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In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*

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Abstract

Fourteen extracts from Brazilian traditional medicinal plants used to treat infectious diseases were used to look for potential antimicrobial activity against multiresistant bacteria of medical importance. *Staphylococcus aureus* strains were susceptible to extracts of *Punica granatum* and *Tabebuia avellanedae*. The minimum inhibitory concentrations (MICs) of the total extracts and of additional fractions of these plants were determined by employing strains of methicillin-resistant (MRSA) and -sensiti ve (MSSA) *S. aureus*, including isolates of the PFGE clone A, which is prevalent in Brazil and two ATCC reference strains. A mixture of ellagitannins isolated from *P. granatum* and two naphthoquinones isolated from *T. avellanedae* demonstrated antibacterial acti vity against all *S. aureus* strains tested. Semi-synthetic furanonaphthoquinones (FNQs) showed lower MICs than those exhibited by natural occurring naphthoquinones. The results indicate that these natural products can be effecti ve potential candidates for the development of new strategies to treat MRSA infections.

Keywords: Brazilian medicinal plants; *Punica granatum*; *Tabebuia avellanedae*; Quinones; Antimicrobial activity; MRSA strains

cus aureus (MRSA) isolates have increased greatly during the last decades in hospital [4,5] and the community [6]. The epidemic clones characterized by

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in traditional medicine for the treatment of gastrointestinal, respiratory, urinary and skin infections. The quinones tested were obtained within a research program of our laboratory that studies the synthesis and evaluation of rare natural quinones from the Brazilian flora and their synthetic analogues against tropical endemic diseases [9].

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2. Materials and methods

2.1. Plant material extraction and fractionation

Fourteen different plant species and plant fractions were used as shown in Table 1. The plants were extracted by maceration in ethanol for 2 days at room temperature and the process was repeated twice. The total ethanolic extracts (te) were concentrated in a rotational evaporator under reduced pressure and the residues were then successively partitioned between water (w) and *n*-hexane (h), followed by chloroform

2.2. Synthesis of naphthoquinones

Compounds I–IV (Fig. 1) were semi-synthetically obtained from lapachol, which was isolated in this study by extraction of *T. avellanedae* sawdust, following the original procedure of Paternó [10]. Quinone I was conveniently synthesized according to Hooker [11]. Quinones II, III and IV were synthesized using our original procedure [12]. The last two quinones were synthesized for the first time in our laboratory [13].

2.3. Test organisms

mixture of ellagitannins (PG_{F1}).

The hexane fraction of the wooden part of *Tabebuia avellanedae* was purified over a silica gel 230–400 mesh-ASTM (Merck, Darmstadt, Germany) column, eluted with solvent gradients from hexane to ethyl acetate and ethyl acetate to methanol. Fractions eluted with 20% ethyl acetate in hexane yielded the naphthoquinones lapachol, α-lapachone and α-xyloidone after further purification on preparative silica gel 60 (PF 254-366, Merck) TLC plates, eluted with 5% of ethyl acetate in hexane.

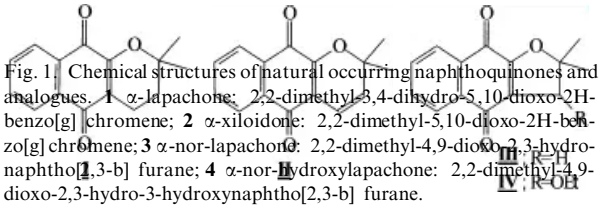

Fig. 1. Chemical structures of natural occurring naphthoquinones and analogues. **1** α-lapachone: 2,2-dimethyl-3,4-dihydro-5,10-dioxo-2H-benzo[g] chromene; **2** α-xiloidone: 2,2-dimethyl-5,10-dioxo-2H-benzo[g] chromene; **3** α-nor-lapachone: 2,2-dimethyl-4,9-dioxo-2,3-hydro-naphtho[2,3-b] furane; **4** α-nor-hydroxylapachone: 2,2-dimethyl-4,9-dioxo-2,3-hydro-3-hydroxynaphtho[2,3-b] furane.

Table 1
Brazilian medicinal plants assayed against multiresistant hospital bacteria

Botanical name	Family	Plant part used	Extracts and fractions assayed
P. granatum	Punicaceae	Seeds	te
		Fruit-pericarp	te, h, c, ea, b, w
T. avellanedae	Bignoneaceae	Wood	te, h, c, ea, b, w
Cissampelos sympodialis	Menispermaceae	Leaf	h, c, ea, b, w
Croton salutaris	Euphorbiaceae	Wood bark	w
Piper nigrum	Piperaceae	Leaf	te
Zanthoxylum rhoifolia	Rutaceae	Wood bark	te, c
Tibouchina granulosa	Melatomataceae	Leaf	te
Alpinia zerumbet	Zingiberaceae	Leaf	d
Amburana cearensis	Fabaceae	Leaf	c
Lobelia thapsoides	Campanulacea	Leaf	te
Melissa officinalis	Lamiaceae	Leaf	te
Spilanthes oleraceae	Asteraceae	Leaf	te
Schinus sp	Anacardiaceae	Leaf	te
Eugenia sp	Myrtaceae	Leaf	te

te, total ethanol extract; h, hexane; d, dichloromethane; c, chloroform; ea, ethyl acetate; b, butanol; w, water.

The antibacterial activity of the above mentioned extracts and fractions was separately determined using the disk diffusion method [16]. Petri dishes containing 20 ml of Mueller–Hinton agar medium (Oxoid, Hampshire, England) were seeded with a 24 h culture of the bacterial strains in Trypticase Soy Broth (TSB, Oxoid). The inoculum size was adjusted to approximately 10^8 colony-forming units (CFU)/ml. The solutions of the plant extracts and fractions were applied to sterile filter paper disks (Whatman No.1; 5 mm in diameter) to give the final concentrations of 250 and 500 μg and placed on the surface of the inoculated medium. The plates were incubated at 35°C for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the disk.

2.5. Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method in Mueller–Hinton agar medium (Oxoid), according to NCCLS [17]. Before gelling, 20 ml of agar medium were added to each of the Petri dishes containing either the plant extract, a specific fraction, or purified compounds and the Petri dishes were swirled carefully until the agar began to set. Concentrations ranging from 0.97 to 250 μg/ml were used for each plant sample. Subsequently, bacteria (10^4 CFU/ml) were inoculated using a Steers replicator that placed 2 μl of each bacterial strain on the Mueller–Hinton agar surface.

3. Results

3.1. Preliminary evaluation of antibacterial activity

naphthoquinones and analogues from *T. avellanedae* by using the same 26 *S. aureus* strains tested by the disk diffusion method. The results reported in Table 2 show lower MICs for naphthoquinone analogues from *T. avellanedae* (III and IV), ranging from 15.6 to 31.2 mg/l. A regular pattern of growth inhibition was also obtained for α-lapachone (I) and PG_{F1}, exhibiting an MIC of 62.5 mg/l for all tested strains. The total ethanolic extracts and its fractions presented MICs ranging from 125 to ≥ 250 mg/l.

4. Discussion

Infections caused by methicillin-resistant *S. aureus* (MRSA) have increased over the last years. The percentage of MRSA isolated in hospitals and reported to the NNIS system ranged from 15 to 45% in 1991 [2]. By 1990, MRSA strains represented between 38 and 78% of all *S. aureus* strains isolated in tertiary hospitals in Brazil [3]. Its resistance has been related to the predominance of the single PFGE clone A of MRSA isolated from Brazilian hospitals [5,8]. The presence of this prevalent clone makes the control of MRSA nosocomial infections difficult because it spreads more easily in hospital institutions than other clones [18].

The increasing occurrence, particularly in hospitals, of *S. aureus* resistant not only to methicillin but to a wide range of antimicrobial agents, including vancomycin, made therapy more difficult [19,20]. Although strategies have been proposed in an attempt to control the spread [19], the search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections.

In this study the analysis of the growth inhibition activity by the disk diffusion method showed that the principal chemical constituents with antimicrobial activity were concentrated in the polar fractions of *P. granatum*, whereas in *T. avellanedae* the major activity

Table 2
MIC (mg/l) of extracts, fractions and isolated compounds of *P. granatum* and *T. avellanedae* by the agar dilution method

S. aureus strains	PFGE clone	PG te	PG c	PG ea	PG b	PG w	TA te	TA h	TA c	TA b	PG_{F1}	I	II	III	IV
MRSA															
1	A	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	31.2	15.6
2	A	250	>250	250	250	250	125	>250	250	250	62.5	62.5	125	31.2	15.6
3	A	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	31.2	15.6
4	A	250	>250	250	250	250	125	>250	250	>250	62.5	62.5	125	31.2	62.5
5	A	250	250	250	250	250	250	>250	250	250	62.5	62.5	125	31.2	15.6
6	A	250	>250	250	250	250	125	>250	250	>250	62.5	62.5	125	31.2	15.6
7	A	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	31.2	15.6
8	B	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	15.6	15.6
9	B	250	>250	250	250	250	125	>250	250	>250	62.5	62.5	125	31.2	15.6
10	C	250	250	250	250	250	125	>250	250	>250	62.5	62.5	250	31.2	15.6
11	D	250	>250	250	250	250	125	>250	250	>250	62.5	62.5	125	31.2	15.6
12	E	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	15.6	15.6
13	F	250	>250	250	250	250	125	>250	>250	>250	62.5	62.5	125	31.2	62.5
14	H	250	>250	250	250	250	125	>250	>250	250	62.5	62.5	125	31.2	62.5
15	I	250	250	250	250	250	250	>250	250	250	62.5	62.5	125	31.2	62.5
16	J	250	250	250	250	250	125	>250	250	250	62.5	62.5	125	31.2	31.2
ATCC 33591		250	250	250	250	250	250	>250	>250	250	62.5	62.5	125	31.2	62.5