

In vitro Antimicrobial Activity of Traditional Plant Used in Mestizo Shamanism from the Peruvian Amazon in Case of Infectious Diseases

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ABSTRACT

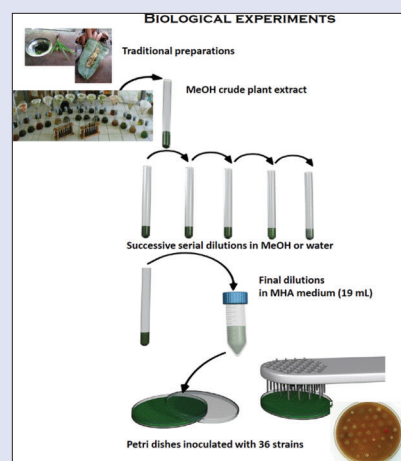
Context: Our survey was performed near Iquitos (Peruvian Amazon) and its surroundings and leads us to consider Mestizo ethnomedical practices. The plant species reported here are traditionally used for ailments related to microbial infections. Inhabitants of various ethnic origins were interviewed, and 52 selected plants extracts were evaluated for their antimicrobial properties against a panel of 36 sensitive and multi-resistant bacteria or yeast. The study aimed at providing information on antimicrobial plant extract activities and the ethnomedical context of Mestizo riverine populations from Loreto (Peru). **Material and Method:** The minimum inhibitory concentrations (MICs) of the plant crude extracts were carried out using the agar dilution method and ranged between 0.075 and 5.0 mg/ml. **Results:** Of the 40 plants analyzed, 9 species showed MIC ≤ 0.3 mg/ml (*Anacardium occidentale*, *Couropita guianensis*, *Croton lechleri*, *Davilla rugosa*, *Erythrina amazonica*, *Jacaranda copaia* subsp. *Spectabilis*, *Oenocarpus bataua*, *Peperomia macrostachya*, and *Phyllanthus urinaria*) for one or several of the 36 microorganisms and only 6 drug extracts were inactive. Among the 40 plants, 13 were evaluated for the first time for an antibacterial activity. **Conclusion:** This evaluation of the antimicrobial activity of 40 plants using an approved standard methodology allowed comparing those activities against various microbes to establish antimicrobial spectra of standardized plant extracts, and give support to the traditional use of these plants. It may also help discovering new chemical classes of antimicrobial agents that could serve against multi-resistant bacteria.

Key words: Antimicrobial activity, Iquitos, Loreto, medicinal plant, traditional use

SUMMARY

- This study leads us to consider Mestizo ethnomedical practices near Iquitos (Peruvian Amazon) and its surroundings. The plant species reported here are traditionally used for ailments related to microbial infections. 52 selected plants extracts were evaluated for their antimicrobial properties against a

panel of 36 sensitive and multi resistant bacteria or yeast. The study aimed at providing information on antimicrobial plant extract activities and the ethnomedical context of Mestizo riverine populations from Loreto.



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DOI : 10.4103/0973-1296.172975

Access this article online

Website: www.phcog.com

Quick Response Code:



INTRODUCTION

Resistance to antibiotics in numerous pathogens has become a real public health problem, it concerns specific pathogens causing diseases such as tuberculosis or gonorrhea but also opportunistic pathogens involved in nosocomial infections.^[1-5] Recently, the WHO expressed urgent need for the development of new antibacterial compounds.^[6-8] But the number of new drugs in development is low, raising the question for alternative research.

As plants have evolved since hundreds of millions of years in the presence of bacteria and develop rarely bacterial diseases (even in warm and damp conditions, which are favorable for bacterial development), this provides a rational basis for the search for vegetal antibacterial compounds. In addition, the long standing empirical use of herbal medicine against infectious ailments is consistent with the previous observation. Our study took place

in the Northern region of Peru called Loreto (one of the Peruvian biggest Amazonian province) with a high level of both biodiversity and traditional knowledge. This knowledge has been transmitted and has circulated

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Cite this article as: Roumy V, Gutierrez-Choquevilca AL, Lopez Mesia JP, Ruiz L, Ruiz Macedo JC, Abedini A, et al. *In vitro* antimicrobial activity of traditional plant used in mestizo shamanism from the Peruvian amazon in case of infectious diseases. Phcog Mag 2015;11:625-33.

through constant interactions and exchanges between Indigenous groups and Mestizo populations, in particular during the Rubber Boom era, leading to a large repository of medicinal practices of various origins.

Our ethnographic survey was performed in Iquitos and its surroundings (Tamshi Yacu) and leads us to consider Mestizo ethnomedical practices as part of a larger ethnomedical system including neighboring Amerindian knowledge and practices. On the basis of traditional medicinal reports gathered during our ethnopharmacological survey, 40 different plants used in the Amazonian district of Peru were selected, and their antimicrobial activity was assessed. The minimal inhibitory concentration (MIC) was determined *in vitro* against 36 microbial strains belonging to 21 different species.

MATERIALS AND METHODS

Ethnomedical context

An intensive ethnographic survey was necessary to understand the relevance and the variable selective criteria of traditional herbal medicines. A “participant-observation” methodology was adopted. Many interviews were performed in the Belén Market in Iquitos, where Mestizo local people commercialized most of the plant species studied, for medical use. The medicinal plants market of Belén is located on the shore of the Itaya River. It started in the late sixties with two women: María de Marreros and María de Yap. In the late nineties, the majority of the “puestos” (around 30 in total) were under the responsibility of women engaged in the commercialization of medicinal plants to urban population and mainly to local shamans called “curanderos”, “vegetalistas” or “medicos” in local Spanish. The local shamans were identified as belonging to different indigenous origins: Mainly Cocama (A. Curico Murayari), Lowland Napo Quechua or Lamista Quechua and Shipibo from the Ucayali. The majority of the 140 commercialized plant species do not come from cultivated areas but from more distant primary forest areas. The three main zones of extraction are the River Napo, the River Nanay, and the rural surroundings of the main road stretching from Iquitos to Nauta.^[9,10]

Although Mestizo shamanism and plant therapy have been mentioned as playing an important role in the medical system of Iquitos and its rural surroundings, an extensive documentation on medicinal plants use linked with ethnomedical practices has never been fully undertaken.^[11-13] Why such a lack of interest for disclosing an ethnomedical overview of Mestizo traditional therapy? Mestizo culture has for a long time appeared as less “traditional” than other indigenous systems, such as the neighboring Shipibo groups, where more extensive ethnopharmacological studies were performed.^[14-16] However, the Mestizo riverine population dwelling the area of study have been, since the early colonial period until nowadays, engaged in constant communication and exchanges with Indigenous population: There are the Shipibo from the Ucayali River (Panoan linguistic family), the Quechua Napu Runa from the Napo and Tigre River, the Quechua Lamista from the Huallaga, or the Cocama from the Amazon and Marañon River. The interactions between Mestizo and Amerindian groups did not restrict to the exchanges of manufactured goods during the Rubber Boom era (1875-1914) and after, but both population have influenced each other from the perspective of medical knowledge and more specifically plant therapy.^[17,18]

In this ethnopharmacological investigation, a close attention was paid to the practices of plant therapy among the Mestizo population. Speaking of mestizo pharmacopoeia without giving a short insight on their conception of the therapeutic process and their representations of illness could be missing the chore of ethnomedical practices in the Peruvian Amazon. For Mestizo riverine populations, most severe diseases etiologies such as strong skin infections, malaria, leishmaniasis, or other physical disorders are often classified in the category of sorcery: “brujería” in local Spanish. In these contexts, traditional ritual specialists are called to diagnose the

spiritual causes of physical disorders, thanks to their dreams and visionary power. Most of the Mestizo ritual specialists refer to themselves as “curanderos” or “vegetalistas” – specialized in the use of medicinal plants.

Ethnobotanical survey

The ethnobotanical study was conducted during June-August 2011 and 2013. Claims of effective therapy for the treatment of cough, diarrhea, dysentery, piles, skin diseases, conjunctivitis, chronic bronchitis, tuberculosis, genito-urinary diseases, and sore throat by traditional herbalists have prompted our interest in this scientific investigation. Information presented here was compiled through general conversations, informal interviews, and rainforest walks with healers, midwives, and local people with knowledge in self-medication using plants.

Plant material

The plants were collected (in forest or in the market), identified, and deposited in the herbarium of the Universidad Nacional de la Amazonia Peruana (UNAP, Iquitos) by the botanist Juan Celidonio Ruiz Macedo, then a second collection was made in higher quantities in the surroundings of Iquitos (voucher specimen, Table 1) for biological essay. In few cases, the collected part of the plant did not match to the drug traditionally used, due to the collection opportunities.

The plant samples were dried, finely grounded with a hammer mill, and extracted overnight with methanol at room temperature, with gentle shaking (methanol provide a more complete extraction, including less polar compounds, and still more representative of the traditional preparations as aqueous or ethanolic extracts). The extracts were filtered through filter paper, dried under reduced pressure at 40°C, and weighed. Thus, crude extracts were dissolved in methanol to a final concentration of 10 mg/ml.

Minimal inhibitory concentration determination

Selected microorganisms

In the setting of malnutrition, HIV, aging, organ transplantation, other immunodeficiency etiologies, and opportunistic infections are common causes of morbidity and mortality. That is the reason why we selected various bacteria, some of which display resistance to antibiotics or can be involved in opportunistic or nosocomial infections or in diseases cited by the informants. The various microorganisms (Gram-positive, Gram-negative, yeast) were all able to grow aerobically in Mueller Hinton Agar (MHA) media.

The investigations use standardized methodology with internationally recognized protocols (CLSI, 2006), American Type Culture Collection strains (reference strains from the ATCC), and drug multi-resistant clinical strains (that reflect what is encountered today in hospital from the CHRU Lille, France).^[19] For each strain, we added the registration number in the CFPL collection, followed by the mentions of antimicrobial resistance or the type of enzymes implicated in resistance. Species identification (determined by mass spectrometry) and antibiogram of each bacteria (cultivated or cryopreserved) are evaluated 3 times in the year.

Bacteria could be divided into several groups

The first three groups include enterobacteria (Gram-negative Bacilli)

The first of those includes lactose-positive, VP negative enterobacteria (which are usually poorly resistant and pathogenic): *Escherichia coli* (8137; 8138: Penicillin resistance; 8157 penicillin and fluoroquinolone resistances; ATCC 25922: NA) and *Citrobacter freundii* (11041; 11042: Cephalosporin resistance; 11043: TEM 3) [Table 2]. The second group of enterobacteria includes lactose-positive, VP positive enterobacteria with frequent implication in nosocomial infections and high antibiotic

Table 1: Alphabetic list and traditional anti-infectious uses of the species investigated

Scientific name, (family)	Part used	Voucher specimen	Local name	Popular uses to treat infectious diseases
<i>Aegiphila filipes</i> Mart and Schauer (Lamiaceae)	L	025657	Arco sacha	Infusion used to clean sores
<i>Ambelania acida</i> Aubl. (Apocynaceae)	L	038479	Pepino del monte	Infusion against diarrhea
<i>Anacardium occidentale</i> L. (Anacardiaceae)	B, L	034216	Casho, m	Crushed leaves or bark decoction applied on skin and genital infections or juice against diarrhea and stomach disorder
<i>Andropogon citratus</i> DC. ex Nees (Poaceae)	L	033669	Yerba Luisa, m	Infusion of leaves against throat infection and nervousness
<i>Bidens pilosa</i> L. (Asteraceae)	Ep, L	035565	Amor seco, m	Infusion of entire plant against cystitis or sore throat. Ointment or ablution with leaves against sore
<i>Bixa orellana</i> L. (Bixaceae)	L, Fr	026035	Achiote, m	Fruit and leaves used to treat sores and skin infections. Decoction of leaves against conjunctivitis dysentery, gonorrhea, or for liver, stomach, and kidney purification
<i>Brugmansia suaveolens</i> (H. and B. ex Wild.) (Solanaceae)	L	033864	Toé, m	Decoction against dermatitis and hurts (psychotropic use in shamanism)
<i>Cedrela odorata</i> L. (Meliaceae)	B	033958	Cedro rojo (amarillo), m	Decoction against sores and cut infections, diarrhea, or malaria
<i>Ceiba samauma</i> (Mart.) K. Schum. (Malvaceae)	L	029810	Huimba	Ointment of leaves against skin infection
<i>Cissus sicyoides</i> L. (Vitaceae)	Re	026513	Sapo huasca	Ointment of resin against sores and hernia
<i>Couroupita guianensis</i> Aubl. (Lecythidaceae)	L, B	033949	Ayahuma, m	Ablution against skin infection due to insect bites, plague, and animals infections. Decoction against digestive infections. Shamanic use to cure sorcery aggression
<i>Croton lechleri</i> Müll. Arg. (Euphorbiaceae)	Re	025080	Sangre de grado, m	Diluted in warm water against throat and stomach or genital infections or directly applied on skin (against acne...)
<i>Davilla kunthii</i> A. St.-Hil. (Dilleniaceae)	Re	036507	Hoja chigue	Section of the stem provides an aqueous resin used against thirst, cystitis, and to wash tools
<i>Davilla rugosa</i> Poir. (Dilleniaceae)	B; L	027099	Chaparo	Decoction of the liana against cystitis and stomach disorder
<i>Desmodium adscendens</i> (Sw.) (Fabaceae)	L	039768	Amor seco, m	Infusion against lungs or skin and genital infections
<i>Echites macranthus</i> Wild. Ex Roem and Schult. (Apocynaceae)	B	024164	Sapo huasca	Bark decoction ointment against skin infection
<i>Erythrina amazonica</i> Krukoff (Fabaceae)	L; B	037513	Amasisa	Bark decoction to wash skin infections (dermatitis, herpes...). Resin applied on skin and genital infections. Bark and root decoction against fever
<i>Erythrina fusca</i> Lour. (Fabaceae)	L; B	035812	See <i>Erythrina amazonica</i> , m	
<i>Jacaranda copaia</i> subsp. <i>spectabilis</i> (Mart. ex A. DC.) A.H. Gentry (Bignoniaceae)	L	027134	Chichara caspi	Crushed, applied on skin and mouth infections
<i>Jatropha gossypifolia</i> L. (Euphorbiaceae)	L	033699	Piñón rojo, m	Infusion against throat infection. Stem decoction against children diarrhea. Fruit and leaves crushed against sore and skin infections. Shamanic use to cure sorcery aggression
<i>Laportea aestuans</i> (L.) Chew (Urticaceae)	R	033692	Ishanga colorada, m	Decoction against measles
<i>Loranthus pyrifolius</i> Kunth (Loranthaceae)	Ap	038873	Suelda con suelda, m	Ointment to cure open bone fracture
<i>Oenocarpus bataua</i> Mart. (Arecaceae)	R	033549	Ungurahui	Decoction against diarrhea
<i>Oryctanthus alveolatus</i> (Kunth) Kuijt (Loranthaceae)	L	038636	Suelda con suelda	Macerated leaves applied on skin infections
<i>Passiflora coccinea</i> Aubl. (Passifloraceae)	L	028536	Granadilla, m	Infusion for "blood purification", fever, conjunctivitis, and as an antiseptic
<i>Passiflora guazumaifolia</i> Juss. (Passifloraceae)	L	029498	Sacha granadilla	Infusion as an antiseptic
<i>Peperomia macrostachya</i> (Vahl) A.Dietr. (Piperaceae)	Ap	039900	Lancetilla, m	Crushed leaves against mouth infection. Ablution with crushed leaves to cure fever
<i>Petiveria alliacea</i> L. (Phytolaccaceae)	L	035371	Mucura, m	Ablution with crushed leaves against skin infection and sorcery aggression. Leaf decoction to wash sores and conjunctivitis
<i>Phlebodium decumanum</i> (Willd.) J. Sm (Polypodiaceae)	R	035991	Cotochupa, m	Infusion of ground roots against cystitis, fever, coughs. Root plaster against sore
<i>Phyllanthus urinaria</i> L. (Euphorbiaceae)	Ep, R	024633	Chanca piedra, m	Decoction against cystitis
<i>Piper arboreum</i> Aubl. (Piperaceae)	L	025910	See <i>Piper tuberculatum</i>	
<i>Piper peltatum</i> L. (Piperaceae)	L	037570	Santa Maria, m	Soaked leaves against fever and headache. Leaf infusion to wash the parturient woman's body
<i>Piper tuberculatum</i> Jacq. Aubl. (Piperaceae)	L	020115	Cordoncillo	Infusion against bronchitis and cystitis
<i>Pseudobombax munguba</i> (Mart. and Zucc.) Dugand (Bombacaceae)	B	028310	Punga	Decoction against bronchitis and rheumatism
<i>Senna bacillaris</i> (L. f.) H.S. Irwin and Barneby (Fabaceae)	Fr	038907	Mataro	Ointment of ground fruit against sores and fungal infections
<i>Senna reticulata</i> (Willd.) H.S. Irwin and Barneby (Fabaceae)	Fl, L	033702	Retama, m	Infusion against intestinal infections and purge
<i>Spondias mombin</i> L. (Anacardiaceae)	B, L	033882	Ubos, m	Root decoction against alcoholism
				Leaf or bark plaster as an antiseptic and aqueous preparation against diarrhea and genital infections or sores (e.g., due to leishmania)

Contd...

Table 1: Contd...

Scientific name, (family)	Part used	Voucher specimen	Local name	Popular uses to treat infectious diseases
<i>Tabernaemontana sananho</i> Ruiz and Pav (Apocynaceae)	L, R	028045	(Lobo) sanango	Leaf infusion against conjunctivitis, rheumatism and fever. Plaster of carbonized roots against sores
<i>Tripogandra serrulata</i> (Vahl)	Ap	037971	Trapograpa	Masticated leaves against mouth infections, caries
<i>Handlos</i> (Commelinaceae)				
<i>Unonopsis floribunda</i> Diels (Annonaceae)	B	034628	Icoja, m	Alcoholic or urinary macerate of the bark against diarrhea. Bark decoction against dermatitis and fever

Ap: Aerial parts; B: Stem bark; Br: Branch; Ep: Entire plant; Fl: Flower; Fr: Fruit; L: Leaves; R: Root; Re: Resin; m: Present in the market (depending from the season)

Table 2: MIC concentrations (mg/ml) for the bacteria of the first group of enterobacteria

Species	Part	Strains						
		<i>C. freundii</i> (11041)	<i>C. freundii</i> (11042)	<i>C. freundii</i> (11043)	<i>E. coli</i> (8137)	<i>E. coli</i> (8138)	<i>E. coli</i> (8157)	<i>E. coli</i> (ATCC 25922)
<i>Anacardium occidentale</i>	B	2.5	2.5	2.1				
<i>Couroupita guianensis</i>	B	2.5	2.5	2.5	5	5		2.5
	L	1.7	1.2	2.5		2.5		1.7
<i>Croton lechleri</i>	Re	0.6	0.6	0.6	1.2	1.1	1.2	0.6
<i>Davilla kunthii</i>	Br	2.5	2.5	-	5			
<i>Davilla rugosa</i>	Br	5	5	2.5	-			

C. freundii: *Citrobacter freundii*; *E. coli*: *Escherichia coli*; MIC: Minimal inhibitory concentration; ATCC: American Type Culture Collection; Br: Branch; B: Stem bark; L: Leaves; Re: Resin

Table 3: MIC concentrations (mg/ml) for the bacteria of the second group of enterobacteria

Species	Part	Strains							
		<i>E. aerogenes</i> (9004)	<i>E. cloacae</i> (11050)	<i>E. cloacae</i> (11051)	<i>E. cloacae</i> (11053)	<i>K. pneumoniae</i> (11016)	<i>K. pneumoniae</i> (11017)	<i>S. marcescens</i> (11056)	<i>S. marcescens</i> (11057)
<i>Anacardium occidentale</i>	B	2.5	2.5	1.7	2.5		0.6	2.5	1.7
<i>Couroupita guianensis</i>	B	2.5	2.5	2.5	2.5		0.6	2.5	2.5
<i>Couroupita guianensis</i>	L	1.7	2.5	2.5	2.5		0.6	2.5	2.5
<i>Croton lechleri</i>	Re	0.6	0.6	0.6	0.6	1.2	0.6	0.6	0.6
<i>Davilla kunthii</i>	Br						1.2	2.5	0.6
<i>Davilla rugosa</i>	Br	5	5	5	5		0.6	5	5
<i>Loranthus pyrifolius</i>	Ap	-					1.2		
<i>Jacaranda copaia</i>	B						2.5		
	L						1.2		
<i>Passiflora guazumaifolia</i>	L						2.5		
<i>Spondias mombin</i>	B					2.5	0.6		
	L						5		
<i>Unonopsis floribunda</i>	B						1.2		

MIC: Minimal inhibitory concentration; B: Stem bark; L: Leaves; Re: Resin; Br: Branch; Ap: Aerial parts; *E. aerogenes*: *Enterobacter aerogenes*; *E. cloacae*: *Enterobacter cloacae*; *K. pneumoniae*: *Klebsiella pneumoniae*; *S. marcescens*: *Serratia marcescens*

Table 4: MIC concentrations (mg/ml) for the bacteria of the third group of enterobacteria

Species	Part	Strains			
		<i>P. mirabilis</i> (11060)	<i>P. stuartii</i> (11038)	<i>Salmonella</i> sp. (11033)	<i>Salmonella</i> sp. (11037)
<i>Anacardium occidentale</i>	B	0.6	0.6		2.5
<i>Bixa orellana</i>	Fr	2.5	2.5		-
<i>Couroupita guianensis</i>	B	1.2	1.2	2.5	2.5
	L	0.6	0.6	2.5	2.5
<i>Croton lechleri</i>	Re	0.3	0.3	0.6	0.6
<i>Davilla kunthii</i>	Br	1.2	1.2		
<i>Davilla rugosa</i>	Br	5	0.6	5	5
<i>Jacaranda copaia</i>	B	2.5	2.5		
<i>Jacaranda copaia</i>	L	5	5		
<i>Loranthus pyrifolius</i>	Ap	1.2	2.5		
<i>Oryctanthus alveolatus</i>	Br	5	5		
<i>Spondias mombin</i>	B	2.5	2.5		

MIC: Minimal inhibitory concentration; B: Stem bark; Fr: Fruit; L: Leaves; Re: Resin; Ap: Aerial parts; Br: Branch; *P. mirabilis*: *Proteus mirabilis*; *P. stuartii*: *Providencia stuartii*

resistance: *Klebsiella pneumoniae* (11016; 11017: Penicillin resistance), *Enterobacter cloacae* (11050; 11051: Cephalosporin resistance; 11053: NDM-1), *Enterobacter aerogenes* (9004: BLSE) and *Serratia marcescens* (11056; 11057: Cephalosporin resistance) [Table 3]. The third group includes lactose-negative and more pathogenic enterobacteria: *Proteus mirabilis* (11060), *Providencia stuartii* (11038), *Salmonella* spp. (11033; 11037: CMY 2: Penicillin resistance) [Table 4].

The fourth group includes other Gram-negative bacteria also found with a high frequency in nosocomial infections: *Pseudomonas aeruginosa* (8131; ATCC 27583), *Acinetobacter baumannii* (9010: VEB-1; 9011: Multi-resistant), *Stenotrophomonas maltophilia* [Table 5].

The fifth group includes Gram-positive cocci (that can be involved in external infections): *Staphylococcus aureus* (8146: Meticillin- and kanamycin-resistant; 8147), *Staphylococcus epidermidis* (5001, 10282), *Staphylococcus lugdunensis* (T26A3), *Staphylococcus warneri* (T12A12), *Enterococcus* spp. (8152 aminoglycoside-resistant; 8153: erythromycin- and clindamycin-resistant), and *Enterococcus faecalis* (C159-6 vancomycin-susceptible) [Table 6].

Table 5: MIC concentrations (mg/mL) for the bacteria of the fourth group (nonenterobacteria Gram-negative)

Species	Part	Strains				
		<i>A. baumannii</i> (9010)	<i>A. baumannii</i> (9011)	<i>P. aeruginosa</i> (8131)	<i>P. aeruginosa</i> (ATCC 27583)	<i>S. maltophilia</i> (TP)
<i>Anacardium occidentale</i>	B	1.2	1.7	2.5	1.2	0.3
<i>Anacardium occidentale</i>	L		5			0.3
<i>Andropogon citratus</i>	Ep		5	5		
<i>Bidens pilosa</i>	L					5
<i>Bixa orellana</i>	Fr	2.5			2.5	0.6
	L					1.2
<i>Cedrela odorata</i>	B					2.5
<i>Couroupita guianensis</i>	B	1.2	1.2	2.5	1.2	0.6
	L	1.2	1.2	2.5	1.2	0.6
<i>Croton lechleri</i>	Re	0.3	0.6	0.6	0.3	2.1
<i>Davilla kunthii</i>	Br	2.5	2.5	2.5	1.2	0.6
<i>Davilla rugosa</i>	L					0.6
	Br	2.1	1.2	1.2	2.1	0.3
<i>Jacaranda copaia</i>	B	-			2.5	2.5
	L	0.5			2.5	1.2
<i>Loranthus pyrifolius</i>	Ap	-	2.5		2.5	0.6
<i>Oryctanthus alveolatus</i>	Br	5	5		5	2.5
	L					5
<i>Oenocarpus bataua</i>	Fr					2.5
<i>Peperomia macrostachya</i>	Ap		0.6			-
<i>Phyllanthus urinaria</i>	Ep		2.5			1.2
<i>Piper peltatum</i>	L					2.5
<i>Pseudobombax munguba</i>	B					5
	H					5
<i>Senna reticulata</i>	Fl					5
	L					5
<i>Spondias mombin</i>	B	5	5	2.5	2.5	1.2
	L			5		2.5

MIC: Minimal inhibitory concentration; B: Stem bark; Fr: Fruit; L: Leaves; Ep: Entire plant; Re: Resin; Br: Branch; Ap: Aerial parts; Fl: Flower; *A. baumannii*: *Acinetobacter baumannii*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. maltophilia*: *Stenotrophomonas maltophilia*; ATCC: American Type Culture Collection

The last group contains other miscellaneous microorganisms: *Mycobacterium smegmatis* (5003), *Corynebacterium striatum* (T25-17), *Candida albicans* (10286) [Table 7].

Antimicrobial tests

MIC determinations of the plant crude extracts were carried out using the agar dilution method stipulated by the CLSI agar dilution methods (CLSI2006).

The plant extracts and the antimicrobial drugs were evaluated, the MIC of the extracts was determined for 35 bacterial strains and 1 yeast by diluting the extracts in MHA media. The inhibitory concentrations ranged between 0.075 and 5.0 mg/ml in seven dilutions (5.0, 2.5, 1.2, 0.6, 0.3, 0.15, and 0.075 mg/ml), 0.075 mg was considered as the lower concentration for a preliminary screening and MIC >5.0 mg/ml were considered as nonactive against tested strains.^[20] Petri dishes (controls and extracts), were inoculated with strains (10^4 CFU, obtained by dilution in brain heart) using a Steer's replicator and were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of extract without bacterial growth after incubation. Control antibiotics were evaluated, and specific anti-biograms were available for each strain (see additional file). Moreover, if we rely on the fact that antibiotics do not have a vegetal origin, we cannot expect that plant extracts could have the same efficiency as that of pure antibiotics compounds. For each group of bacteria, only plants showing an activity are mentioned. The extracts that exhibited MIC ≤ 0.3 mg/ml were tested in triplicate at lower concentrations (down to 0.075 mg/ml, and standard deviation are done for values: 4.2 ± 1.7 ; 2.1 ± 0.8 ; 1.7 ± 0.8 ; 1.1 ± 0.2 ; 0.5 ± 0.1 ; 0.4 ± 0.1 , and <0.0 for other values).

Thin-layer chromatography bioautography

Plates (Silica gel 60 Xtra Sil G/UV254) were developed with ethyl acetate:methanol:water (16:2:1). Tannins were revealed with FeCl_3 aqueous solution. Other developed thin-layer chromatography (TLC) plates were dried and overlaid by nutrient agar seeded with an overnight culture of *S. epidermidis* (5001). The plate was incubated for 24 h at 37°C and then sprayed with a solution of p-iodonitrotetrazolium violet (1-h later, clear zones appears corresponding to bacterial inhibition).

RESULTS

Ethnobotanical study

Bark or roots are also often prepared as hydro-alcoholic maceration and need special diet and cannot be mixed with other plants or food.^[21] Some common names, such as Sapo huasca, Amor seco, Masisa, Suelda con suelda, and Cordoncillo, can refer to different species [Table 1]; this may be ascribed to a loss in traditional medical knowledge in the district of Iquitos. For few species (*Oryctanthus alveolatus*, *Pseudobombax munguba*, *Tabernaemontana sananho*), the plant organ tested do not exactly match with the part used (cause of collection opportunities).

Biological screening methodology and activities

As the authors well known the difficulties to ascribe any specific identified "bacteria" to a so-called "traditional use" they decided to test plant extracts on a large panel of 36 bacteria or yeast, in order to be more representative of the infectious pathogens enhanced in the described disease. The agar dilution method (macrodilution) with methanolic extract enabled to test the antibiotic activity of all our plants without

Table 6: MIC concentrations (mg/mL) for the bacteria of the fifth group (Gram-positive cocci)

Species	Part	Strains								
		<i>E. faecalis</i> (C159-6)	<i>Enterococcus</i> <i>sp.</i> (8152)	<i>Enterococcus</i> <i>sp.</i> (8153)	<i>S. aureus</i> (8146)	<i>S. aureus</i> (8147)	<i>S. epidermidis</i> (5001)	<i>S. epidermidis</i> (10282)	<i>S. lugdunensis</i> (T26A3)	<i>S. warneri</i> (T12A12)
<i>Aegiphila filipes</i>	L				-	5	-	1.2	2.5	2.5
<i>Ambelania acida</i>	B				5			2.5	5	5
<i>Anacardium occidentale</i>	B	1.2	1.7	1.2	0.6	0.6	0.5	0.15	0.4	0.6
<i>Anacardium occidentale</i>	L	2.5	2.5	2.5	1.2	1.2	2.5	0.6	1.2	1.2
<i>Andropogon citratus</i>	Ep				2.5	2.5	1.2	0.6	2.5	
<i>Bidens pilosa</i>	L		2.5	2.5					5	
<i>Bixa orellana</i>	Fr			5	2.5	2.5	5	0.6	1.2	1.2
	L				2.5	1.2	5	0.6	0.6	1.2
<i>Brugmansia suaveolens</i>	L	5	5	5						
<i>Cedrela odorata</i>	B	2.5	1.2	2.5	1.2	1.2	2.5	1.2	0.6	1.2
<i>Ceiba samauma</i>	L	5	2.5	2.5	1.2	1.2	2.5	5	2.5	2.5
<i>Couroupita guianensis</i>	B	1.2	2.5	1.2	1.2	1.2	2.5	0.3	0.3	1.2
	L	2.5	2.5	1.2	0.6	0.6	1.2	0.3	0.6	0.6
<i>Croton lechleri</i>	Re	0.6	1.2	0.6	0.3	0.3	0.6	0.075	0.15	0.15
<i>Davilla kunthii</i>	Br	1.2	0.6	2.5	0.6	0.6	0.6	0.6	0.6	0.6
<i>Davilla rugosa</i>	L	1.2	0.6	0.6	0.6	0.6	0.6	2.5	0.6	1.2
	Br	2.5	2.5	2.5	0.6	0.6	1.2	0.3	0.3	0.6
<i>Desmodium adscendens</i>	Ep	5	5		5	5		1.2		
<i>Echites macranthus</i>	B	2.5	2.5		2.5	2.5	5	2.5	1.2	2.5
	L	2.5	2.5		2.5	2.5	5	0.3	2.5	2.5
<i>Erythrina amazonica</i>	B	0.3	0.3	0.3	0.075	0.15	0.3	0.15	0.15	0.6
<i>Erythrina amazonica</i>	L	2.5			5	2.5		1.2	2.5	5
<i>Jacaranda copaia</i>	B				2.5	2.5	5	0.6	1.2	2.5
<i>Jacaranda copaia</i>	L				5	1.2	5		0.2	1.2
<i>Loranthus pyrifolius</i>	Ap	5	5		2.5	1.2	2.5	5	1.2	1.2
<i>Oryctanthus alveolatus</i>	Br	2.5	2.5		2.5	2.5	2.5	0.6	2.5	2.5
	L	2.5	2.5		2.5	1.2	2.5	1.2	2.5	2.5
<i>Oenocarpus bataua</i>	Fr	1.2	1.1	0.6	0.6	0.6	0.6	1.1	0.6	1.2
<i>Passiflora coccinea</i>	L			5				2.5	5	
<i>Passiflora guazumaifolia</i>	L	2.5	5		2.5	2.5	5	1.2	2.5	5
<i>Peperomia macrostachya</i>	Ap	0.075	0.15	0.15	0.3	0.4	0.075	0.3	0.075	0.15
<i>Phyllanthus urinaria</i>	Ep	5	5		2.5	2.5	5	0.3	2.5	2.5
<i>Piper arboreum</i>	L	5	2.5		5	5	5	2.5	5	5
<i>Piper peltatum</i>	L	5	5		2.5	2.5		2.5	2.5	
<i>Pseudobombax munguba</i>	B	1.2	1.2		1.2	1.2	2.5	5	2.5	2.5
	H	1.2	1.2		1.2	0.6	2.5	2.5	1.2	2.5
<i>Senna reticulata</i>	Fl	1.2	1.2		1.2	1.2	2.5	2.5	1.2	2.5
	L	5	5			2.5		0.6	2.5	5
<i>Spondias mombin</i>	B	1.2	1.2		1.2	1.2	1.2	5	1.2	1.2
	L	1.2	1.2				1.2	2.5	5	5
<i>Unonopsis floribunda</i>	B	2.5	2.5		2.5	2.5	5	1.2	2.5	5

MIC: Minimal inhibitory concentration; L: Leaves; B: Stem bark; Ep: Entire plant; Fr: Fruit; Re: Resin; Ap: Aerial parts; Br: Branch; Fl: Flower; *E. faecalis*: *Enterococcus faecalis*; *S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. lugdunensis*: *Staphylococcus lugdunensis*; *S. warneri*: *Staphylococcus warneri*

problem of solubility, to obtain standardized numeric value (MIC) for each of the 36 microorganisms.

Only six plant extracts were active against the first group of enterobacteria [Table 2] with the lowest MIC values for *Croton lechleri* on all strains. As concerns *E. coli*, only four extracts have an activity against at least one of the four strain species.

Twenty-one plants show activities against the second group of enterobacteria, especially against the *Enterobacter cloacae* strain 11053 [Table 3]. We also observed the global activity of the *C. lechleri* extract and the multi-resistance of *K. pneumonia* (inhibited by only two extracts).

Concerning the last group of enterobacteria [Table 4], we also observed the highest antibacterial activity for *C. lechleri* and the highest level of resistance for the *Salmonella* spp. strains.

Usually, Plant extracts were more active against the fourth group of bacteria (nonenterobacteria Gram-negative) that are involved in nosocomial infections and often highly resistant to antibiotics. We can

underline a high sensitivity of the *S. maltophilia* strain that is emerging pathogen in hospital with life-threatening infections [Table 5]. Best antibacterial activities were obtained for *C. lechleri*, *Anacardium occidentale*, *Couroupita guianensis*, *Davilla rugosa*, and *Spondias mombin* extracts.

A majority of plant extracts showed good activity against Gram-positive cocci especially against the genera *Enterococcus* and *Staphylococcus*, which validates the use against skin infections frequently stated in the survey [Table 6]. *Peperomia macrostachya* shows an unexpected activity with low MIC concentrations for both *Enterococcus* and *S. aureus*, even for the meticillin-resistant (8146) strain whereas it has never been studied before for its biological or chemical properties.

The sixth group also contains the nonpathogenic species *M. smegmatis*, which is close in structure to the highly pathogenic *Mycobacterium tuberculosis* [Table 7]. An activity on this strain is a good argument for further investigation, as drug resistance is on the rise and primarily in HIV-infected patients, is associated with a significantly higher

Table 7: MIC concentrations (mg/mL) for the bacteria of the sixth group comprising miscellaneous strains

Species	Part	Strains		
		<i>C. albicans</i> (10286)	<i>C. striatum</i> (T25-17)	<i>M. smegmatis</i> (5003)
<i>Aegiphila filipes</i>	L		5	5
<i>Ambelania acida</i>	B	5	5	
<i>Anacardium occidentale</i>	B	1.1	0.3	0.3
	L			0.6
<i>Andropogon citratus</i>	Ep		0.6	2.5
<i>Bixa orellana</i>	Fr		0.6	1.2
	L		0.3	0.6
<i>Brugmansia suaveolens</i>	L		5	
<i>Cedrela odorata</i>	B		0.6	0.6
<i>Ceiba samauma</i>	L		1.2	0.6
<i>Couroupita guianensis</i>	B	2.5	0.6	0.6
<i>Couroupita guianensis</i>	L	2.5	0.6	0.4
<i>Croton lechleri</i>	Re	0.15	0.075	0.15
<i>Davilla kunthii</i>	Br	1.2	0.6	0.6
<i>Davilla rugosa</i>	L	5	5	1.2
<i>Davilla rugosa</i>	Br	1.2	0.2	0.6
<i>Desmodium adscendens</i>	Ep		2.5	5
<i>Echites macranthus</i>	B		0.6	0.6
<i>Echites macranthus</i>	L		0.6	1.2
<i>Erythrina amazonica</i>	B	0.15	0.075	0.15
<i>Erythrina amazonica</i>	L		0.6	5
<i>Erythrina fusca</i>	B		1.2	
<i>Jacaranda copaia</i>	B		1.2	1.2
<i>Jacaranda copaia</i>	L		1.2	1.2
<i>Loranthus pyrifolius</i>	Ap	0.6	0.3	1.2
<i>Oryctanthus alveolatus</i>	Br		0.6	0.6
<i>Oryctanthus alveolatus</i>	L		1.2	1.2
<i>Oenocarpus bataua</i>	R		2.5	5
<i>Oenocarpus bataua</i>	Fr		0.2	0.6
<i>Passiflora guazumaifolia</i>	L		1.2	1.2
<i>Peperomia macrostachya</i>	Ap	2.5	0.075	0.075
<i>Phyllanthus urinaria</i>	Ep		-	1.2
<i>Piper arboreum</i>	L		2.5	5
<i>Piper peltatum</i>	L		1.2	2.5
<i>Pseudobombax munguba</i>	B		0.6	1.2
<i>Pseudobombax munguba</i>	H		0.6	5
<i>Senna reticulata</i>	Fl		1.2	2.5
<i>Senna reticulata</i>	L		1.2	2.5
<i>Spondias mombin</i>	B	1.2	0.6	
<i>Spondias mombin</i>	L		2.5	
<i>Tabernaemontana sananho</i>	Br		2.5	
<i>Unonopsis floribunda</i>	B		1.2	

MIC: Minimal inhibitory concentration; L: Leaves; B: Stem bark; Ep: Entire plant; Fr: Fruit; Re: Resin; Br: Branch; Fl: Flower; Ap: Aerial parts; *C. albicans*: *Candida albicans*; *C. striatum*: *Corynebacterium striatum*; *M. smegmatis*: *Mycobacterium smegmatis*

mortality rate and short survival period, which makes the search for new anti-tuberculosis molecules of great interest.

Of the 40 plants analyzed (52 methanolic extracts), 9 species showed MIC ≤ 0.3 mg/ml (*A. occidentale*, *C. guianensis*, *C. lechleri*, *D. rugosa*, *Erythrina amazonica*, *Jacaranda copaia* subsp. *Spectabilis*, *Oenocarpus bataua*, *P. macrostachya*, *Phyllanthus urinaria*) for one or several of the 36 microorganisms and only 7 extracts were inactive (*Bidens pilosa*, *Jatropha gossypifolia*, *Laportea aestuans*, *Petiveria alliacea*, *Phlebodium decumanum*, *Piper tuberculatum*, *Tripogandra serrulata*).

Examination by bio-autography of the *Davilla kunthii* and *D. rugosa* (chemically unknown species) revealed an antibacterial activity due to polar compounds. Revelation with FeCl_3 aqueous solution of the TLC permitted to identify hydrolysable tannins as responsible of the antibacterial activity.

Among the 40 plants, 13 of them were evaluated for the first time for an antibacterial activity (*Aegiphila filipes*, *Ceiba samauma*, *Echites machrantus*, *O. bataua*, *O. alveolatus*, *Passiflora coccinea*, *Passiflora guazumaefolia*, *P. macrostachya*, *P. decumanum*, *P. munguba*, *Senna bacillaris*, *T. sananho*, *T. serrulata*). The more sensitive microorganisms were Gram-positive: *C. striatum*, *P. aeruginosa*, *S. epidermidis*, *S. lugdunensis*, *S. warneri*.

DISCUSSION/CONCLUSION

This is the first time that those plants were tested on so many different bacteria in a same standardized experiment with a MIC value. It permitted a comparative evaluation of the plant activities in absolutely equal conditions of assessment on microbial species. Strains of the same species (with very different anti-biograms) displayed only marginal differences (*K. pneumoniae* resistant strains were more sensible to plant extracts than the nonresistant strain) which is in favor of very different thus readily complementary-modes of action of such extracts in comparison with antibiotics (similar plant extract activities are observed for resistant and sensitive strains from *C. Freundi*, *E. coli*, *E. cloacae*: Gram-negative; *S. epidermidis*, *S. aureus*: Gram-positive).

Bibliographic and experimental studies of the more active species (MIC ≤ 0.3 mg/ml) permitted to describe their potential active compounds:

A. occidentale: Tannins, alkaloids, saponins, alkyl phenols, and flavonoids from leaves and bark may be responsible of bactericidal activity.^[22]

C. guianensis: Benzyl benzoate (from leaves against Gram-positive bacteria) and saponins, flavonoids, and tanins from leaves and bark may be active compounds.^[23-25]

E. amazónica: Phytochemical reports have never been published for this species but alkaloids and isoflavonoids are suspected to be responsible of antibacterial activity in other *Erythrina* species.^[26]

C. lechleri: Diterpenoids (antibacterial compounds) but steroids, procyanidins, and alkaloids are considered to be the most active constituents of the sap.^[27]

D. rugosa: Experimental data as bio-autography of this chemically unknown species revealed an antibacterial activity due to hydrolysable tannins.

J. copaia: Benzoquinole (jacaranone) was isolated from the leaves and exhibited anti-infectious activities.^[28]

O. bataua: Anthocyanins, condensed tannins, stilbenes, and phenolic acids are suspected to be the biological active compounds of the fruit.^[29]

P. urinaria: Sesquiterpene as phyllanthocin is known to have antibacterial activity in this genus.^[30]

P. macrostachya: Essential oil (with bisabolol, caryophyllene oxide, myristicin, and limonene) from aerial parts may be responsible of the *in vitro* bactericidal activity.^[31]

Some of the plants assayed were quite well-studied (*C. lechleri*, *C. guianensis*) (Jones, Al-Dhabi) but their activity had never been evaluated on all those bacteria (or yeast) and permitted to confirm our method and results. Other plants have specific scientific interest for further chemical investigations because they have never been tested on bacteria and their antimicrobial compounds still yet unknown (*A. filipes*, *C. samauma*, *E. machrantus*, *O. bataua*, *O. alveolatus*, *P. guazumaifolia*, *P. macrostachya*, *P. munguba*, *T. sananho*).

Regarding this ethnopharmacological study allowed to compare the antimicrobial activities of various plant species, to establish the antimicrobial spectra of standardized plant extracts, and give support to the traditional use of these plants.^[32-38] Further examination by bio-autography is ongoing in order to determine new active constituents. When observing the antimicrobial versatility of *C. lechleri*

on enterobacteria, it should be kept in mind that proanthocyanidins in this resin also display anti-secretoty activity, as has been well established and put into use with the recently marketed crofelemer (Fulyzaq[®]), in perfect consistency with the traditional uses of numerous tannin-contain plants.^[8] This reminds us of the up-to-now unavoidable narrow scope and insufficiencies of screening studies, but it also warns us against too straightforward analysis of numeric values and harsh discarding of traditional remedies, as these assays may only partly highlight the potential usefulness of herbal medicines.

Acknowledgments

The authors gratefully acknowledge: Séverine Mahieux (from the laboratory of bacteriology, Lille 2), the ARPIA Peru association (Association pour la Recherche sur les Pharmacopées indigènes d'Amazonie), the EDF foundation (Electricité De France), the Nord Pas de Calais department (SISA: Solidarité Ici Solidarité Ailleurs), as well as Clara Mori, Corina Güivinsinti and other Peruvian people who were willing to share with us their knowledge about medicinal plants. The authors declare that they have no conflict of interest.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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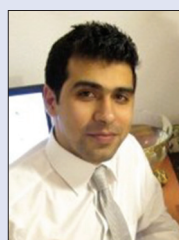
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Lastenia Ruiz



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Minimum inhibitory concentration (MIC) of active plants (mg/mL)

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Family, species Part	Gram-negative bacilli (Enterobacteria)																Gram-negative (non enterobacteria)										Gram-positive cocci										Miscellaneous strains							
	C. freundii		E. Coli		E. aerogenes		E. cloacae		K. pneumoniae		S. marcescens		P.mirabilis		P.stuartii		Salmonella sp.		A. baumannii		P. aeruginosa		S. maltophilia		E. faecalis		Enterococcus sp.				S. aureus		S. epidermidis		S. lugdunensis		S. warneri		C. striatum		M. smegmatis		C. albicans	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36								
Malvaceae, Ceiba samauma	L																																											
Meliaceae, Cedrela odorata	B																																											
Passifloraceae, Passiflora coccinea	L																																											
Passifloraceae, Passiflora guazumaefolia	L																																											
Piperaceae, Peperomia macrostachya	AP																																											
Piperaceae, Piper arboreum	L																																											
Piperaceae, Piper pellatum	L																																											
Piperaceae, Piper tuberculatum	L																																											
Poaceae, Andropogon citratus	EP																																											
Solanaceae, Brugmansia suaveolens	L																																											
Antibiotics																																												
Gentamicin	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	R	S	R	I	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nt	
Vancomycin	-	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	nt	
Amoxicillin	-	S	R	R	I	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nt	
Amphotericin B	-	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	S

1,2,3: *Citrobacter freundii* (11041; 11042, 11043); 4, 5, 6, 7: *Escherichia coli* (8137; 8138, 8157, ATCC 25922); 8: *Enterobacter aerogenes* (9004); 9, 10, 11: *Enterobacter cloacae* (11050; 11051, 11053); 12, 13: *Klebsiella pneumoniae* (11016; 11017); 14, 15: *Serratia marcescens* (11056, 11057); 16: *Proteus mirabilis* (11038); 18, 19: *Salmonella sp.* (11033; 11037); 20, 21: *Acinetobacter baumannii* (9010, 9011); 22, 23: *Pseudomonas aeruginosa* (8131; ATCC 27583); 24: *Stenotrophomonas maltophilia* (TP); 25: *Enterococcus faecalis* (C159-6); 26, 27: *Enterococcus sp.* (8152, 8153); 28, 29: *Staphylococcus aureus* (8146, 8147); 30, 31: *Staphylococcus epidermidis* (5001, 10282); 32: *Staphylococcus lugdunensis* (T26A3); 33: *Staphylococcus warneri* (T12A12); 34: *Corynebacterium striatum* (T25-17); 35: *Mycobacterium smegmatis* (5003); 36: *Candida albicans* (10286). AP: aerial parts, B: stem bark, EP: entire plant, Fl: flower, Fr: fruit, L: leaves, R: root, Re: resin. MIC ($\mu\text{g/ml}$) of positive controls: nt: not tested, gentamicin, S: ≤ 4 , R: > 8 ; vancomycin, S: ≤ 4 , R: > 16 ; amoxicillin, S: ≤ 4 , R: > 16 ; amphotericin B, S: ≤ 1 , R: > 4 , S: sensitive, I: intermediate, R: resistant. Standard deviation for values: 4.2 \pm 1.7; 2.1 \pm 0.8; 1.7 \pm 0.8; 1.1 \pm 0.2; 0.5 \pm 0.1; 0.4 \pm 0.1 (0 for other value)