Antibacterial Activity of Brazilian Amazon Plant Extracts

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Infections caused by multiresistant bacteria are a widespread problem, especially in intensive care units. New antibiotics are necessary, and we need to search for alternatives, including natural products. Brazil is one of the hottest spots in the world in terms of biodiversity, but little is known about the chemical and pharmacological properties of most of the plants found in the Amazon rain forest and the Atlantic Forest. We screened 1,220 organic and aqueous extracts, obtained from Amazon and Atlantic rain forest plants, against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *E. coli*. Seventeen organic and aqueous extracts obtained from 16 plants showed activity against both Gram-positive bacteria. None of the extracts showed relevant activity against the Gram-negative *E. coli* and *Pseudomonas aeruginosa*.

Key Words: Plant extract, antibacterial activity, Amazon rain forest, Atlantic forest.

Multiresistant bacteria outbreaks have recently been reported in some Brazilian hospitals, particularly in intensive care units. A strain of methicillin resistant *Staphylococcus aureus* (MRSA) was responsible for pneumonia in an 11-year old male patient in Southern Brazil. The patient did not respond to vancomycin treatment [1]. In another case, clinical failure of antibiotic treatment was reported in Southeast Brazil. *Pseudomonas aeruginosa* was isolated from conjunctival cultures obtained from seven babies who were in a new-born intensive care unit. This strain of *Pseudomonas aeruginosa* was found to be resistant to ceftazidime and aminoglycosides, and patients only recovered after treatment with imipenem and ciprofloxacin [2]. Those examples illustrate how introduction of new antibiotics is essential for fighting infectious diseases.

Tropical forests concentrate the highest biodiversity in the world, particularly those located in Brazil, whose species richness is estimated to account for 20% of the total world figures [3]. However, little is known about the chemical and pharmacological potentialities of Brazilian tropical forests, including the Amazon rain forest and the Atlantic forest. Based on these facts and since approximately 60% of the new antibiotics introduced in the market from 1983 and 1994 were from natural sources, or were synthesized or semi-synthesized based on natural products [4], our group screened 1,220 extracts obtained from more than 350 species belonging to 71 different plant families native to the Brazilian Amazon rain forest and to the Atlantic forest; they were tested for their antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *E. coli*. The complete list of species can be obtained from the corresponding author. Here we list the 17 antibacterial active extracts selected from the primary screening and report the corresponding minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC).

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Material and Methods

Plant collection and extract preparation

Plants were collected from the Amazon rain forest and from the Atlantic forest from 1997 to 2001. A voucher for each specimen was deposited in the Herbarium UNIP (Universidade Paulista, São Paulo, SP).

Plant organs were separated according to their biomass availability, dried and ground before being submitted to 24-h maceration with dichloromethane : methanol (1:1), followed by a 24-h maceration with water, resulting in two extracts from each plant material. Further details on this technique can be found elsewhere [5], as well as the list of species used in the primary screening [6].

Antimicrobial assay

The broth microdilution method was used for primary screening of 1,220 plant extracts and was also used for the determination of MIC's and MBC's of the active organic and aqueous plant extracts. Each inoculum was obtained from fresh colonies of *Staphylococcus aureus* ATCC 29213 (Sau), *E. coli* ATCC 25922 (Ecol), *Enterococcus faecalis* ATCC 29212 (Efae) and *Pseudomonas aeruginosa* ATCC 27853 (Psa). Further information on this technique can be found elsewhere [6]. Results from the primary screening procedures are available elsewhere; only MiC's and MBC's obtained for the 17 active antibacterial extracts are reported here.

Results and Discussion

Results can be seen in Table 1. Screening methodology is one of the fastest means of testing a large number of extracts or compounds [7,8], to evaluate the traditional uses of plants [9] and to identify new uses. We report the MIC's and MBC's

Table 1. Determination of minimal inhibitory concentrations and minimal bactericidal concentrations of plant extracts from Brazilian rain forests against *Staphylococcus aureus* and *Enterococcus faecalis*

Collection number	Extract number	Family	Species	Plant organs	Bacteria	MIC*	MBC*
AAO3562	1093	Annonaceae	*Guatteria riparia* R.E.Fries	LF	Sau	100	120
AAP3589	1089	Annonaceae	*Guatteria schomburgkiana* Mart.	WS	Sau	80	120
IBS66	1263	Combretaceae	n.d.	WS	Sau	100	120
AAO3687	1229	Gnetaceae	*Gnetum leyboldii* Tul.	WS	Sau	40	80
PSC192	457	Lauraceae	*Ocotea* sp	AO	Sau	180	>200
PSC83	326	Leguminosae	*Mora paraensis* (Ducke) Ducke	LF, WS	Sau	≥200	≥200
IBS9	352	Leguminosae	*Stryphnodendron pulcherrimum* (Willd.) Hochr.	WS	Efae	60	60
AAO3416	973	Myristicaceae	*Virola theiodora* Warb.	LF	Efae	40	100
AAO3458	841	Myrsinaceae	*Rapanea parvifolia* (A.DC.) Mez.	AO	Efae	30	60
PSC414	321	Myrtaceae	*Psidium densicomum* Mart. Ex DC.	LF, FL	Sau	40	40
PSC414	321	Myrtaceae	*Psidium densicomum* Mart. Ex DC.	LF, FL	Efae	60	>200
IBS53	1247	Myrtaceae	*Psidium densicomum* Mart. Ex DC.	AO	Sau	60	80
IBS53	1247	Myrtaceae	*Psidium densicomum* Mart. Ex DC.	AO	Efae	60	180
IBS56	1259	Piperaceae	*Piper arboreum* Aubl.	AO	Sau	60	80
IBS56	1259	Piperaceae	*Piper arboreum* Aubl	AO	Efae	80	>200
PSC144	429	Proteaceae	*Roupala* sp.	WS	Sau	60	60
PSC144	429	Proteaceae	*Roupala* sp.	WS	Efae	100	140
AAO3717	1257	Rubiaceae	*Palicourea guianensis* Aubl.	AO	Sau	40	40
AAO3717	1257	Rubiaceae	*Palicourea guianensis* Aubl	AO	Efae	40	40
AAO3299	632	Rutaceae	*Zanthoxylum* sp.	WS	Sau	60	60
PSC381	53	Smilacaceae	*Smilax rufescens* Griseb.	AO	Efae	80	90
PSC401	581	Vochysiaceae	*Ruizterania retusa* (Spruce ex Warm.) Marc.-Berti	WD	Sau	140	160
			Tetracycline		Sau	0.50	0.50
			Tetracycline		Efae	32.0	32.0
			Gentamycin		Sau	0.20	0.20
			Gentamycin		Efae	8.0	8.0

WS = stem; WD = wood; AO = aerial organs; LF = leaves; FL = flowers; Efae = *Enterococcus faecalis*; Sau = *Staphylococcus aureus*; n.d. = not determined; * = minimal inhibitory concentrations and minimal bactericidal concentrations are given in μg/mL; tetracycline and gentamycin were used as standard drugs for comparison.

obtained for 17 antibacterial active organic and aqueous Brazilian plant extracts, selected from a primary screening of more than 1,000 Brazilian plant extracts [6].

The first relevant aspect of the results obtained from the 17 extracts is the inefficacy of both aqueous and organic extracts to fight Gram-negative Ecoli and Psa, in contrast with the efficiency of their action against Gram-positive Sau and Efae, at the doses ≤ 200 μg/mL. This may be due to general physiological differences in the membrane constitution of Gram-negative and Gram-positive bacteria.

As the MIC's cut point was established as ≤ 200 μg/mL, all the extracts listed in Table 1 needed to be bioguide fractionated in order to have their respective active compounds determined. Conversely, the organic extract obtained from the aerial organs of *Rapanea parvifolia* showed exceptional MIC = 30 μg/mL and MBC = 60 μg/mL against Efae. Four organic extracts obtained from *Gnetum leyboldii*, *Virola theiodora*, *Psidium densicomum* and *Palicourea guianensis* showed expressive activity against Sau and Efae (MIC = 40 μg/mL, MBC's ≤ 100 μg/mL, depending on the

extract, see Table 1 for specific results). Six aqueous or organic extracts obtained from *Stryphnodendron pulcherrimum*, *Psidium densicomum*, *Piper arboretum*, *Roupala* sp. and *Zanthoxylum* sp. also gave significant antibacterial activity (MIC = 60 μg/mL, MBC's ≤ 200 μg/mL, depending on the extract) (Table 1). Three organic extracts obtained from *Guatteria schomburgkiana*, *Piper arboretum* and *Smilax rufescens* showed MIC = 80 μg/mL and MBC ranging from 90 to > 200 μg/mL, depending on the extract. Three organic extracts obtained from *Guatteria riparia*, *Roupala* sp and Combretaceae species showed MIC = 100 μg/mL and MBC = 120, 120 and 140 μg/mL, respectively. The organic extract of *Ruizterania retusa* showed MIC = 140 μg/mL and MBC = 160 μg/mL, the organic extract of *Ocotea* sp. showed MIC = 180 μg/mL and MBC > 200 μg/mL and the aqueous extract obtained from *Mora paraensis* showed MIC and MBC > 200 μg/mL.

The 17 extracts can be considered potential sources of new antibiotic natural products, because of their low MICs and MBCs. These extracts will now be pharmacologically studied, using bioguide fractionation as a tool to select active compounds and to discard extracts whose activity is related to a synergic effect of two or more compounds.

Tetracycline and gentamycin were used as reference drugs. Although it is not recommended to compare the biological activity of an isolated compound, such as these standard drugs, with the activity of a complex mixture of substances, such as these 17 extracts, the antibacterial activity of the extracts whose MIC is 30-40 μg/mL is comparable to that of tetracycline against Efae, based on the parameters established in our assay.

Seventeen plant extracts obtained from 16 different species belonging to *Annonaceae*, *Combretaceae*, *Gnetaceae*, *Lauraceae*, *Leguminosae*, *Myristicaceae*, *Myrsinaceae*, *Myrtaceae*, *Piperaceae*, *Proteaceae*, *Rubiaceae*, *Rutaceae*, *Smilacaceae* and *Vochysiaceae* showed potent antibacterial activity against Sau and Efae and will be phytochemically evaluated.

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