

## Antimicrobial activity of medicinal plants of the Caatinga (semi-arid) vegetation of NE Brazil

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### ABSTRACT

The traditional medicine still plays an important role in the primary health care in Brazil. In this report, 23 plants were selected and collected in different physiognomy from Parque Nacional do Catimbau, Northeastern Brazil. The dried plants were separated into leaves, fruit, stem-bark and roots. Each part was extracted with aqueous, ethanolic and hydroalcoholic extracts, and 54 crude extracts were prepared. The extracts were tested for their antimicrobial activity using three Gram-positive bacterial strains (*Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*), two Gram-negative bacterial strains (*Escherichia coli* and *Klebsiella pneumoniae*) and *Candida albicans*. In addition, a phytochemical screening of ethanolic extracts of five species was done. The highest antimicrobial activity was exhibited by the hydroalcoholic extracts of leaves of *Buchenavia tetraphylla* and ethanolic extracts of fruits of *Libidibia ferrea* var. *ferrea* against *Mycrococcus luteus*. The phytochemical screening demonstrated the presence of different types of compounds like flavonoids, terpenoids and others, which could be responsible for the obtained activities. The presence

of antibacterial activity could be confirmed in species used in traditional medicine in Caatinga which were assayed in this study. This is the first report about antimicrobial activities of some plants namely *Chamaecrista desvauxii*, *Melocactus zehntneri* and *Stigmaphyllon paralias*.

**KEYWORDS:** antibacterial activity, antifungal activity, Northeastern Brazilian plants, Caatinga

### INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China and India, but it is doubtless an art as old as mankind [1]. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller.

Secondary metabolites produced by plants constitute the largest source of bioactive substances [2]. Scientific interest in these metabolites have increased today with research into novel therapeutic agents of plant origin, which is due to increased resistance patterns of

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microorganisms to the various drugs used today [3]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine [4, 5].

Brazil has the world's scenario by having a greater richness than any other country, with about 43,345 species of plants or 20% of the world flora [6], and the huge diversity of landscapes. In this scenario, there is the Caatinga biome with over 900 recorded species of vascular plants [7]. The Caatinga biome is a mosaic of thorn bushes and seasonally dry forests, with enclaves of montane rain forests and savannah, which covers most of the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and northeastern part of Minas Gerais in Jequitinhonha Valley, occupying an area of approximately 735,000 km<sup>2</sup>. Researchers have mentioned many species that might possess great phytochemical and pharmacological potential for various medical purposes but that require studies to prove their scientific activities [8-14].

This study aimed to investigate the antimicrobial properties of crude extracts from plants of Brazilian Caatinga against Gram-positive and Gram-negative bacteria as well as against pathogenic yeasts.

## METHODS

### Study site

The Parque Nacional do Catimbau (PARNA do Catimbau) was created in 2002, being one of the seven National Parks that includes Caatinga vegetation, and the only National Park in state of Pernambuco (excluding the marine National Park of Fernando de Noronha). With an extension of 62,554 ha, the PARNA do Catimbau occupies part of Buíque, Ibimirim and Tupanatinga municipalities, situated in the transition between the "agreste" and the "sertão", in the São Francisco River basin.

The climate in the PARNA do Catimbau is semi-arid tropical, with annual median temperature of 23°C, and annual median precipitation of 500 to 1098 mm. Vegetation is typical of Caatinga, with high diversity of species and structure. However,

the area also includes species from Cerrado, Rupestrian Fields, Atlantic Forest and Restinga along with those typical from the Caatinga. Shrub trees characteristic to Chapada Diamantina rupestrian fields (state of Bahia) also occurs in that region, as well as some bromeliads, cacti and "babaçu", and "buriti" palm trees.

### Plant materials

The selected plant species were collected in the PARNA do Catimbau at different physiognomy. Botanical identification was performed at the Instituto Agronômico de Pernambuco Herbarium (IPA), where voucher specimens of each species have been deposited (Table 1). The aerial parts of the plants (leaf, fruit and brunch) and roots were used.

### Extraction of plant material

Based on the ethnobotanical use, for each species tested, various parts of the plants e.g., leaf, root, stem-bark, fruit or seeds were separated and ground into a fine powder. The finely powdered dry leaves were soaked in enough ethanol for one week. Then it was filtered and the filtrate was concentrated under vacuum using rotary evaporator. The crude extracts were stored at -20°C until used. Aqueous extracts (AE) were prepared by submerging ground dried plant material in sterile distilled water, kept at 37°C for 24 h, filtered and stored at -70°C before lyophilization. The extracts were then filtered and concentrated under vacuum in rotary evaporator to give (as a percentage of powdered plant materials) 6-11% gummy residue. All the extracts were kept in tightly stoppered bottle in a refrigerator until used for the anti-microbial testing.

### Test organism

The following microorganisms were used as test organism: *Bacillus subtilis* (UFPEDA 86), *Escherichia coli* (UFPEDA 224), *Klebsiella pneumoniae* (UFPEDA 396), *Micrococcus luteus* (UFPEDA 100), *Staphylococcus aureus* (UFPEDA 02) and *Candida albicans* (UFPEDA 1007) were used for the antimicrobial tests.

### Antimicrobial assay

The paper disc diffusion method was used to determine antibacterial activity [15]. Bacterial

**Table 1.** Selected plant species from Brazilian Caatinga for the evaluation of antimicrobial activity.

Família	Scientific name	Popular name(s)	Voucher	Indication and form of use
Anacardiaceae	<i>Myracrodruon urundeuva</i> Alemão	Aroeira, aroeira-do-sertão	IPA 84059	In inflammations of ovaries. A decoction or maceration of a handful in a liter of water. It is drunk "as water". Against external ulcers. The same recipe as above. It is used to wash the ulcerative affections. This species has many other medicinal indication (Agra <i>et al.</i> 2007a e b).
Apocynaceae	<i>Allamanda blanchetii</i> A.DC.	Quatro-patacas-roxa, leiteiro	IPA 84112	Latex is used as laxative, emetic, cathartic and vermifuge. One teaspoon of the latex in a cup of water. It is drunk after meals. It is referred to as poisonous. (Agra <i>et al.</i> 2007a e b).
Burseraceae	<i>Commiphora leptophloeos</i> (Mart.) J.B.Gillett	Imburana, amburana, imburana de cambão	IPA 84037	In treatment of gripes, coughs, bronchitis, treat urinary and liver diseases. A decoction of a handful in a liter of water and made with sugar as syrup. A spoonful is drunk 5-6 times a day. The external use against ulcers in washes or baths against vaginal ulcers (Agra <i>et al.</i> 2007a e b).
Cactaceae	<i>Melocactus zehntneri</i> (Britton & Rose) Luetzelb.	Coroa-de-frade	IPA 85039	Stem pulp is used mashed with sugar. A spoonful is drunk three times (Agra <i>et al.</i> 2007b).
Combretaceae	<i>Buchenavia tetraphylla</i> (Aubl.) R. A. Howard	Esparrada	IPA 84104	Is used as infusion as digestive. It is drunk after meals (Agra <i>et al.</i> 2007b).
Euphorbiaceae	<i>Jatropha mutabilis</i> (Polh) Baill.	Pinhão, pinhão bravo	IPA 84887	Leaves and flowers are used against intestinal diseases (Agra <i>et al.</i> 2007b).
Leguminosae - Caesalpinioideae	<i>Apuleia leiocharpa</i> (Vogel) J. F. Macbr.		IPA 84889	Not recorded in the literature. In communication with local people was informed that whole plant is used against infections.
Leguminosae - Caesalpinioideae	<i>Bauhinia acuruana</i> Moric.	Mororó, pata de vaca	IPA 84042	Not recorded in the literature. In communication with local people was informed that whole plant is used as a tonic and depurative and against diabetes. An infusion or decoction of a handful in a liter of water. It is drunk during the meals until the symptoms disappear.
Leguminosae - Caesalpinioideae	<i>Chamaecrista cytisoides</i> (DC. ex Collad.) H.S. Irwin & Barneby	Vassourinha	IPA 84103	Not recorded in the literature. In communication with local people was informed that whole plant is used as healing.

Table 1 continued..

Leguminosae - Caesalpinoideae	<i>Chamaecrista desvauxii</i> (Collad.) Killip	Vassourinha	IPA 84064	Not recorded in the literature. In communication with local people was informed that whole plant is used as healing.
Leguminosae - Caesalpinoideae	<i>Hymenaea courbaril</i> var. <i>courbaril</i> L.	Jatobá	IPA 84888	The stem-bark is used as decoction or as syrup. It is drunk against coughs and anemia. The resin is used against sinusitis and abdominal spasms. The epicarp is used as the syrup is drunk as tonic against anemia (Agra <i>et al.</i> 2007b).
Leguminosae - Caesalpinoideae	<i>Libidibia ferrea</i> var. <i>ferrea</i> (Mart. ex Tul.) L.P. Queiroz	Pau ferro, jucá	IPA 84035	The stem-bark in "cachaça" (garrafada) is used against anemia, diarrheas and dysenteries (Agra <i>et al.</i> 2007b).
Leguminosae - Caesalpinoideae	<i>Poincianella microphylla</i> (Mart. ex G. Don) L.P. Queiroz	Catingueira	IPA 84880	The decoction is drunk as digestive and sedative (Agra <i>et al.</i> 2007b).
Leguminosae - Mimosoideae	<i>Anadenanthera colubrina</i> var. <i>colubrina</i> (Griseb.) Altschul	Angico	IPA 84039	The stem-bark is used against coughs, whooping coughs and bronchitis. A maceration of a handful in a liter of wine or "cachaça". It is drunk until the is drunk three times a day until the symptoms disappear. (Agra <i>et al.</i> 2007a e b).
Leguminosae - Mimosoideae	<i>Pityrocarpa moniliformis</i> (Benth.) Luckow & R. W. Jobson	Canzenzo, angico de bezerro, quipembe	IPA 84048	Not recorded in the literature. In communication with local people was informed that the stem bark and root are used as healing.
Leguminosae - Papilionoideae	<i>Stylosanthes viscosa</i> (L.) Sw.	Pegajoso	IPA 84886	Not recorded in the literature. In communication with local people was informed that the leaf is used against infections.
Malpighiaceae	<i>Stigmaphyllon paralias</i> A. Juss.	Amarelinho	IPA 84041	Not recorded in the literature. In communication with local people was informed that the leaf is used as healing.
Myrtaceae	<i>Eugenia brejoensis</i> Mazine	Cutia	IPA 84033	Plant endemic. In communication with local people was informed that the plant is used as healing.
Ochnaceae	<i>Ouratea blanchetiana</i> Engl.	Batiputá	IPA 84044	Not recorded in the literature. In communication with local people was informed that the oil extracted from the fruits by the heat against ear pains. It is dropped into the ears until the pain disappears.
Sapotaceae	<i>Manilkara rufula</i> (Miq.) H. J. Lam	Massaranduba	IPA 84889	In communication with local people was informed that the plant is used as healing.

Table 1 continued..

Sapotaceae	<i>Sideroxylon obtusifolium</i> (Roem. & Schult.) T.D. Penn.	Quixaba. rompe-gibão	IPA 84076	Against ovarian inflammations and diabetes. A decoction or maceration of a handful in a liter of water. It is drunk "as water" or tea until the symptoms disappear. (Agra et al. 2007a e b).
Turneraceae	<i>Piriqueta guianensis</i> N.E. Br.	Malva de vassoura. malva branca	IPA 85036	The entire plant is used as emollient. It is used externally. The root is used in decoction against amenorrhea and as abortive (Agra et al. 2007b).
Turneraceae	<i>Turnera subulata</i> Sm.	Chanana	IPA 84060	The root is used against amenorrhea and dysmenorrhea. A decoction of a handful in a liter of water. It is drunk as tea. As expectorant against bronchitis and coughs. As syrup (Agra et al. 2007a e b).

strains grown on Mueller-Hinton Agar (MHA) at 37°C for 18 h were suspended and adjusted to a turbidity of 0.5 in the McFarland standard scale (approximately  $1.5 \times 10^8$  CFU/mL). The MHA was poured into petri dishes and inoculated with 100  $\mu$ L of the suspension. Sterile paper discs of diameter 6 mm (Whatman paper n° 1) were punched in the agar. The plates were cultured at 37°C for 18 h. Positive controls were performed using paper disc loaded with antibiotics. Negative controls were performed using paper disc loaded with 20  $\mu$ L of DMSO. At the end of the incubation period the antimicrobial activity was evaluated by measuring the inhibition zone (diameter of inhibition zone plus diameter of the disc). An inhibition zone of 14 mm or more was considered as high antibacterial activity [16].

#### **Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)**

The minimal inhibitory concentrations (MICs) of all extracts and reference antibiotic (tetracycline, vancomycin and penicillin from Sigma Chemical Co., Louis, MO, USA) were determined by microdilution techniques in Mueller-Hinton broth (Merck) following the protocol established by the CLSI for bacteria [17]. Inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard [108 colony-forming units (CFU)/ml] and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37°C and the MICs were recorded after 24 h of incubation. Minimum inhibitory concentration corresponded to the minimum extract concentration that inhibited visible bacterial growth. Afterwards, cultures were seeded onto MHA and incubated for 24 h at 37°C to determine the minimum bactericidal concentration (MBC) which corresponded to the minimum concentration of extract that caused the bacteria elimination. When the ratio of MBC/MIC is  $\leq 2$ , the active fractions were considered as bactericidal otherwise as bacteriostatic. If the ratio is  $\geq 16$  the fractions were considered as ineffective [18-21].

#### **Phytochemical screening of the hydroalcoholic extracts**

For plant extracts that showed Inhibition Zone  $>14$  mm, phytochemical analysis was carried out.

The screening of chemical constituents was carried out with the methanol extracts using chemical methods and thin-layer chromatography (TLC) according to the methodology given in [22].

## **RESULTS AND DISCUSSION**

Nowadays, many biological activities have been evaluated for numerous species of plants. This demonstrates that compounds from medicinal plants are indeed useful as alternative therapy, either directly or as models for new synthetic substances [21]. The selection of plants for this study was based on ethnobotanical data and on their traditional use in the treatment of infectious diseases and in treatment of gripes, coughs, bronchitis, urinary tract and liver diseases. This article describes the antimicrobial activities of a number of plants from PARNA do Catimbau used in Brazilian traditional medicine. A total of 54 extracts representing 23 species belonging to seven families were submitted in the screening. Table 1 shows the botanical names, plant part used and the traditional use of plants.

The results of the antimicrobial activity of the investigated extracts are shown in Table 2. The hydroalcoholic/ethanolic extracts exhibited higher antibacterial effects than the corresponding aqueous extracts. Among the 23 plants screened, the largest inhibitory zones were observed in the hydroalcoholic extracts of leaves of *Buchenavia tetraphylla* (30 mm) and ethanolic extracts of fruits of *Libidibia ferrea* var. *ferrea* (21 mm), against *Mycrococcus luteus* (Table 2).

The minimum inhibitory concentrations (MIC's) of crude extracts are described in Table 3. The MIC values ranged from 0.20 mg/mL as the most potent to 12.5 mg/mL as the least potent. The best activity against bacteria was observed for the plants *Buchenavia tetraphylla* (MIC  $\leq 0.20$  mg/mL), *Libidibia ferrea* var. *ferrea* and *Pityrocarpa moniliformis*, against *Mycrococcus luteus* (MIC = 0.39 mg/mL). *L. ferrea* var. *ferrea* was previously screened by other investigators, without proven antimicrobial activity. According to some authors the concentrations and proportions of the active compounds in essential oils and other substance extract components are affected by abiotic factors (soil, temperature, precipitation), time of harvest, season, etc [23].

**Table 2.** Results of antimicrobial activity of 56 extract crude of 23 medicinal plants of Brazilian Caatinga, determined by agar disc diffusion method (inhibition zone in mm).

Plant species	Plant part	Solv.	B.s.	M.I.	Microorganisms			
					S.a.	E.c.	K.p.	C.a.
<i>Apuleia leiiocharpa</i>	Lv.	Ha	-	15±0	-	-	-	-
<i>Allamanda blanchetii</i>	Lv.	Ha.	-	-	16±1	-	-	-
<i>Anadenanthera colubrina</i> var. <i>colubrina</i>	Fr.	Aq.	-	-	-	-	-	-
		Et.	-	-	-	-	-	-
		Ha.	-	-	-	-	-	-
	Lv.	Aq.	-	-	-	-	-	-
		Et.	13±1	14.67±0.57	8.67±1.15	-	-	-
		Ha.	17.33±1.15	-	14.67±0.57	-	-	-
<i>Buchenavia tetraphylla</i>	Lv.	Aq.	-	-	-	-	-	-
		Et.	-	24.6±0.58	-	-	-	-
		Ha.	-	29.33 ±2.08	-	-	-	-
<i>Bauhinia acuruana</i>	Lv.	Aq.	-	-	-	-	-	-
		Et.	9±0	13.33±0.57	10.67±0.57	-	-	-
		Ha.	10.33±0.57	13.33±0.57	9.33±1.15	-	-	-
<i>Chamaecrista cytisooides</i>	Lv.	Aq.	-	-	-	-	-	-
		Et.	-	-	-	-	-	-
		Ha.	14.33±0.58	21±0	-	11±0	-	-
<i>Chamaecrista desvauxii</i>	Lv.	Aq.	-	-	-	-	-	-
		Et.	-	-	-	-	-	15±0
		Ha.	-	-	-	-	-	-
	Fr.	Aq.	-	-	-	-	-	19±0
		Et.	-	-	-	-	-	-
		Ha.	-	-	-	-	-	-

Table 2 continued..

<i>Commiphora leptophloeos</i>	Sb.	Aq.	-	-	-	-	-	-	-		
<i>Eugenia brejoensis</i>		Et.	-	-	-	14.67±2.08	-	-	-		
		Ha.	-	-	-	15.33±2.08	-	-	-		
	Fr.	Aq.	-	-	-	-	-	-	-		
		Et.	-	-	-	-	-	-	-		
		Ha.	-	12±0	-	-	-	-	-		
<i>Jatropha mutabilis</i>	Lv.	Aq.	-	12±0	-	-	-	-	13±1		
		Et.	-	-	-	-	-	-	-		
		Ha.	9±0	14±0	15±0	8±0	10±0	-	-		
	Rt.	Ha.	28±1	26±1	21±0	-	-	-	-		
	<i>Libidibia ferrea</i> var. <i>ferrea</i>	Fr.	Aq.	-	-	-	-	-	-	-	
		Et.	12.67±1.15	19.67±1.52	12.33±1.52	-	-	-	-		
		Ha.	12.67±0.57	19±1	15.33±3.78	13.33±0.57	10.67±1.15	-	-		
Lv.		Aq.	-	-	-	-	-	-	-		
		Et.	-	-	-	-	-	-	-		
<i>Manilkara rufula</i>		Ha.	-	-	-	-	-	-	-		
	Lv.	Ha.	-	21±0	19±1	-	-	-	-		
	<i>Melocactus zehntneri</i>	Rt.	Aq.	-	-	-	-	-	-	-	
			Et.	11.67±0.57	-	-	-	-	-	-	
			Ha.	17.33±1.52	-	-	-	-	-	-	
Sb.		Ha.	16±0	22±1	17±0	-	-	-	-		
Lv.		Ha.	-	19±1	23±1	-	-	-	-		
<i>Myroxylon</i>	Lv.	Ha.	-	16±1	16±0	-	-	-	-		
	<i>Ouratea blanchetiana</i>	Lv.	Ha.	-	18±1	16±1	-	-	-	-	
		Lv.	Ha.	14±0	25±0	18±1	-	-	12±1	-	
		<i>Poincianella microphylla</i>	Fr.	Ha.	19±1	-	30±1	-	-	-	-



Table 2 continued..

<i>Piriqueta guianensis</i>	Rt.	Ha	15±0	14±0	-	-	-
<i>Pityrocarpa moniliformis</i>		Aq.	-	-	-	-	-
	Fr.	Et.	-	-	-	-	-
		Ha.	-	-	15±1	-	-
	Lv.	Aq.	-	-	-	-	-
<i>Stylosanthes viscosa</i>		Et.	10.67±0.57	15±1.73	13±1	-	-
		Ha.	10.67±0.57	17.67±1.52	15±0	-	-
<i>Turnera subulata</i>	Br.+ Lv	Ha.	16±0	10±0	-	-	-
	Br.	Ha.	-	19±1	15±0	-	-

Br. = branches; Fr. = fruit; Lv. = leaves; Rt. = root; Sb. = stem-bark; Solv. = Solvent; Aq. = Aqueous extracts; Et. = Ethanolic extracts; Ha. = Hydroalcoholic extracts; - = no activity B.s. = *Bacillus subtilis*; M.l. = *Micrococcus luteus*; S.a. = *Staphylococcus aureus*; E.c. = *Escherichia coli*; K.p. = *Klebsiella pneumoniae*; C.a. = *Candida albicans*.

Solvente: Aq. = Aqueous extracts; Et. = Ethanolic extracts; Ha. = Hydroalcoholic extracts.







The lower activity of tested plants against Gram-negative bacteria could be attributed to the presence of an extra outer membrane in their cell wall acting as a barrier for substances including antibiotics. The extracts were bactericidal in most cases. The aqueous extractions were less active than tinctures against Gram-positive and Gram-negative bacteria with MICs values between 600 and 2400 µg/mL.

The results of the phytochemical screening of the investigated hydroalcoholic extract showed the presence of different types of active constituents like flavonoids, terpenoids, tannins, etc. The results of our screening assays confirmed the use of the Brazilian traditional medicine. It is the first report about antimicrobial activities of some plants namely *Chamaecrista desvauxii*, *Melocactus zehntneri* and *Stigmaphyllon paralias*. Whereas other plants like *Anadenanthera colubrina* var. *cebil*, *Libidibia ferrea* var. *ferrea*, *Eugenia brejoensis* and *Buchenavia tetraphylla* are partly well investigated by our group [25-27].

These results indicate that different parts of same plant species have different antimicrobial effects. Even if extracts are prepared using the same parts (leaves or fruits or seeds) of the same plant species, it is possible that the bacteria on which they are effective and the degrees of the effect may vary. Factors such as the structure of soil, daily and seasonal changes during the collection of the plant material, and the physiological growth cycle of the plant may cause variations in the chemical compounds of the plant. In addition to these factors, the parts of plants, extraction process, solvent, and the species of bacteria that are used are also variables [28, 29].

In conclusion, the results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. *Buchenavia tetraphylla*, *Libidibia ferrea* var. *ferrea*, *Melocactus zehntneri* and *Pityrocarpa moniliformis* could be a source for antibacterial drugs against Gram-positive bacteria. Effective compounds to be obtained by the determination of the active compound in the plant can provide new resources for chemotherapeutics to be

synthesized. It will be a base to our further investigations on advanced purification.

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