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Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against *Staphylococcus epidermidis* planktonic and biofilm lifestyles

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ABSTRACT

Ethnopharmacological relevance: Medicinal plants from the Caatinga, a Brazilian xeric shrubland, are used in folk medicine to treat infections. These ethnopharmacological data can contribute to obtaining new antimicrobial/antibiofilm extracts and natural product prototypes for the development of new drugs. The aim of this study was to investigate the antibiofilm and antibacterial activities of 45 aqueous extracts from 24 Caatinga plant species.
Materials and methods: The effect of aqueous extracts on planktonic cells and on biofilm formation by *Staphylococcus epidermidis* was studied by the OD_{600} absorbance and by the crystal violet assay, respectively. Scanning electron microscopy (SEM) was used to generate comparative images of extract-treated and untreated biofilms. Chromatographic analyses were performed to characterize the active extracts.
Results: The *in vitro* screening, at 0.4 mg/mL and 4.0 mg/mL, showed 20 plants effective in preventing biofilm formation and 13 plants able to inhibit planktonic bacterial growth. SEM images demonstrated distinct profiles of bacterial adhesion, matrix production and cell morphology according to different treatments and surfaces. The phytochemical analysis of the selected active extracts indicates the polyphenols, coumarins, steroids and terpenes as possible active compounds.
Conclusion: This study describes the first antibiofilm and antibacterial screening of Caatinga plants against *S. epidermidis*. The evaluation presented in this study confirms several ethnopharmacological reports and can be utilized to identify new antibiofilm and antibacterial products against *S. epidermidis* from traditional Brazilian medicine.

1. Introduction

The Caatinga, a xeric shrub-dominated biome of northeastern Brazil, supports a high, yet poorly studied, diversity of plant resources. Caatinga is a matrix of thorn shrublands and seasonally dry forests, with pockets of montane rain forests and savannah. It covers most of the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and the northeastern part of Minas Gerais in Jequitinhonha Valley, occupying an area of approximately 735,000 km^2 (Basso et al., 2005). The annual mean temperature in the region is 27.5 °C (Alves et al., 2011), the humidity is low, and the average annual rainfall is 250–500 mm (Basso et al., 2005). With respect to soils, there is a predominance of luvisols, eutrophic inceptisols and vertisols, in addition to sparsely scattered rocky outcroppings (Ab'Saber, 1974). They are fertile, well drained, and oxygenated (Basso et al., 2005). The dry season lasts seven months or more and the winter is the rainy season, in which temperatures are not as high. These characteristics making the Caatinga a peculiar kind of vegetation adapted to the prevailing local ecological conditions.

A review of the literature from the northeast of Brazil reveals that traditional medicinal practices have been used to treat a variety of illnesses including skin and gastrointestinal disorders, tuberculosis and urinary tract infections (Agra et al., 2007a,b). However, some of them still have not been subjected to scientific study to confirm their effectiveness for a given disease (Cartaxo et al., 2010). Taking into account current concerns over bacterial multi-resistance, the

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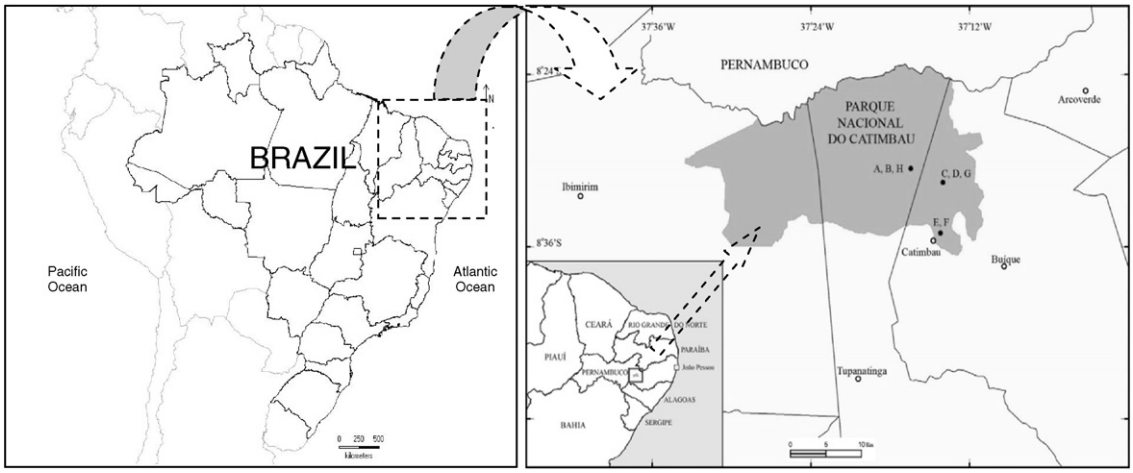

Fig. 1. Geographical localization of PARNA do Catimbau (Buíque, Tupanatinga and Ibimirim municipalities), Pernambuco state, Brazil.

screening of potential new antibiofilm and antibacterial agents as therapeutic alternatives is an important issue.

Staphylococcus epidermidis has attracted substantial interest in recent years because it has become the most important cause of nosocomial infections (Vuong and Otto, 2002). Its pathogenicity is mainly due to the ability to form biofilms (an important virulence factor), a process in which planktonic bacteria adhere to a surface and initiate the development of sessile microcolonies surrounded by an extracellular matrix, existing as a bacterial community (Otto, 2009). Biofilm-associated microorganisms have been shown to be related to more than 65% of all medical infections, including endocarditis, otitis, prostatitis, periodontitis, conjunctivitis, vaginitis, and infections related to cystic fibrosis. In addition, biofilms are important colonizers of a wide variety of medical devices, such as catheters and prostheses (Donlan and Costerton, 2002). The costs linked to vascular catheter-related blood-stream infections caused by *S. epidermidis* amount to an estimated US$2 billion annually in the United States (Otto, 2009). In a biofilm, the bacterium is protected against attacks from both, the immune system and antibacterial treatment, making *S. epidermidis* infections difficult to eradicate (Vuong and Otto, 2002; Davies, 2003; Antunes et al., 2011). The inhibition of virulence targets could bring new antibacterial molecules with radically new mechanism of action and represent an innovative therapeutic concept (Escaich, 2008).

Therefore, we screened 45 aqueous extracts from 24 Caatinga plants, used in local traditional medicine, for the potential to inhibit the biofilm formation and the planktonic bacterial growth of *S. epidermidis*.

2. Materials and methods

2.1. Plant material: source, sampling and identification

Based on ethnobotanical information (Agra et al., 2007a,b and local community information) the plants were collected from several habitats at Parque Nacional do Catimbau (PARNA do Catimbau), Pernambuco, Brazil (Fig. 1), between July and August 2009. Voucher specimens were identified at the herbarium of the Instituto Agronômico de Pernambuco (IPA), where a voucher of each species was deposited (Table 1).

The aerial parts of the plants (leaf, fruit, inflorescence, branch and cephalium – the flowering adult phase of a cactus), stem bark and roots were dried in an incubator at 45 °C, for 2–3 days. The dried material was ground into powder using a grinder followed by a blender (Waring Laboratory, USA).

2.2. Extracts

The powdered dried material was mixed for 15 min with water [1:9; (w:v)] and kept in the dark at room temperature (22 °C) for 24 h. After this period the mixture was centrifuged for 10 min, at 10,000 rpm, and the supernatant was collected; the water was then evaporated at 40 °C, during 96–120 h. A 1 mg/mL and 10 mg/mL aqueous solution were filtered through a 0.2 μm pore membrane and stored at −20 °C.

2.3. Phytochemical analysis

A preliminary phytochemical analysis to detect the major components of the extracts was carried out using thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC).

The different extracts were developed by TLC plates (Kieselgel 60 F254 0.2 mm, Merck, Germany) using dichloromethane:methanol (8:2) as eluent and visualized under UV light (254 and 365 nm, Handheld UV Lamp Model 9403E, BioAmerica Inc., USA). Polyphenol compounds were detected with aluminum chloride 1% and ferric chloride 2%, potassium hydroxide 5% was used for coumarins, 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 and Bladt, 1996).

The HPLC analyses were carried out on a Shimadzu LC-20AT coupled to a Shimadzu SPD M20A diode array detector, and a reversed-phase column Shim-pack VP-ODS (250 mm × 6 mm I.D., Shimadzu) was used. Gradient elution was performed with solution A, acetonitrile:water (5:95 [v:v]), and solution B comprising 100% of acetonitrile, delivered at a flow rate of 0.6 mL/min as follows: initially 5% of solution B increasing up to 100% for 60 min and finally 100% of B for 15 min. The injection volume was 20 μL from a solution at 2.5 mg/mL.

2.4. Surfaces, bacterial strain and culture conditions

Bacterial adhesion is determined both by the type of microorganism and by the properties of the surface of the material involved (Pavithra and Doble, 2008). Two surfaces were studied: sterile 96-well polystyrene flat-bottom microtiter plates (Costar 3599) purchased from Corning Inc. (USA) as a model of a hydrophobic material, and glass as a model of a hydrophilic material. *Staphylococcus epidermidis* ATCC 35984 was grown in Mueller Hinton agar (Oxoid Ltd., England) overnight, at 37 °C, and a bacterial suspension

Table 1
List of plant species from the Brazilian Caatinga for tested against *S. epidermidis*.

Family	Scientific name	Popular name	Voucher	Usage forms, preparation and therapeutic indication
Anacardiaceae	*Myracrodruon urundeuva* Alemão	Aroeira, aroeira-do-sertão	IPA 84059	Drink or wash the affected site. A decoction or maceration of a handful of stem bark in a liter of water. Used in cases of ovarian inflammation and in ulcerative external afflictions This species has many other medicinal indications (Agra et al., 2007b).
Apocynaceae	*Allamanda blanchetii* A.DC.	Quatro-patacas-roxa, leiteiro	IPA 84112	Drink. One teaspoon of the latex in a cup of water. Latex is used as a laxative, emetic, cathartic and vermifuge. It is described as poisonous (Agra et al., 2007a,b).
Burseraceae	*Commiphora leptophloeos* (Mart.) J.B.Gillett	Imburana, amburana, imburana de cambão	IPA 84037	Drink or wash/bathe the affected site. A decoction of a handful of roots in a liter of water is prepared with sugar as syrup. Used in the treatment of influenza, coughs, bronchitis, to treat urinary and liver diseases. Also, external use against vaginal ulcers and others (Agra et al., 2007a).
Cactaceae	*Melocactus zehntneri* (Britton & Rose) Luetzelb.	Coroa-de-frade	IPA 85028	Drink. Stem pulp is used mashed with sugar to treat bronchitis, coughs and physical debility (Agra et al., 2007a,b).
Combretaceae	*Buchenavia tetraphylla* (Aubl.) R.A. Howard	Caicaró	IPA 84104	Drink. An infusion of stem bark or leaves is used as a digestive after meals (Agra et al., 2007b).
Euphorbiaceae	*Jatropha mutabilis* (Pohl) Baill.	Pinhão bravo, pinhão manso	IPA 84054	Eat and drink. The oil extracted from seeds is ingested as a veterinary vermifuge and the latex is drunk to treat snake bites. A spoonful is used once only (Agra et al., 2007b).
Fabaceae Cercideae	*Bauhinia acuruana* Moric.	Mororó, pata de vaca	IPA 84042	Drink. An infusion or decoction of a handful of leaves in a liter of water it is drunk during the meals until the symptoms disappear. Used as tonic, depurative and against diabetes (Agra et al., 2007a,b).
Fabaceae "Caesalpinioideae"	*Chamaecrista cytisoides* (DC. ex Collad.) H.S. Irwin & Barneby	Vassourinha	IPA 84103	Drink. According to a local community the whole plant is used as a laxative and purgative.
Fabaceae "Caesalpinioideae"	*Chamaecrista desvauxii* (Collad.) Killip	Vassourinha	IPA 84064	Drink. According to the local community the whole plant is used as a laxative and purgative.
Fabaceae "Caesalpinioideae"	*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz *var. ferrea*	Pau ferro, jucá	IPA 84035	Drink. The stem bark in "cachaça" (bottled) is used against anemia, diarrhea and dysentery (Agra et al., 2007b).
Fabaceae "Caesalpinioideae"	*Pakinsonia aculeata* L.	Turco, tangerim	IPA 84113	Drink. The seeds are roasted, powdered, prepared as coffee and drunk as tea until the symptoms disappear; used to treat fevers and malaria. Prepared as an infusion or decoction of a handful in a liter of water and drunk as antiepileptic, febrifuge, and to treat snake bites (Agra et al., 2007a,b).
Fabaceae "Caesalpinioideae"	*Senna macranthera* (Collad.) H.S. Irwin & Barneby *var. macranthera*	Pau fava, fedegoso	IPA 84045	Drink. According to the local community a decoction of a spoonful of the fruit in a cup of water is used against influenza and colds four times a day.
Fabaceae "Caesalpinioideae"	*Senna splendida* (Vogel.) H.S. Irwin & Barneby	Feijão-brabo	IPA 84040	Drink. According to the local community the fruits are roasted, powdered and prepared as coffee to be used against anemia. A cup is drunk after meals until the symptoms disappear.
Fabaceae Mimosoideae	*Anadenanthera colubrina* (Vell.) Brenan *var. colubrina*	Angico	IPA 84039	Drink. A maceration of a handful of stem bark in a liter of wine or "cachaça" is used against coughs, whooping cough and bronchitis (Agra et al., 2007a,b).
Fabaceae Mimosoideae	*Piptadenia viridiflora* (Kunth) Benth.	Jacurutu, espinheiro-preto	IPA 84036	Drink. The stem bark is used in a decoction against asthma, intestinal spasms and toothaches (Agra et al., 2007b).
Fabaceae Mimosoideae	*Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson	Canzenzo, angico de bezerro, quipembe	IPA 84048	In communication with local people was informed that the stem bark and root are used as healing.
Fabaceae Faboideae	*Dioclea grandiflora* Mart. ex Benth.	Mucunã, parreira-brava	IPA 84057	Drink. A decoction of a handful of roots in a liter of water is used against prostate inflammation. It is drunk in place of water until the symptoms disappear (Agra et al., 2007a,b).
Fabaceae Faboideae	*Myroxylon peruiferum* L.f.	Bálsamo	IPA 84110	Topic. In communication with local community was informed that the stem-bark is used like as anti-inflammatory.
Malpighiaceae	*Stigmaphyllon paralias* A. Juss.	Amarelinho	IPA 84041	Drink. According to the local community the leaf is used as a decoction against fevers and diarrhea, syphilis and kidney diseases.
Malvaceae	*Sida galheirensis* Ulbr.	Malva-veludo, malva-branca, malva, malvão	IPA 84078	Drink or wash the affected site. According to the local community an infusion of a spoonful of stem bark and root in a cup of water are used against acne, coughs and leucorrhea. Also used for skin afflictions (Agra et al., 2007b).
Myrtaceae	*Eugenia brejoensis* Mazine	Cutia	IPA 84033	Drink. According to the local community a decoction or infusion of the leaves is drunk against diarrhea and dysentery. (Endemic plant).
Ochnaceae	*Ouratea blanchetiana* Engl.	Batiputá	IPA 84044	Topical. The oil extracted by heat from the fruits is used against earache. It is dropped into the ears until the pain disappears (Agra et al., 2007b).
Polygalaceae	*Polygala boliviensis* A.W.Benn.	Arrozinho	IPA 84066	Drink or eat and topic. An infusion or decoction of a handful of leaves in water is used as diuretic, emetic, expectorant and against blenorrheas. The roots are eaten and placed above the affected area to treat snake bites (Agra et al., 2007a,b).
Polygalaceae	*Polygala violacea* Aubl.	Erva-iodeque	IPA 84051	Drink or eat and topical. An infusion or decoction of a handful of roots in water is used as a diuretic, emetic, expectorant and against blenorrheas. The roots are eaten and placed over the affected area to treat snake bites. (Agra et al., 2007b).

in 0.9% NaCl, corresponding to 1 McFarland scale (3×10^8 CFU/mL), was used in the assays.

2.5. Antibiofilm formation assay

A protocol adapted from Antunes et al. (2010), employing crystal violet in 96-well microtiter plates and glass tubes was used. In the case of microtiter plates, 80 μL of the bacterial suspension, 80 μL of the aqueous extract (concentration of 0.4 mg/mL or 4.0 mg/mL in the wells) and 40 μL of tryptone soya broth (TSB) (Oxoid Ltd., England) were added. In the case of glass tubes, 800 μL of the bacterial suspension, 800 μL of the aqueous extract (concentration of 0.4 mg/mL or 4.0 mg/mL in the tubes) and 400 μL of TSB were added. In both experiments, following the incubation period (37 °C for 24 h) the content of the wells/tubes was removed and the wells/tubes were washed three times with sterile saline. The remaining attached bacteria were heat-fixed at 60 °C for 1 h. The adherent biofilm layer formed was stained with 0.4% crystal violet for 15 min at room temperature. The stain bound to the cells was solubilized with 99.5% DMSO (Sigma–Aldrich Co., USA) and absorbance was measured at 570 nm (Spectramax M2e Multimode Microplate Reader, Molecular Devices, USA). The biofilm formation control was considered to represent 100% of biofilm formation, and the extracts were replaced by 80 μL and 800 μL of water in 96-well microtiter plates and glass tubes, respectively. Values higher than 100% represent a stimulation of biofilm formation in comparison to the control.

2.6. Scanning electron microscopy

Biofilms of *S. epidermidis* ATCC 35984 were grown in 96-well microtiter plates, as described in Section 2.5, with a piece of Permanox™ slide (Nalge Nunc International, USA) or a glass coverslip in each well. After 24 h of incubation at 37 °C, the samples were fixed in 2.5% glutaraldehyde for 2.5 h, washed with 100 mM cacodylate buffer pH 7.2, treated for 2 h with 2% osmium tetroxide and dehydrated in increasing concentrations of acetone. The Permanox™ slides and glass coverslips were dried by the CO_2

Table 2
Biological activity of 45 aqueous extracts of Caatinga plants against biofilm formation and growth of *S. epidermidis* ATCC 35984.

Species	Part used	*S. epidermidis*			
		0.4 mg/mL		4.0 mg/mL	
		Biofilm formation (%)	Bacterial growth (%)	Biofilm formation (%)	Bacterial growth (%)
Allamanda blanchetii	Branches	110.9 ± 0.1	100.7 ± 4.6	183.2 ± 1.7*	33.0 ± 31.7*
Allamanda blanchetii	Leaves	93.1 ± 6.2	108.1 ± 7.0	74.2 ± 7.6	170.6 ± 12.5*
Anadenanthera colubrina var *cebil*	Fruits	118.0 ± 14.1	91.9 ± 5.4	101.0 ± 6.8	131.2 ± 13.8*
Anadenanthera colubrina var *cebil*	Leaves	167.1 ± 1.1	93.8 ± 14.4	119.4 ± 2.6*	257.6 ± 4.7*
Anadenanthera colubrina var *cebil*	Branches	113.2 ± 12.3	102.2 ± 4.3	130.8 ± 5.6*	207.5 ± 0.6*
Anadenanthera colubrina var *cebil*	Stem bark	136.8 ± 10.2	95.2 ± 7.5	11.6 ± 1.7*	63.9 ± 5.8*
Bauhinia acuruana	Branches	99.0 ± 7.2	83.1 ± 9.6	18.3 ± 3.7*	184.0 ± 8.0*
Bauhinia acuruana	Fruits	121.4 ± 12.2	81.6 ± 7.2	22.2 ± 5.0*	135.4 ± 3.1*
Bauhinia acuruana	Leaves	128.0 ± 14.5	74.1 ± 1.6*	144.5 ± 3.9*	343.1 ± 7.0*
Buchenavia tetraphylla	Leaves	179.4 ± 6.5*	136.0 ± 5.9*	177.4 ± 9.4*	268.5 ± 19.5*
Chamaecrista desvauxii	Leaves	103.8 ± 4.9	91.4 ± 10.5	126.2 ± 12.9*	153.6 ± 12.4*
Chamaecrista desvauxii	Fruits	68.3 ± 2.7*	154.7 ± 12.5*	12.6 ± 1.3*	201.3 ± 9.9*
Chamaecrista cytisoides	Branches	108.4 ± 17.5	113.0 ± 6.9	131.1 ± 9.0*	122.5 ± 6.6*
Commiphora leptophloeos	Branches	153.8 ± 9.6	97.2 ± 8.3	174.1 ± 0.8*	144.6 ± 3.1*
Commiphora leptophloeos	Stem bark	32.7 ± 8.5*	93.3 ± 5.1	15.3 ± 1.3*	0 ± 4.5*
Dioclea grandiflora	Leaves	166.1 ± 6.5	109.9 ± 13.9	172.4 ± 12.0*	178.4 ± 6.6*
Dioclea grandiflora	Branches	110.9 ± 4.5	92.7 ± 4.6	177.8 ± 18.0*	99.2 ± 4.4
Dioclea grandiflora	Fruits	86.5 ± 5.1	107.6 ± 5.4	68.5 ± 1.9*	127.5 ± 11.9*
Eugenia brejoensis	Leaves	140.3 ± 23.1	91.2 ± 2.4	76.0 ± 6.9	131.8 ± 1.2*
Jatropha mutabilis	Roots	110.3 ± 17.5	87.6 ± 4.3*	227.3 ± 7.8*	130.6 ± 8.5*
Jatropha mutabilis	Branches	102.0 ± 7.2	82.3 ± 7.1*	201.7 ± 7.4*	177.4 ± 16.9*
Libidibia ferrea var ferrea	Leaves	106.0 ± 13.5	113.6 ± 14.5	122.0 ± 11.0*	160.5 ± 1.1*
Libidibia ferrea var ferrea	Fruits	120.7 ± 12.2	133.0 ± 3.4	30.0 ± 1.2*	178.9 ± 0.5*
Melocactus zehntneri	Roots	66.5 ± 6.8*	122.4 ± 3.1	86.2 ± 9.9	139.0 ± 5.5*
Melocactus zehntneri	Cephalium	68.8 ± 7.8*	100.8 ± 5.0	154.4 ± 5.3*	206.0 ± 7.7*
Myracrodruoun urundeuva	Branches	81.5 ± 14.5	82.6 ± 11.9	159.1 ± 7.9*	158.1 ± 12.2*
Myracrodruoun urundeuva	Leaves	71.4 ± 5.8*	90.3 ± 5.1	171.5 ± 8.4*	161.4 ± 20.3*
Myracrodruoun urundeuva	Stem bark	141.2 ± 7.2*	99.7 ± 1.6	16.1 ± 0.4*	62.7 ± 3.4*
Myroxylon peruiferum	Leaves	71.6 ± 2.4*	146.1 ± 4.1*	53.6 ± 6.7*	195.4 ± 6.1*
Ouratea blanchetiana	Branches	195.5 ± 10.2	117.2 ± 9.8	122.2 ± 2.7*	239.9 ± 8.6*
Ouratea blanchetiana	Leaves	172.8 ± 14.2	130.4 ± 11.2	50.0 ± 2.5*	273.1 ± 9.8*
Parkinsonia aculeata	Leaves	90.2 ± 11.4	102.9 ± 12.9	47.2 ± 12.0*	85.2 ± 2.7*
Piptadenia viridiflora	Branches	91.4 ± 17.0	107.3 ± 9.4	117.2 ± 26.0	102.4 ± 1.4
Piptadenia viridiflora	Fruits	85.3 ± 11.6	98.6 ± 10.2	61.3 ± 9.2*	218.5 ± 17.7*
Pityrocarpa moniliformis	Leaves	108.7 ± 3.8	126.1 ± 11.2	23.0 ± 0.7*	266.7 ± 6.2*
Polygala boliviensis	Inflorescences	105.9 ± 5.0	101.1 ± 4.9	112.2 ± 7.6	244.3 ± 1.7*
Polygala boliviensis	Leaves	111.1 ± 8.7	92.7 ± 9.5	93.0 ± 10.1	134.3 ± 5.8*
Polygala boliviensis	Branches	75.7 ± 12.4	83.1 ± 10.1*	96.5 ± 6.3	154.6 ± 12.8*
Polygala violacea	Leaves	93.8 ± 2.1	113.9 ± 2.7*	86.2 ± 4.7*	166.5 ± 8.2*
Polygala violacea	Roots	82.8 ± 10.2	90.5 ± 9.0	52.6 ± 7.4*	138.1 ± 4.1*
Senna macranthera	Fruits	43.3 ± 3.1*	152.8 ± 8.7	103.4 ± 13.8	88.9 ± 10.6
Senna splendida	Branches	98.0 ± 0.7	104.8 ± 0.5	58.8 ± 2.3*	164.5 ± 1.3*
Sida galheirensis	Branches	88.2 ± 14.0	126.7 ± 10.6	110.0 ± 24.8	112.0 ± 3.6
Sida galheirensis	Leaves	84.8 ± 8.0	105.8 ± 7.8	95.0 ± 8.3	107.3 ± 8.6
Stigmaphyllon paralias	Leaves	138.6 ± 10.9*	113.5 ± 4.3	197.5 ± 7.7*	319.4 ± 6.3*

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

critical point technique (CPD 030 Balzers, Liechtenstein), fixed on aluminum stubs, covered with gold film and examined in a JEOL JSM-6060 scanning electron microscope.

2.7. Bacterial growth assay

The planktonic bacterial growth was evaluated by the difference between the OD_{600} absorbance measured at the end and at the beginning of the incubation time in polystyrene 96-well microtiter plates. As a control for bacterial growth the extracts were replaced by 80 μL of water, with this being considered to represent 100% of planktonic bacterial growth. Values higher than 100% represent a stimulation of bacterial growth in comparison to the control. The minimum inhibitory concentration (MIC) to kill 100% of bacterial cells was determined and 50 μL of a serial dilution was spread on to Mueller Hinton agar plates. After incubation (37 °C, 24 h), the number of colony-forming units was counted to determine the bacteriostatic or bactericidal effect of the extract. Rifampicin 8 μg/mL (Sigma–Aldrich Co., USA) was used as a control for the inhibition of bacterial growth.

2.8. Statistical analysis

All microbiological experiments were carried out at least in triplicate and data are presented as percentage mean ± S.D. Differences between groups were evaluated by Student's *t*-test and a $p \leq 0.05$ was considered significant.

3. Results

3.1. Screening of bioactive Caatinga plant aqueous extracts

Table 2 shows the screening of 45 aqueous extracts against *S. epidermidis* on polystyrene.

Considering the extracts at 0.4 mg/mL, stem bark of *Commiphora leptophloeos* and fruits of *Senna macranthera* allowed a biofilm formation of 32.7% and 43.3%, respectively, although the screening did not reveal any significant antibacterial activity at this concentration. At 4.0 mg/mL, the lowest rates of biofilm formation were obtained using: stem bark of *Anadenanthera colubrina* (11.6%), *Commiphora leptophloeos* (15.3%) and *Myracrodruoun urundeuva* (16.1%); fruits of *Bauhinia acuruana* (22.2%), *Chamaecrista desvauxii* (12.6%) and *Libidibia ferrea* (30.0%); leaves of *Parkinsonia aculeata* (47.2%) and *Pityrocarpa moniliformis* (23.0%), and branches of *Bauhinia acuruana* (18.3%). At this concentration, two extracts presented marked inhibition of planktonic bacterial growth since, branches of *Allamanda blanchetii* and stem bark of *Commiphora leptophloeos* permitted just 33.0% and none of bacterial growth, respectively (Table 2).

Table 3
Percentage effect of Caatinga plant extracts against *S. epidermidis*.

	0.4 mg/mL		4 mg/mL	
	Biofilm (%)	Growth (%)	Biofilm (%)	Growth (%)
Stimulation	46.7	35.5	48.8	82.2
No effect	11.1	24.4	4.4	4.4
Up to 25% inhibition	26.7	37.8	11.1	4.4
From 26 to 50% inhibition	11.1	2.2	15.5	4.4
From 51 to 75% inhibition	4.4	0	4.4	2.2
From 76 to 100% inhibition	0	0	15.5	2.2

We chose four active extracts for further investigation (branches of *Bauhinia acuruana* – BBA11; stem bark of *Commiphora leptophloeos* – SBCL33; fruits of *Bauhinia acuruana* – FBA35 and leaves of *Pityrocarpa moniliformis* – LPM45), which were obtained in higher amounts during preparation and represent the different parts of a plant (branches, stem bark, fruits and leaves). The extracts BBA11, FBA35 and LPM45 inhibited biofilm formation by a mechanism that did not involve bacterial death, but instead they stimulated cell growth (Table 2). At 0.4 mg/mL, these extracts did not affect biofilm formation or bacterial growth (Table 2). However, the SBCL33 extract did kill *S. epidermidis* at the highest concentration, although at 0.4 mg/mL the discrete biofilm formation (32.7%) was not associated with bacterial death (Table 2).

To compare the various extracts at both concentrations, they were classified into six categories according to their effect against *S. epidermidis* (Table 3).

3.2. Evaluation of S. epidermidis biofilm on glass

The four active extracts selected after screening were also tested for their capacity to inhibit the formation of a biofilm on a glass surface. The results were similar to those obtained using polystyrene plates (Section 3.1 and Table 2), but showed a discrete decrease in antibiofilm activity (Table 4).

Table 4
Biological activities of four Caatinga aqueous extracts against *S. epidermidis* ATCC 35984.

	Biofilm inhibition					Growth inhibition	
	0.4 mg/mL	4.0 mg/mL				0.4 mg/mL	4.0 mg/mL
	Polystyrene	Polystyrene	SEM Permanox™	Glass	SEM glass	Polystyrene	
Branches of *Bauhinia acuruana*	–	81.7 ± 3.7%*	Small clusters/single cells; DC; MO	69.0 ± 11.9%*	Small clusters/single cells; DC; MO	16.9 ± 9.6%	–
Fruits of *Bauhinia acuruana*	–	77.8 ± 5.0%*	Small clusters/single cells; DC; MO	68.5 ± 7.4%*	Small clusters/single cells; MO	18.4 ± 7.2%	–
Leaves of *Pityrocarpa moniliformis*	–	77.0 ± 0.7%*	Small clusters	71.0 ± 0.6%*	Small clusters/single cells; MO	–	–
Stem bark of *Commiphora leptophloeos*	67.3 ± 8.5%	84.7 ± 1.3%*	Small clusters; DC	82.8 ± 5.6%*	Small clusters/single cells	6.7 ± 5.1%	100 ± 4.5%*

Results represent mean ± standard deviation of 3 experiments. DC represents deformation of the cells and MO represents matrix overproduction.
* Represents significant difference in relation to control ($p < 0.05$).

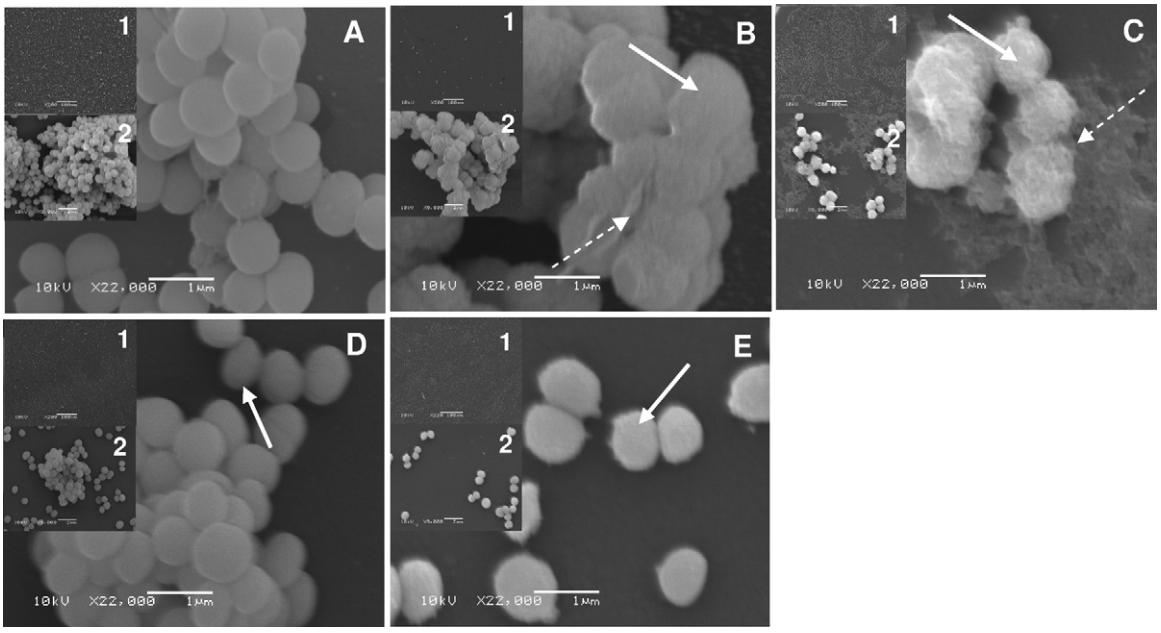

Fig. 2. Scanning electron microscopy images of the biofilms upon Permanox™. Untreated biofilms (control for biofilm formation) (A); Extract-treated biofilms: BBA11 (B); FBA35 (C); LPM45 (D); and SBCL33 (E). Scale bars: 22,000× magnification (in the images: insert 1 200× magnification and insert 2 9000× magnification). Solid arrows: cell deformation. Dotted arrows: matrix overproduction.

3.3. *Scanning electron microscopy: biofilm visualization*

The inhibition of *S. epidermidis* biofilm formation by the selected active extracts was observed by SEM using Permanox™, in order to mimic polystyrene, and glass coverslips. Images showed a dense and uniform staphylococcal biofilm covered the Permanox™ and glass surfaces in untreated biofilms (control for biofilm formation) (Figs. 2 and 3, panel A). By contrast, treated biofilms displayed a significant reduction in the number of adherent bacteria and in the size of aggregates, which were reduced to small clusters or even single cells (Figs. 2 and 3, panels B–E, respectively) and confirmed the results obtained by crystal violet assay.

Extract of BBA11 almost completely prevented bacterial adherence on both surfaces (Figs. 2 and 3, panel B), whereas LPM45 and SBCL33 extracts were similarly effective on glass coverslips only (Fig. 2, panels D and E, respectively). Regarding the FBA35 extract, the bacteria were equally able to form a biofilm on both surfaces, although to a lesser extent than the positive control (Figs. 2 and 3, panels C and A, respectively). Interestingly, the images indicated that some extract-treated biofilms presented a structural deformation in the bacterial cells and/or an overproduction of the exopolymeric matrix, the latter probably as a way of protecting themselves against the aggression of the extract (Figs. 2 and 3 and Table 4).

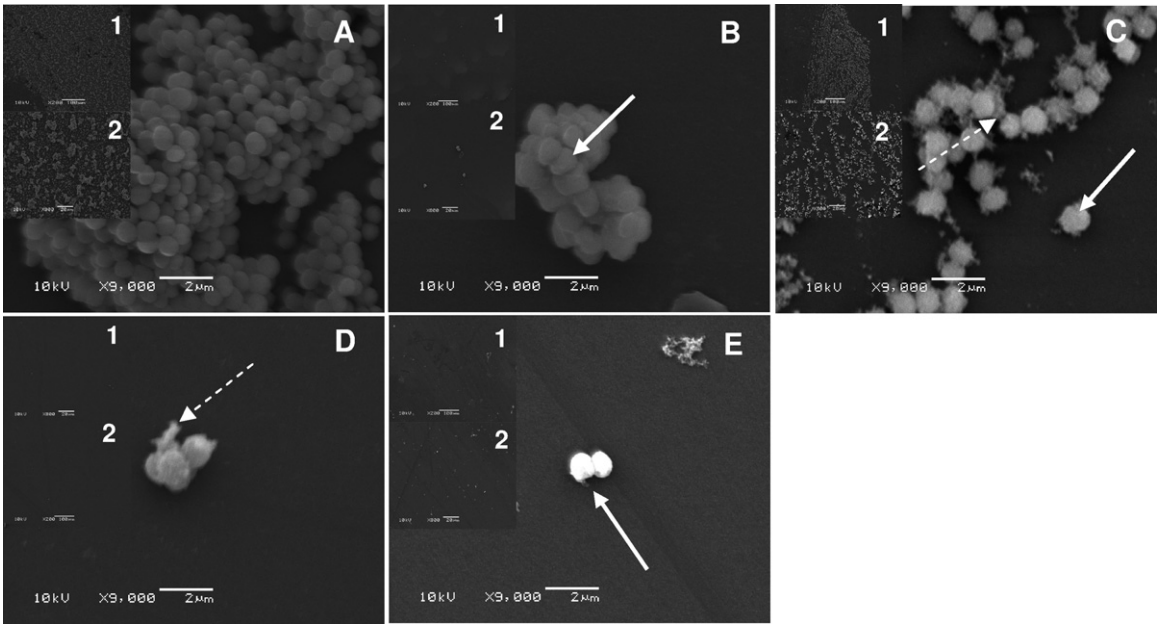

Fig. 3. Scanning electron microscopy images of the biofilms upon glass coverslips. Untreated biofilms (control for biofilm formation) (A); extract-treated biofilms: BBA11 (B); FBA35 (C); LPM45 (D); and SBCL33 (E). Scale bars: 9000× magnification (in the images: insert 1 200× magnification and insert 2 800× magnification). Solid arrows: cell deformation. Dotted arrows: matrix overproduction.

3.4. Antibacterial activity of Commiphora leptophloeos stem bark

Extract of SBCL33, at 4.0 mg/mL, caused 100% bacterial death according to OD_{600} measurements. The serial dilution (from 4.0 to 0.4 mg/mL) showed that the MIC was 1.0 mg/mL and the counting of the colony-forming units confirmed the bactericidal effect.

3.5. Phytochemical analysis

A preliminary qualitative phytochemical screening was carried out with the four extracts chosen. The TLC analysis revealed the absence of alkaloids and amines/aminoacids in the extracts and indicated the presence of polyphenols, coumarins, steroids and terpenes.

Although each sample was obtained from different species (3 plants) and from distinct plant structures (4 parts), the HPLC analysis presented similar chromatographic profiles among these four extracts. Each extract was monitored at 210, 254, 273 and 365 nm and compared by overlay (Fig. 4A–D). In addition, an overlay of the chromatograms from the differents extracts was obtained at 210 nm (Fig. 4E).

4. Discussion

Ethnopharmacology rescues the historical use of plants by people who have acquired great knowledge through family tradition. The information that is held and that can be accumulated about the use of plants in folk health care systems around the world is of inestimable importance. For example, the ethnobotanical and medicinal knowledge of the native people who live in or near tropical forests is recognized as valuable to bioprospecting (Quave et al., 2008). Likewise, ethnopharmacological data from residents of the Caatinga as well as from the literature pointed to the species collected and investigated in this study (Table 1). In addition, the

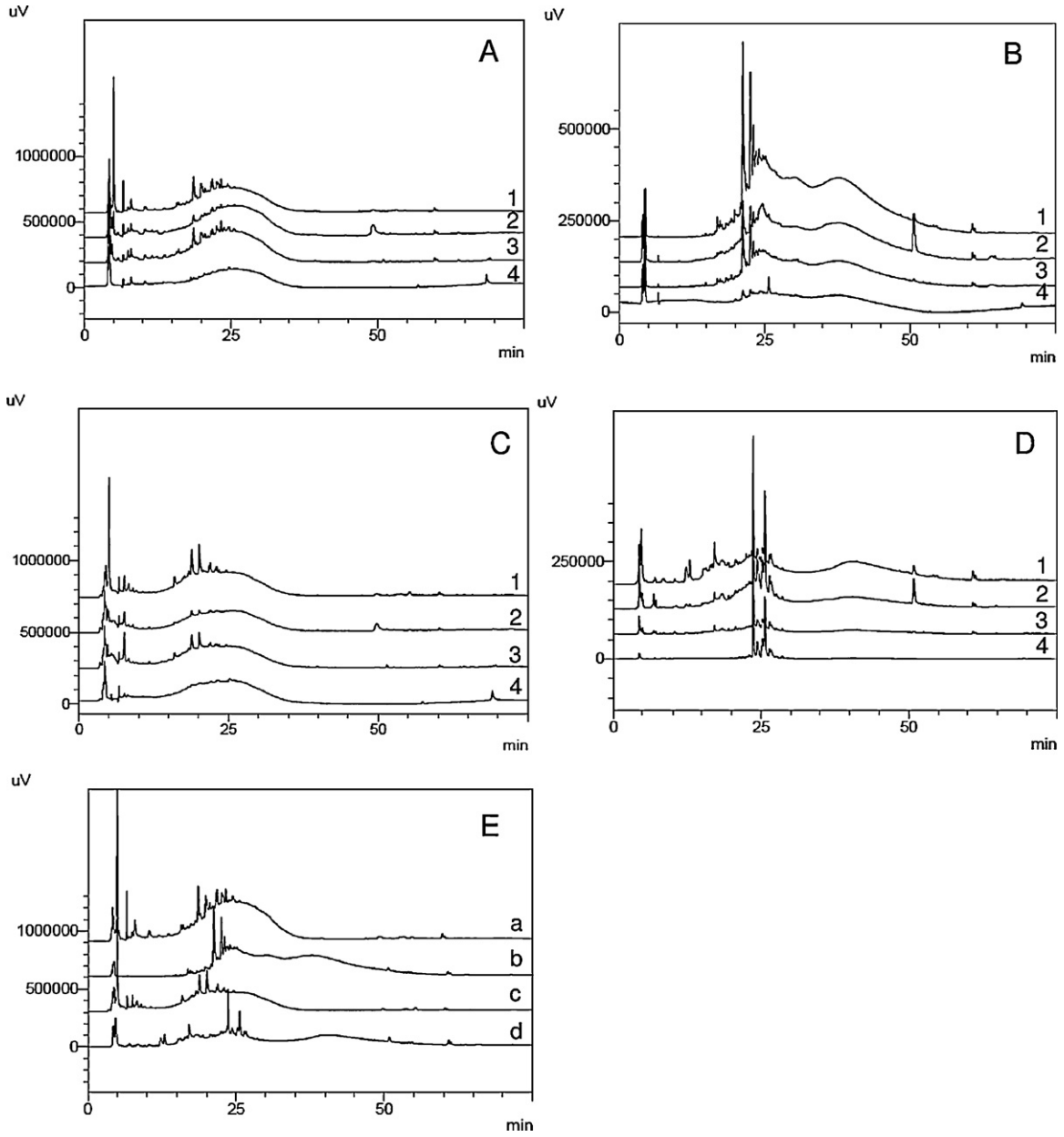

Fig. 4. HPLC-DAD chromatograms of the aqueous extracts. Extract BBA11 (A); SBCL33 (B); FBA35 (C); and LPM45 (D). Chromatograms 1, 2, 3, and 4 represent wavelengths of 210, 254, 273 and 365 nm, respectively and (E) represents an overlay of BBA11 (a); SBCL33 (b); FBA35 (c); and LPM45 (d) at 210 nm.

preparation of the aqueous extracts aimed to reproduce the form of use in traditional medicine and thereby enable us to confirm or refute their effectiveness, considering antibacterial/antibiofilm properties.

Among the 24 collected plants, 17 (70.8%) of them presented antimicrobial potential because they have been used in someway as antiseptics, anti-inflammatory or healing agents by folk medicine (Table 1). When tested *in vitro* against *S. epidermidis*, the most important cause of nosocomial infections, our results identified 13 plants (54.2% of the collection) with antibacterial properties, corresponding to 10 plants (58.6%) of those with antimicrobial potential. However, considering antibiofilm activity, 20 plants (83.3% of the collection), were active, corresponding to 15 plants (88.2%) used in folk medicine with antimicrobial potential. *Chamaecrista cystisoides* (branches) and *Stigmaphyllon paralias* (leaves) were the unique plants that not presented antibacterial neither antibiofilm activity, the last one was indicated by Caatinga community against diarrheas, syphilis and renal disorders. Interestingly, we found that 78% of the extracts that presented a high ability to inhibit *S. epidermidis* biofilm on polystyrene (Section 3.1), without killing the bacteria, came from plants that belong to the Fabaceae family (Table 2).

Of the four active extracts chosen for further investigation (BBA11, SBCL33, FBA35 and LPM45), one presented antibacterial activity (SBCL33), demonstrating a bactericidal effect against *Staphylococcus epidermidis* with a MIC of 1.0 mg/mL. Consequently, the biofilm structure was poorly formed when examined at 4.0 mg/mL on polystyrene and glass (more than 80% of antibiofilm activity) (Table 4). However, at 0.4 mg/mL, SBCL33 permitted a biofilm formation of 32.7% without antibacterial activity (Table 2). Additionally, the other three extracts selected for a more detailed study (BBA11, FBA35 and LPM45) demonstrated marked antibiofilm activity on polystyrene and glass surfaces without causing bacterial death (Table 4). This observation points out the wide potential of *Bauhinia acuruana* as a source of antivirulence compounds, since both branches (BBA11) and fruits (FBA35) prevented *S. epidermidis* biofilm formation on the two surfaces.

The notable activity demonstrated by the extracts was visualized by SEM, a valuable tool to improve understanding of the qualitative and quantitative impact of the samples upon the bacteria. Based on SEM images all four selected extracts affected the bacterial adhesion on Permanox™ and glass, two surfaces with distinct characteristics of hydrophobicity (Figs. 2 and 3 and Table 4). We found that the cells which adhered to surfaces presented an overproduction of extracellular matrix (BBA11 on Permanox™; FBA35 on both surfaces and LPM45 on glass), suggesting that molecules involved in the activity of the extracts did not exert their effect through extracellular matrix inhibition. Also, we observed bacterial cells with deformed morphology (BBA11, SBCL33 and FBA35 on both surfaces, and LPM45 on Permanox™), which could indeed be related to the extracts mechanism of action (Figs. 2 and 3 and Table 4).

Impairment of bacterial adhesion and biofilm formation by a pathway that does not involve bacterial death is a remarkable characteristic of a new concept in antivirulence therapies. Importantly, it explores new mechanisms of action that may difficult the rapid development of bacterial resistance. Moreover, in this alternative approach, which does not affect bacterial growth and maintains the cells in a planktonic state, the switching off of virulence expression and attenuation of the pathogen make microorganisms more susceptible to other antimicrobials and to the immune system (Clatworthy et al., 2007; Martin et al., 2008; Macedo and Abraham, 2009).

In this context, natural products are an important source of bioactive molecules and the medicinal plants used in popular medicine could facilitate the search for new agents. The results of TLC phytochemical screening of the four extracts chosen enabled us to exclude alkaloids and amines/aminoacids from the compounds potentially responsible for the bioactivity. In contrast, polyphenols, coumarins, steroids and terpenes do appear to be among the compounds involved with the extracts effects. HPLC-DAD analyses were carried out to characterize the four extracts selected and the results demonstrated similar profiles of compounds among them, especially at 210 nm where the highest number of compounds was detected (Fig. 4).

Polyphenols have received some attention recently regarding their antimicrobial effect upon microorganisms in biofilms, including a small number of studies involving *S. epidermidis* (Ferrazzano et al., 2009; Prabhakar et al., 2010; Schito et al., 2010). Sampaio et al. (2009) showed that polyphenol-rich extracts of fruits from *Caesalpiniea ferrea* had antibiofilm activity in a multispecies biofilm model involving *Streptococcus* sp., *Candida albicans* and *Lactobacillus casei*. This agrees with our results for fruits of the synonym *Libidibia ferrea*, which inhibited the formation of a biofilm by *S. epidermidis* by 70.0% (Table 2). Considering coumarins, we found only a few studies involving biofilm inhibition (Girennavar et al., 2008; Praud-Tabaries et al., 2009). However, antimicrobial activity of coumarins, terpenes (excluding volatile oils) and steroids against bacteria and fungi has been more widely described (Ojala et al., 2000; Sparg et al., 2004; Zhang et al., 2008; Popova et al., 2009; Smyth et al., 2009).

The antivirulence therapy is one of the most promise alternative to combat pathogenic microorganisms. The study presented herein show the Caatinga plants potential as a new and valuable source of prototype compounds. Our efforts now are concerned with the isolation and elucidation of active molecules from the four selected extracts.

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