements in actual pharmaceutical practice (VANDEBROEK et al., 2011, MEDEIROS; ALBUQUERQUE, 2012). Therefore, there is an urgent call to collect information on and to identify the best health practices all over the world, and use them cost-effectively to enhance health care delivery (ORTEGA, 2006).

The Caatinga, typical semi-arid vegetation, represents the fourth largest area covered by a single vegetation form in Brazil and accounts account for about 60% of the northeast territory (ANDRADE-LIMA, 1981, SAMPAIO et al., 2002). Several publications describe the rich flora in this region as having many medicinal purposes and great phytochemical potential (AGRA et al., 2007; ALBUQUERQUE et al., 2007a; 2012). In addition to being widely known and used by local communities, many medicinal species in the Caatinga are sold as herbal products (ALBUQUERQUE et al., 2007b; CARTAXO et al., 2010; MONTEIRO et al., 2011).

Pseudomonas aeruginosa is a major opportunistic human pathogen and an important causative agent of morbidity and mortality. In addition antimicrobial resistance of *P. aeruginosa* remains a challenge for clinical treatment, antibiotic selection and effectiveness. This bacterium is able to express all known mechanisms of antimicrobial resistance, such as the low outer membrane permeability, the expression of broad multidrug efflux systems and the horizontal gene transfer (STRATEVA; YORDANOV, 2009). Therefore, *P. aeruginosa* often exhibits a multidrug-resistant or even pan drug-resistant phenotype, warranting its reputation as a 'superbug' and highlighting its clinical significance (SOULI et al., 2008; XIAO et al., 2012). Nosocomial infections having multidrug-resistant *P. aeruginosa* as ethiologic agent is frequently worldwide, accounting for about of 25% of isolated strains from two nationwide surveillance programmes in China (XIAO et al., 2012). In addition, *P. aeruginosa* has emerged as a pathogen adept at adhering to surfaces and forming biofilms, a bacterial lifestyle which cells are significantly more resistant to antimicrobials agents than as planktonic cells, causing chronic and very difficult to eradicate infections (RYBTKE et al., 2011). *P. aeruginosa* ranks first among all nosocomial pathogens related to pneumonia in intensive care in Brazil (ROSSI, 2011) and in United States of America (RICHARDS et al. 1999) – a usually biofilm-associated infection.

Targeting bacterial virulence (like biofilm formation) is an alternative approach to antibacterial therapy that offers promising opportunities to inhibit pathogenesis without threatening bacterial existence, resulting in a reduced selection pressure for drug-resistant mutations (CEGELSKI et al., 2008). Plants have been used as medicine in all cultures and the interest in research involving antimicrobial activity and medicinal properties of plants has increased. Given the great importance of biofilm formation in the infectious process, the association of screening of medicinal plants and the modulation of *P. aeruginosa* biofilm started to be considered (DING et al., 2011). We used aqueous extracts to reproduce the traditional medicine preparation, and thereby, it enables us to evaluate their effectiveness, considering *in vitro* antibacterial/antibiofilm properties against this important pathogen. In this context, we investigated 24 species of plants from Caatinga vegetation (45 aqueous extracts) for their ability to inhibit planktonic growth and to prevent biofilm formation by *P. aeruginosa*. Also, we performed preliminary phytochemical analysis of the most active extracts.

MATERIALS AND METHODS

The plants were collected at the Parque Nacional do Catimbau (PARNA from Catimbau), Pernambuco State, Brazil (8°30'0.07"S and 37° 26'41.00"W), in July and August 2009. The species were chosen according to the published ethnopharmacological data and using information from the local community. The taxonomic identifications were performed by the MSc. Alexandre Gomes da Silva from the Federal University of Pernambuco, Pernambuco, Brazil. Voucher specimens were deposited in the herbarium Dárdano de Andrade-Lima at Agronomic Institute of Pernambuco (IPA), Brazil (Table 1). Aqueous extracts were prepared by static maceration of the powdered dried material with sterilized water [1:9; (w:v)] at 22 °C during 24 h (TRENTIN et al., 2011) and stock solutions (1.0 and 10 mg mL^{-1}) were prepared by solubilization of the dried extract in water and sterilized by filtration (0.22 µm).

The preliminary phytochemical analysis was developed through analyzing the extracts by thin-layer chromatography - TLC (Kieselgel 60 GF_{254}, 0.2 mm, Merck, Germany) using ethyl acetate:water:acetic acid:formic acid [9:2.3:1:1 (v:v:v)] as eluent. The plates were visualized under UV light (254 and 365 nm) and using standard procedures: ferric chloride 2% for polyphenol compounds, Dragendorff's reagent for alkaloids, anisaldehyde/ sulfuric acid for steroids and terpenes, ninhydrin for amines and aminoacids, and iodine vapor as an universal reagent (WAGNER; BLAT, 1996). In addi-

tion, HPLC analyses were carried out on a Shimadzu LC-20AT coupled to a diode array detector. A reversed-phase column Shim-pack VP-ODS (250 mm × 4.6 mm I.D., 5µm, Shimadzu) was used. The gradient elution was performed with solution A - acetonitrile: water (5:95, [v/v]), and solution B - comprising 100% acetonitrile, at a flow rate of 0.6 mL min^{-1}. Solution B increasing up from 5% to 100% in 60 min. The injection volume was 20 µL (2.5 mg mL^{-1} solution).

Biological experiments were developed using *Pseudomonas aeruginosa* ATCC 27853 which was cultured overnight at 37 °C in Mueller Hinton agar (Oxoid Ltd., England). Colonies were suspended in 0.9% saline to obtain a bacterial suspension (3×10^8 CFU mL^{-1}). The biofilm formation and the bacterial growth were evaluated employing 96-well polystyrene plates. To each well we added 80 µL of the bacterial suspension, 80 µL of the extract (0.4 mg mL^{-1} or 4.0 mg mL^{-1} in the wells) and 40 µL of TSB (Oxoid Ltd., England), and the microplate was incubated at 37 °C for 6 h. After, the content was removed and the plates were washed with saline solution. The remaining attached bacteria were fixed and stained with crystal violet. The stain was solubilized with DMSO and the OD at 570 nm was determined. Bacterial growth was evaluated measuring the difference between OD_{600} absorbance at initial time and after 6 h (incubation time). Gentamicin sulfate 8 µg/mL (Sigma-Aldrich Co., USA) was used as a control for the inhibition of bacterial growth. Since does not exist a commercially available non-biocidal compound possessing antibiofilm activity, we cannot apply a positive control to antibiofilm activity. Extracts were replaced with sterile water to represent 100% of biofilm formation and planktonic bacterial growth (untreated control). Values higher than 100% represent a stimulation of biofilm formation or bacterial growth in comparison to the untreated control. Experiments were carried out in triplicate. The results were expressed as percentual mean ± standard deviation and analyzed using the Student's t-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Pseudomonas aeruginosa was exposed to the 45 extracts of several plant species from Caatinga, as showed in Table 1. The different parts used in the preparation of the extracts demonstrated differentiated responses to the inhibition of planktonic growth and biofilm formation by *P. aeruginosa*. The most significant results were obtained for the extracts produced from stem bark of the species *Anadenanthera colubrina*, *Commiphora leptophloeos* and *Myracrodruoun urundeuva*. Furthermore, it was found that the extract of the leaves of *Pityrocarpa moniliformis* presented moderate activity against growth and biofilm formation.

At 0.4 mg mL^{-1} no extract showed remarkable activity, and the stem bark of *C. leptophloeos* was the most effective extract to inhibit the *P. aeruginosa*, allowing about 65% of growth and 75% of biofilm formation. Regarding extracts at 4.0 mg mL^{-1}, the lowest rates of biofilm formation, when compared to untreated control, were found using *A. colubrina* (11%), *M. urundeuva* (21%), and *C. leptophloeos* (44%) stem barks and *P. moniliformis* (32%) leaves.

Table 2 summarizes the effect of the extracts against *P. aeruginosa*, classifying them according to the activity range. At 4.0 mg mL^{-1}, the results demonstrated that among all tested extracts, only 2 (4.4% - stem bark of *A. colubrina* and *M. urundeuva*) possess high ability to prevent biofilm formation and that 3 (6.7% - stem bark of *A. colubrina*, *C. leptophloeos* and *M. urundeuva*) present high ability to inhibit *P. aeruginosa* growth (Table 2). These most active plants are traditionally used in folk medicine as anti-inflammatory and antiseptic agents (AGRA et al., 2007) and, regarding these 3 species (stem bark), the formation of biofilm by *P. aeruginosa* seems to be avoided through antibacterial properties, since their extracts strongly affected bacterial growth (Table 1). Considering *P. moniliformis*, it is used by Caatinga communities as healing agent (TRENTIN et al., 2011) and the leaves extract allowed about 32% of biofilm formation while bacterial growth was about 69%. However, our previous screening evaluating the same extracts against *Staphylococcus epidermidis* revealed a different scenario, since 15.5% and 2.2% of them presented high inhibition of biofilm formation and planktonic growth, respectively. In addition, only one extract showed *S. epidermidis* biofilm prevention associated to its bactericidal effect (TRENTIN et al., 2011).

The qualitative phytochemical screening was carried out with these four active extracts. The TLC-fingerprint indicated the presence of polyphenols, steroids, terpenes and amines/aminoacids (data not shown). The chromatograms obtained by HPLC showed a complex and similar profile of the four extracts (Figure 1) in agreement with TLC analysis. The qualitative phytochemical analysis reveals the complexity of these aqueous extracts, presenting several absorption peaks in overlapping retention times even after several attempts of chromatogram optimization, especially in stem bark samples, which is indicative of compounds with high molecular weight. In this sense, Siqueira et al. (2012), studying medicinal plants from Brazilian semiarid, highlighted that the group of plant with antimicrobial potential showed a higher content of tannins compared to a control group. Moreover, the antimicrobial activity of this class of metabolites is well documented. Unfortunately, HPLC-PDA is a limited analytical tool regarding the identification of natural products and it is harder when analyzing an aqueous extract. The strategy of co-injection of both standards compounds

and the extract could induce false results because the chromatogram baseline and the peaks resolution were impaired by sample complexity. Taken together the limitation of PDA detector to provide chemical features for structural elucidation and the aim of this study, a qualitative comparison between the most active aqueous extracts was performed. On this way, (i) the phytochemical analysis combined with (ii) the origin of plant material (stem bark and leaves), (iii) the extraction method applied (aqueous maceration), (iv) the association between tannin content and the effects popularly attributed to wound-healing and anti-inflammatory Caatinga plants (ARAÚJO et al., 2008) and (v) the data about the potential Anacardiaceae, Bursereaceae and Fabaceae to synthesize tannins, particularly by stem bark tissues enables us to suggest the tannins as the possible bioactive secondary metabolites in these samples.

Table 1. Aqueous extracts of the Caatinga plant species against biofilm formation and planktonic growth of *P. aeruginosa* ATCC 27853.

Family Species Voucher	Parts used	*P. aeruginosa*			
		0.4 mg mL^{-1}		4.0 mg mL^{-1}	
		Biofilm formation (%)	Bacterial growth (%)	Biofilm formation (%)	Bacterial growth (%)
Apocynaceae *Allamanda blanchetii* A.DC. IPA 84112	Branches	116.5 ± 4.8*	95.3 ± 0.4	109.4 ± 8.0	123.5 ± 3.0*
	Leaves	100.2 ± 4.4	89.5 ± 0.4	91.8 ± 9.4	99.6 ± 1.4
Anacardiaceae *Myracrodruoun urundeuva* Alemão IPA 84059	Branches	108.3 ± 4.8	83.0 ± 1.7*	100.4 ± 4.5	90.0 ± 1.2
	Leaves	115.5 ± 6.9	75.3 ± 1.0*	109.2 ± 5.9	97.6 ± 2.0
	Stem bark	147.3 ± 3.8*	81.9 ± 3.8*	20.7 ± 2.0*	0.1 ± 2.6*
Burseraceae *Commiphora leptophloeos* (Mart.) J.B.Gillett IPA 84037	Branches	95.8 ± 6.7	87.7 ± 0.2	103.3 ± 7.8	153.6 ± 6.7*
	Stem bark	75.6 ± 0.9*	65.1 ± 2.1*	44.0 ± 2.8*	0.0 ± 15.3*
Cactaceae *Melocactus zehntneri* (Britton & Rose) Luetzelb. IPA 85028	Roots	98.6 ± 0.3	110.6 ± 5.8	103.7 ± 1.2	164.6 ± 0.9*
	Cephalium	85.8 ± 2.4*	102.4 ± 5.8	122.0 ± 5.3	138.3 ± 1.7*
Combretaceae *Buchenavia tetraphylla* (Aubl.) R.A. Howard IPA 84104	Leaves	140.2 ± 6.7*	73.9 ± 4.7*	103.7 ± 2.4	83.5 ± 4.1*
Euphorbiaceae *Jatropha mutabilis* (Pohl) Baill. IPA 84054	Roots	124.6 ± 2.5*	87.6 ± 4.7	126.1 ± 5.2*	110.3 ± 1.7
	Branches	116.5 ± 6.6	84.5 ± 5.2	130.0 ± 5.8	132.1 ± 2.2*
Fabaceae - Caesalpinioideae *Chamaecrista desvauxii* (Collad.) Killip IPA 84064	Leaves	128.4 ± 8.8*	91.7 ± 3.4	96.2 ± 5.5	110.7 ± 2.0*
	Fruits	122.2 ± 1.7*	83.5 ± 4.2	113.9 ± 4.5	97.4 ± 1.1
Fabaceae - Caesalpinioideae *Chamaecrista cytisoides* (DC. ex Collad.) H.S. Irwin & Barneby IPA 84103	Branches	118.6 ± 3.2	103.6 ± 2.7	114.1 ± 17.4	92.8 ± 1.4
Fabaceae - Caesalpinioideae *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea* IPA 84035	Leaves	104.7 ± 5.4	78.4 ± 0.6	163.3 ± 5.3*	84.5 ± 2.4*
	Fruits	91.7 ± 0.6	78.3 ± 2.8	202.3 ± 10.4*	56.1 ± 0.7*
Fabaceae - Caesalpinioideae *Parkinsonia aculeata* L. IPA 84113	Leaves	98.9 ± 0.3	92.4 ± 2.1	101.2 ± 4.4	128.4 ± 7.8*
Fabaceae - Caesalpinioideae *Senna macranthera* (Collad.) H.S. Irwin & Barneby var. *macranthera* IPA 84045	Fruits	93.8 ± 1.5	95.7 ± 1.9	89.9 ± 2.0*	118.8 ± 3.0*
Fabaceae - Caesalpinioideae *Senna splendida* (Vogel.) H.S. Irwin & Barneby IPA 84045	Fruits	93.8 ± 1.5	95.7 ± 1.9	89.9 ± 2.0*	118.8 ± 3.0*
Fabaceae - Cercideae *Bauhinia acuruana* Moric. IPA 84042	Branches	93.1 ± 7.8	94.3 ± 0.1	98.5 ± 8.2	98.3 ± 6.4
	Fruits	155.2 ± 12.1*	82.0 ± 5.0*	119.8 ± 6.0	114.2 ± 3.1*
	Leaves	108.8 ± 1.4	95.1 ± 2.9	102.7 ± 3.6	78.7 ± 1.5*

Results represent mean ± standard deviation of 3 experiments.
* Represents significant difference in relation to control ($p < 0.05$).

Table 1. Continued.

Family Species Voucher	Parts used	*P. aeruginosa*			
		0.4 mg mL^{-1}		4.0 mg mL^{-1}	
		Biofilm formation (%)	Bacterial growth (%)	Biofilm formation (%)	Bacterial growth (%)
Fabaceae - Faboideae *Dioclea grandiflora* Mart. ex Benth IPA 84057	Leaves	122.9 ± 6.9	82.4 ± 6.5	114.3 ± 3.6*	85.4 ± 1.6*
	Branches	113.0 ± 1.7	100.0 ± 3.0	84.5 ± 1.8*	103.9 ± 3.0
	Fruits	86.6 ± 3.9*	92.0 ± 1.4	85.9 ± 5.2*	112.2 ± 3.8
Fabaceae - Faboideae *Myroxylon peruiferum* L.f. IPA 84110	Leaves	100.2 ± 9.0	106.2 ± 4.0	128.0 ± 0.4*	111.5 ± 1.2*
Fabaceae - Mimosoideae *Anadenanthera colubrina* (Vell.) Brenan var. *colubrina* IPA 84039	Fruits	97.6 ± 6.2	88.2 ± 6.3	95.3 ± 0.7	100.2 ± 1.0
	Leaves	127.0 ± 1.8*	86.4 ± 4.3*	95.5 ± 1.4	67.8 ± 0.7*
	Branches	132.1 ± 1.9	104.3 ± 3.1	130.7 ± 31.4	99.3 ± 0.5
	Stem bark	124.1 ± 0.2*	97.8 ± 5.8	10.6 ± 4.1*	0.1 ± 12.5*
Fabaceae - Mimosoideae *Piptadenia viridiflora* (Kunth) Benth. IPA 84036	Branches	109.4 ± 5.6	121.8 ± 7.3	96.7 ± 2.0	118.8 ± 0.3*
	Fruits	71.6 ± 8.6*	92.5 ± 2.0	90.6 ± 2.0	97.9 ± 0.1
Fabaceae - Mimosoideae *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson IPA 84048	Leaves	205.0 ± 13.5*	76.0 ± 3.2*	32.3 ± 6.4*	69.2 ± 10.8*
Malvaceae *Sida galheirensis* Ulbr. IPA 84078	Branches	111.8 ± 4.9*	97.8 ± 2.5	75.7 ± 3.8	98.0 ± 1.4
	Leaves	121.2 ± 0.6*	99.5 ± 3.7	69.9 ± 9.6*	104.7 ± 2.9
Malpighiaceae *Stigmaphyllon paralias* A. Juss. IPA 84041	Leaves	155.2 ± 1.4*	79.3 ± 0.9	126.3 ± 15.1	124.9 ± 9.4*
Myrtaceae *Eugenia brejoensis* Mazine IPA 84033	Leaves	107.9 ± 4.0	98.9 ± 1.2	104.1 ± 12.0	100.3 ± 2.8
Ochnaceae *Ouratea blanchetiana* Engl. IPA 84044	Branches	118.2 ± 6.4	84.4 ± 3.9	119.6 ± 2.1	103.3 ± 2.8
	Leaves	150.7 ± 12.3*	82.5 ± 2.3	104.2 ± 11.7	104.5 ± 5.0
Polygalaceae *Polygala boliviensis* A.W.Benn. IPA 84066	Inflorescences	95.8 ± 4.3	84.9 ± 3.0	119.2 ± 2.1	102.5 ± 0.9
	Leaves	104.2 ± 0.4*	82.2 ± 0.7	91.5 ± 1.9*	88.6 ± 3.0*
	Branches	106.3 ± 4.6	86.7 ± 6.1	115.4 ± 0.3	139.8 ± 9.0*
Polygalaceae *Polygala violacea* Aubl. IPA 84051	Leaves	114.2 ± 0.4*	112.9 ± 4.6	112.4 ± 16.6	116.7 ± 1.0*
	Roots	151.2 ± 13.2*	91.3 ± 5.8	96.1 ± 0.7	107.5 ± 1.6*

Results represent mean ± standard deviation of 3 experiments.
* Represents significant difference in relation to control ($p < 0.05$).

Table 2. Relationship among the number of aqueous extracts (%) and their respective anti-*P. aeruginosa* activities.

	0.4 mg mL^{-1}		4.0 mg mL^{-1}	
	Biofilm (%)	Growth (%)	Biofilm (%)	Growth (%)
Stimulate	53.3	6.7	35.6	33.3
No effect	35.6	35.6	37.8	37.8
Up to 25% inhibition	8.9	53.3	13.3	13.3
From 26 to 50% inhibition	2.2	4.4	4.4	8.9
From 51 to 75% inhibition	-	-	4.4	-
From 76 to 100% inhibition	-	-	4.4	6.7

This is the first study to screen the potential of extracts of Caatinga plant species to prevent the biofilm formation by *P. aeruginosa*. Since just 6.7% of the assayed extracts in this study demonstrated expressive activity against *P. aeruginosa*, the difficulty to control this pathogen with some Brazilian Caatinga plant extracts is stressed. This is in agreement with many studies that investigate antibacterial activity of medicinal plants extracts in several countries, such as Spain, Cuba, Colombia, Siberia, Africa, Arabia and India (HERRERA et al., 1996; MARTÍNEZ et al., 1996; LOPEZ et al., 2001; KOKOSKA et al., 2002; KONÉ et al., 2004; MOTHANA; LINDEQUIST, 2005; KUMAR et al, 2006). Considering that plant secondary metabolism is responsive and modulated by environmental conditions and by plant-pathogen interactions, we might hypothesize that the low number of active extracts observed herein against *P. aeruginosa* could be related to the lower presence of Gram-negative bacteria in semi-arid soil of Northeastern Brazil (GORLACH-LIRA; COUTINHO, 2007).

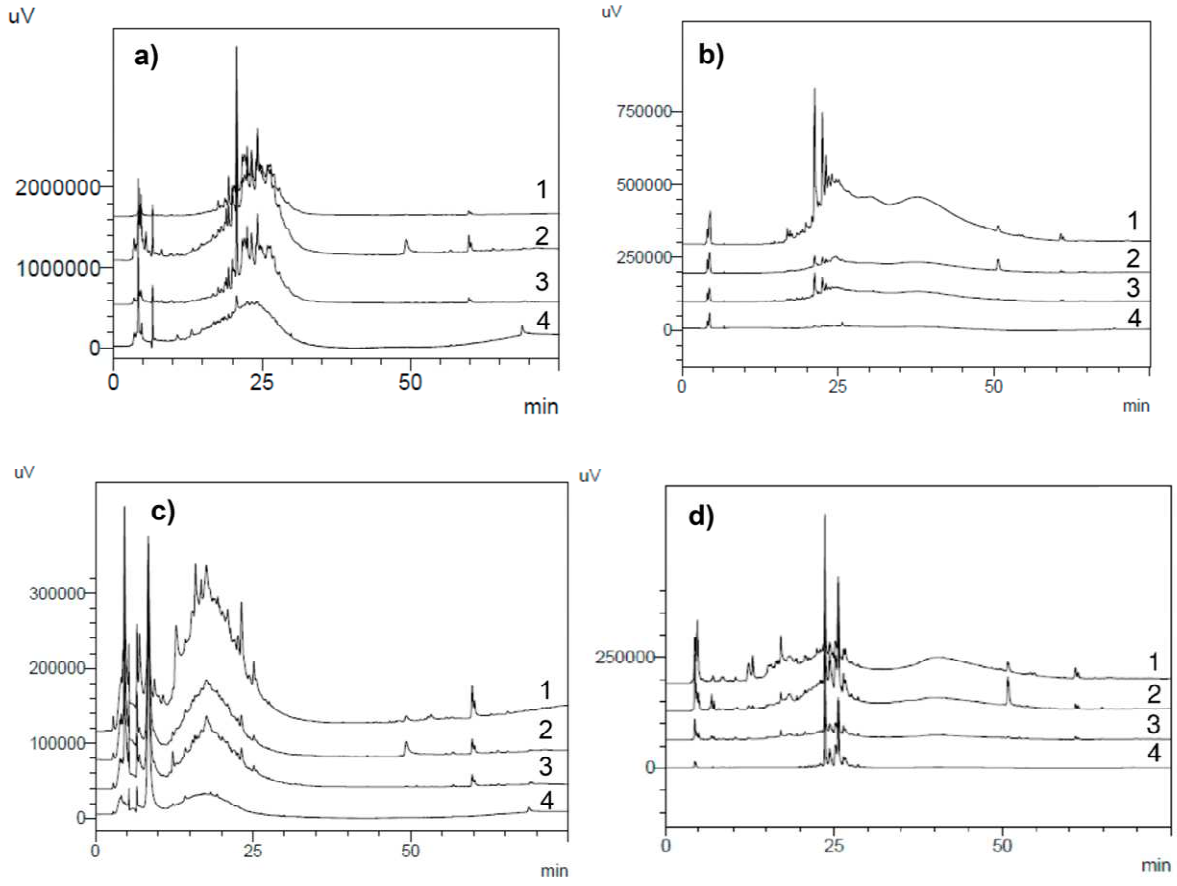

Figure 1. Qualitative HPLC-DAD chromatogram of the aqueous extracts: a) stem bark of *A. colubrina*; b) stem bark of *C. leptophloeos*; c) stem bark of *M. urundeuva*; d) leaves of *P. moniliformis*. Chromatograms 1, 2, 3, and 4 represent wavelengths of 210, 254, 273 and 365 nm, respectively.

CONCLUSION

Overall, this work point to the importance of four aqueous extracts against *P. aeruginosa*, which were produced from plants found in Caatinga vegetation and used by Caatinga community (especially stem bark of *A. colubrina*, *C. leptophloeos* and *M. urundeuva* and also leaves of *P. moniliformis*). The

antibiofilm action of these extracts against *P. aeruginosa* is associated to the inhibition of bacterial growth. The features of the samples (aqueous extraction and part of the plant used) combined with the qualitative phytochemical analysis of the most active extracts allowed us to propose that high molecular weight compounds – like tannins – might be the compounds responsible for the anti-*P. aeruginosa* action.

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