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Full Length Research Paper

Exploring the antibacterial potential of hydroalcoholic extracts from *bignoniaceae* and *moraceae* species

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The search for new substances with antimicrobial properties has become necessary due to the bacterial capacity of resistance. Medicinal plants have become an important alternative since many plants exhibit antimicrobial activity. The Brazilian Atlantic Forest contains a huge diversity of plant species, many of which have not been thoroughly investigated. *Jacaranda puberula* Cham. (Bignoniaceae) and *Sorocea bonplandii* Baill. (Moraceae) are used popularly to treat conditions associated with bacterial infection such as syphilis, as well as, infections of the skin, kidneys and bladder. In this study, the antibacterial activity of the hydroalcoholic extracts of leaves from *J. puberula* and *S. bonplandii* was investigated by the agar-well method. The results obtained show that the hydroalcoholic extracts of *J. puberula* and *S. bonplandii* presented a bacteriostatic action against *Staphylococcus aureus*. *J. puberula* also exhibited bactericidal activity towards *S. aureus* at a concentration of 100 mg/ml. Therefore, these species represent a potential tool for the production of new phytomedicines with antibacterial action against important agents with high resistance to drugs in current use.

Key words: Ethnopharmacology, antibacterials, Brazilian Atlantic Forest, *Staphylococcus aureus*, agar-well method.

INTRODUCTION

The wide acceptance of plant-based medicines has stimulated ethnopharmacological research to confirm the therapeutic properties of plants traditionally used by the population, with the objective of developing new medicines (Schlemper et al., 1998). Worldwide there are an estimated 250,000 higher plants (Wilson, 1988), and although only 5 to 15% have been studied for their potential therapeutic value (Balandrin et al., 1985; Kinghorn, 1992), a large number continue to be investigated (Rojas et al., 2003). The Brazilian Atlantic Forest is home to more than 20 thousand plant species, of which 50% are endemic. Furthermore, it is considered the richest forest in the world in terms of trees per unit area (Shâffer and Prochnow, 2002).

During the last few decades, the search for new anti-infectious agents derived from medicinal plants has been intensified (Ríos and Recio, 2006). This has been stimulated in part by the fact that the efficacy of allopathic antibacterial medicines in current use has decreased markedly, mainly as a result of their incorrect application, which has added to the genetic ability of bacteria to acquire and transmit resistance to these agents (Cohen, 1992).

The use of plant extracts and phytochemical compounds with known antimicrobial activity as therapeutic treatments may take on greater significance in the future. Numerous studies have been carried out, in several countries, to demonstrate their efficacy (Nunan et al., 1985; Locher et al., 1995; Annapurna et al., 1999;

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Djipa et al., 2000; Feresin et al., 2001; Khan et al., 2001; Ramesh et al., 2002) and medicinal plants therefore represent an important tool for the discovery of new antibacterial agents (Nascimento et al., 2000).

Jacaranda puberula Cham. (Bignoniaceae) and *Sorocea bonplandii* Baill. (Moraceae) are tree species native to the Brazilian Atlantic Forest and are used by the local populations as agents with antimicrobial properties. *J. puberula* is used to treat skin conditions, for example as a cleanser and anti-syphilitic (Reitz, 1950; Pavan-Fruehauf, 2000), for dermatoses and scabies (Cervi et al., 1989), as a sudorific, for syphilis, as well as skin disorders in general such as ulcers and condylomas (Marcuzzo, 1998); it is also used for conditions of the kidneys and bladder, intestinal amoebas, rheumatic pain, throat inflammation, and for the treatment of wounds (Pavan-Fruehauf, 2000). *S. bonplandii* is used as an analgesic and anti-ulcerogenic (Fonseca-Kruel and Peixoto, 2004; Silva Junior, 2006; Ruschel et al., 2006), as a tranquiliser, to regulate the blood pressure, is efficacious in baths and for rheumatic pain (Franco and Fontana, 2001), to cleanse the blood, as a tonic, for wound healing, and as a laxative, diuretic, abortive, anti-asthmatic and anti-septic (Ruschel et al., 2006).

Despite its popular usage, however, there are no studies in the literature concerning the antibacterial activity of *J. puberula*, while regarding *S. bonplandii* there is only one study (Agripino et al., 2004), in which its activity was analysed but not successfully demonstrated.

Consequently, the objective of the present study was to investigate the antibacterial properties of hydroalcoholic extracts of the leaves from *J. puberula* and *S. bonplandii* by means of the agar diffusion test modified according to the agar-well method, and the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

MATERIALS AND METHODS

Plant material and preparation of the extracts

Leaves of *J. puberula* and *S. bonplandii* were collected in January 2007 in the area around Barragem do Rio São Bento, in the municipality of Siderópolis, Santa Catarina, southern Brazil. A sample of each plant was identified at the Pe. Raulino Reitz Herbarium (CRI) of the Universidade do Extremo Sul Catarinense (UNESC). The leaves were dried in a ventilated oven at a temperature of 40°C, then triturated and macerated in a 70% hydroalcoholic solution, at room temperature for 10 days. Next, the extracts were filtered and concentrated in a Rota-evaporator at 42°C and 117 turns per min. The concentred extracts were stored in hermetically sealed flasks in a refrigerator at a temperature of 4°C and protected from light.

Microorganisms used

The antibacterial activity was investigated against the lyophilised commercial strains of two gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and two gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The bacterial strains used for the test are registered in the American Type Culture Collection (ATCC).

Culture media

The culture media used in the tests were Mueller Hinton agar (MH), Trypticase Soy broth (TSB) and blood agar (BA). The MH and TSB media were prepared according to the instructions of the manufacturer HIMEDIA, while the blood agar was obtained ready for use from the manufacturer Newprov.

Preparation of bacterial culture

The bacteria were activated by inoculating TSB broth with lyophilised ATCC strains in a laminar flow hood, stirring until complete hydration of the bacterial strain had been achieved and transferring to a bacteriological incubator at a temperature of 35 ± 2°C, for 24 h. Next, when turbidity was observed in the broth, the tubes were transferred to -40°C. A 1 µl nickel-chromium loop was used to seed blood agar by the method of weariness, in such a way as to obtain colonies of isolated bacteria which were then transferred to a bacteriological incubator at a temperature of 35 ± 2°C, for 24 h.

Standardisation of the inoculum

After 24 h of incubation, the isolated colonies were selected for standardisation of the inoculum density. In a laminar flow hood, using a 1 µl nickel-chromium loop, aliquots of the previously selected colonies of each bacterial species were removed and the bacteria were suspended in 5 ml of TSB broth, adjusting the turbidity of the broth to 0.5 on the MacFarland scale to give approximately 10^8 CFU/ml, according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003a, b).

Agar-well method

The antibacterial activity of the crude hydroalcoholic extracts of *J. puberula* and *S. bonplandii* was determined by the Bauer disc-diffusion method, updated by the National Committee for Clinical Laboratory Standards, and modified by the agar-well method (Fyhrquist et al., 2002; Tadeg et al., 2005; Rojas et al., 2006).

The bacterial suspensions, standardised to 10^8 CFU/ml, were distributed over the MH agar using a sterile swab. Wells were then created by perforating the agar with a metal tube, 0.8 mm in diameter, which had been previously flamed. Two wells were made in each plate, with 45 µl of one of the extracts being added to one well at a concentration of 25, 50 or 100 mg/ml, while the same volume of 20% propylene glycol, the solvent for the extracts, was added to the other as a negative control. All procedures were carried out in triplicate. The plates were transferred to a bacteriological incubator at a temperature of 35 ± 2°C, for 24 h. In addition, a plate containing only MH agar was also incubated in the same conditions described as a negative control. Results were obtained by measuring the halo of inhibition, including the 8 mm well itself.

The agar diffusion test was performed with discs of commercial antibiotics as a positive control, using ampicillin (10 µg) as a positive control for *S. aureus* and ciprofloxacin (5 µg) as a positive control for *E. coli*, *P. aeruginosa* and *S. pneumoniae*.

Minimum inhibitory concentration

The MIC was determined with the crude hydroalcoholic extracts of

Table 1. Antimicrobial activities of crude hydroalcoholic extract (CHE) of *Jacaranda puberula* and *Sorocea bonplandii* against selected strains of bacteria.

Sample	Concentration	Zone of inhibition Ø (mm)			
		Ec	Pa	Sa	Sp
CHE of *Jacaranda puberula* Cham. (Bignoniaceae)	25 mg/ml	0	0	nt	0
	50 mg/ml	0	0	130	0
	100 mg/ml	0	0	nt	0
CHE of *Sorocea bonplandii* Baill. (Moraceae)	25 mg/ml	0	0	nt	0
	50 mg/ml	0	0	230	0
	100 mg/ml	0	0	nt	0
Ampicillin	10 µg	nt	nt	570	nt
Ciprofloxacin	5 µg	450	450	nt	260
Propylene glycol	20%	0	0	0	0

nt: not tested, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Sp: *Streptococcus pneumoniae*.

the leaves of trees that presented antibacterial activity towards the bacteria tested. After completing the process of bacterial isolation and standardisation, the extracts were diluted to the concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml. Three millimetre (3 ml) of each concentration were then transferred to separate tubes and 3 ml of the standardised inoculum were added to each tube with the different concentrations of the extracts. The tubes were placed in a bacteriological incubator at a temperature of 35 ± 2°C for 24 h. A tube containing TSB broth alone was also incubated under the same conditions as a negative control, while a tube containing only the standardised inoculum of each bacterium was included as a positive control. The results were obtained by determining the turbidity of the broth.

Minimum bactericidal concentration

After determination and interpretation of the MIC, a 50 µl aliquot of the remaining culture was added to MH plates, with one plate corresponding to each tube, and spread with a Drigalski loop that had been previously flamed. The whole of the previous procedure was carried out with the standardised inoculum of each bacterium as a positive control for the MIC, which corresponded to the total number of colonies. The plates were placed in a bacteriological incubator at a temperature of 35 ± 2°C, for 24 h. The results were determined and interpreted by counting the number of colony forming units (CFUs), using a colony counter. Plates presenting 100% death corresponded to the Minimum Bactericidal Concentration.

RESULTS AND DISCUSSION

From the results obtained with the agar-well modification of the agar diffusion method, it is clear that the hydroalcoholic extracts of *J. puberula* and *S. bonplandii* inhibited the growth of *S. aureus* at the concentration of 50 mg/ml, producing halos of inhibition 130 and 230 mm in diameter, respectively (Table 1). By contrast, in a study by Agripino et al. (2004), the hydroalcoholic extract of leaves of *S. bonplandii* did not show any activity towards *C. albicans*, *E. coli* and *S. aureus* when tested at the concentration of 10 mg/ml by the agar-well diffusion method. It is possible that the difference in concentration was determinant in obtaining the growth inhibition of *S. aureus* seen in the present work. Even so, in the same study *S. bonplandii* did not show any effect against *E. coli*.

Ampicillin 10 µg, the positive control for *S. aureus*, presented a halo of inhibition 570 mm in diameter, while ciprofloxacin 5 µg, the positive control for *S. pneumoniae*, *E. coli* and *P. aeruginosa* presented, respectively, a halo of inhibition 260, 450 and 450 mm in diameter. Meanwhile, the solution of 20% propylene glycol, the negative control for the extracts, did not produce a halo of inhibition, demonstrating that the solvent had not interfered in the results for the extracts.

Table 2 shows the MIC data for the crude hydroalcoholic extracts of leaves from *J. puberula* and *S. bonplandii*, tested against *S. aureus,* a bacterium which presented inhibition in the agar-well diffusion test. MICs are defined as the minimum concentration of an active principle at which no visible growth is observed, resulting in the inhibition of *in vitro* growth of the microorganisms (Yu et al., 2005). The MIC does not represent an absolute value, but rather it falls between the lowest test concentration that inhibits the growth of the organism and the next lowest test concentration (NCCLS, 2003a). From the results for the MIC, it can be seen that *S. aureus* was resistant to the concentrations of 3.125, 6.25, 12.5 and 25 mg/ml of the extract of *J. puberula*, while it was sensitive to the concentrations of 50 and 100 mg/ml. Therefore, the MIC of the extract of *J. puberula* fell between >25 mg/ml and ≤50 mg/ml. *S. aureus* was found to be sensitive to all of the tested concentrations of the extract of *S. bonplandii*; consequently, the MIC is

Table 2. MIC of hydroalcoholic extracts of *J. puberula* and *S. bonplandii* against *S. aureus*.

Extract	Concentration (mg/ml)	Sensitivity of *S. aureus*
Jacaranda puberula	3.125	R
	6.25	R
	12.5	R
	25	R
	50	S
	100	S
Sorocea bonplandii	3.125	S
	6.25	S
	12.5	S
	25	S
	50	S
	100	S

S: sensitive, R: resistant.

Table 3. MBC of crude hydroalcoholic extracts of medicinal trees found in Barragem do Rio São Bento, SC, Brazil.

Extract	Concentration (mg/ml)	Bactericidal activity
		S. aureus
Jacaranda puberula	3.125	-
	6.25	-
	12.5	-
	25	-
	50	-
	100	+
Sorocea bonplandii	3.125	-
	6.25	-
	12.5	-
	25	-
	50	-
	100	-

+: positive bactericidal action, -: negative bactericidal action.

considered to be ≤3.125 mg/ml (Table 2). The MBC is the lowest concentration of an active principle, in µg/ml, which kills the microorganisms under study (Pelczar et al., 1996; Koneman et al., 2001) and represents a subsequent stage of testing after MIC. The hydroalcoholic extract of *J. puberula* presented bactericidal activity against *S. aureus* at the concentration of 100 mg/ml, with its MBC falling between a value >50 and ≤100 mg/ml. The hydroalcoholic extract of *S. bonplandii* did not exhibit bactericidal activity against *S. aureus* at any of the concentrations tested (Table 3).

These results demonstrate that *J. puberula* and *S. bonplandii* possess a bacteriostatic action against the culture of *S. aureus*, a bacterium of major medical importance considering its pathogenicity towards humans and other animals. The extract of *J. puberula* also showed a bactericidal action against *S. aureus*. This is a highly relevant result since *S. aureus* possesses a high rate of mutability, considerably increasing the number of strains resistant to antibiotic agents (Zelante et al., 1983).

The enterotoxins produced by *S. aureus* belong to a large family of pyrogenic toxins produced by bacteria belonging to both the genus *Staphylococcus* and *Streptococcus*. These toxins can cause toxic shock and are commonly associated with food poisoning and various forms of allergy and autoimmune diseases

(Balaban and Rasooly, 2000). It is believed that there is an association between specific enzymatic activity, enterotoxigenicity and the resistance of *S. aureus* to various antibiotics, mainly in those samples that produce more than one type of enterotoxin (Refai et al., 1998).

Over the last decade gram-positive microorganisms, especially *S. aureus* have emerged as important causative agents of blood infections (Salomão et al., 1993; Macgowan, 1985). These infections affect patients young and the elderly, and present a worse prognosis in patients aged over 50 (Shah and Watanakunakorn, 1979; Watanakunakorn et al., 1987). Among hospital infections, sepsis caused by *S. aureus* is responsible for higher morbidity and mortality (Watanakunakorn et al., 1987; Gransden et al., 1984; Mylotte et al., 1987).

The search for new plant-based medicines through scientific investigations is of great importance considering the alarming rise in bacterial resistance to synthetic antibiotics. As such, the results of this study reinforce the value of investigating medicinal plants with a view to the production of new antibacterial agents. The species, *J. puberula* and *S. bonplandii*, native to the Brazilian Atlantic Forest, revealed themselves as promising candidates in reaching this objective and highlight the significance of preserving areas of native vegetation as a sustainable source of natural resources useful to human health.

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