Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr
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Full Length Research Paper

In vitro antimicrobial activity of extracts from *Abarema cochliacarpos* (Gomes) Barneby and J. W. Grimes

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Accepted 5 July, 2017

The usage of *Abarema cochliacarpos* (Mimosaceae) in traditional medicine by many communities in Brazil for diseases such as leucorrhea and dermatitis and as an antiseptic might indicate its antimicrobial activities. In order to assay *in vitro* antimicrobial activity, three extracts (hot aqueous extract, cold aqueous extract and methanol extract) from stem bark of *A. cochliacarpos* were tested against a panel of standard microorganisms (*Staphylococcus aureus* ATTC 6835, *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesuis* ATCC 10708, *Candida albicans* ATCC 10231, *Trichophyton mentagrophytes* ATCC 9533 and *Aspergillus niger* ATCC 16404) and multiresistant clinical isolates (*S. aureus* MR 01, MR02 and MR03). The antimicrobial activity was evaluated through the disk diffusion method, and the minimum inhibitory concentration (MIC) was determined using the micro dilution method. The results indicated that both aqueous extracts are active against gram-positive bacteria (*M. luteus* ATCC 9341, *S. aureus* ATCC 6835, and all clinical multiresistant samples) and against gram -negative bacteria (*S. choleraesuis* ATCC 10708*). MIC values ranged between 5.0 and 15.62 µg/ml for gram-positive bacteria. The methanol extract gave a positive result only for gram-positive bacteria (ATTC standards *M. luteus* and *S. aureus* and all clinical multiresistant samples).

Key words: *Abarema cochliacarpos*, antimicrobial activity, gram-negative bacteria, gram-positive bacteria, medicinal plant, traditional use.

INTRODUCTION

Of the 250 drugs that are considered basic and essential by the World Health Organization (WHO), 11% are produced exclusively from medicinal plants, and a significant number are synthetics developed from natural sources. These include antibiotics that were recently introduced into the market. From 1981 to 2006, ten of 109 new antimicrobial agents that were analyzed by the U.S. Food and Drug Administration (FDA) were derived from natural products (teicoplamin, mupirocin, myocamicin, carumonama, isepamicin, and RV-11), and 67 were semi-synthetic compounds that were based on natural products (Newman and Cragg, 2007). In spite of a number of recent findings in this field, there is still a need for new antimicrobial drugs because of the increased number of deaths caused by microbial infections associated with human immuno virus (HIV) or inadequate hygiene, and also the increasing number of multiresistant microorganisms. According to the 2000 World Health Report of infectious diseases, overcoming resistance to antibiotics is one of the major issues facing the WHO during the present millennium (Mbosso et al., 2010). The systematic selection of antimicrobially active plant extracts requires a continuous effort in the search for new

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compounds that show potential activity, especially against multiresistant bacteria (Suffredini et al., 2004; Zakaria et al., 2010). Although, up to the present, no plant compounds have been found to compete with the antibiotics that are currently in clinical use, the great structural variety found in plants makes them attractive as a source of novel lead compounds (Cowan, 1999).

Abarema cochliacarpos (B. A. Gomes) Barneby and J. W. Grimes is a native Brazilian species belonging to the family Mimosaceae. It grows especially in the Atlantic Forest but is also found in the scrub savanna (Brazilian cerradão) and savanna (cerrado), as well as on rock outcrops (campo rupestre), sometimes up to 1100 meters above sea level (IUCN, 2010). Popularly known as "barbatimão", the decoction of the stem bark is used in traditional medicine as a healing aid and antiseptic, and against leucorrhea and dermatosis (Agra et al., 2008), inflammation and gastric ulcers (Silva et al., 2006), and as an analgesic (Silva, 2006). The presence of triterpenes, catechins, lupeol saponins, tannins, phenols and anthraquinones has been reported for different stem-bark extracts of *A. cochliacarpos* (Araújo et al., 2002; Silva et al., 2009). Previous pharmacological studies demonstrated that different extracts have analgesic and healing effects on gastric and skin lesions and a protective effect in acute experimental colitis (Silva et al., 2006, 2009, 2010). Therefore, this study aimed to evaluate the *in vitro* antimicrobial activity of aqueous and methanol extracts from stem bark of *A. cochliacarpos* against standard ATCC (American Type Culture Collection) bacteria strains and clinical isolates.

MATERIALS AND METHODS

Plant material

Plant material was collected in May 2003 in Sauípe, Bahia, Brazil. It was identified by a specialist of the Rio de Janeiro Botanical Garden Herbarium, where a voucher specimen (RB365914) was preserved. Stem bark collected from the same plant was used to prepare the extracts.

Extract preparation

Air-dried and powdered stem bark was used to prepare the extracts, according to Silva et al. (2009). For the hot aqueous extract (HAE), 130 g of plant powder was boiled for 5 min in 1.5 L distilled water. For the cold aqueous extract (CAE), 130 g of plant powder was macerated in 1.5 L distilled water for 36 h. The extracts were filtered using Whatman filter paper no. 1, frozen and lyophilized. Both aqueous extracts were stored in the dark at 20°C until further use. The methanol extract (MeOH) was obtained from powdered bark (900 g) macerated in methanol at 20°C in the dark for three weeks. The extract was filtered and evaporated to dryness using a rotary evaporator (Fitosan-820) at 45°C, and the resulting extract was stored under the same conditions described above.

Microorganisms

Standard strains of ATCC microorganisms: *Staphylococcus aureus* ATTC 6835, *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesuis* ATCC 10708, *Candida albicans* ATCC 10231, *Trichophyton mentagrophytes* ATCC 9533, and *Aspergillus niger* ATCC 16404.

Multiresistant clinical isolates: *S. aureus* MR01 (resistant to tetracycline + clavulanic acid, ampicillin, cephalothin, ceftazidine, ciprofloxacin, cefoxitin, erythromycin, oxacillin, and penicillin, and sensitive to clindamycin, gentamicin, rifampicin, tetracycline, vancomycin, and sulfazotrin); *S. aureus* MR02 (resistant to amoxicillin + clavulanic acid, ampicillin, cephalothin, ceftazidine, ciprofloxacin, cefoxitin, erythromycin, oxacillin, penicillin, clindamycin, gentamicin, tetracycline, and sulfamethoxazole, and sensitive to vancomycin and rifampicin); *S. aureus* MR03 (resistant to amoxicillin + clavulanic acid, ampicillin/sulbactam, cefazolin, ceftraxone, ciprofloxacin, erythromycin, levofloxacin, and oxacillin, intermediate resistance to gentamicin and gatifloxacin, and sensitive to clindamycin, rifampicin, and synercid). All strains were obtained from clinical samples from the Hospital Santo Amaro, Salvador, Bahia. The strains were identified by the use of biochemical profiles, according to the recommendations of the Manual of Clinical Microbiology (Murray et al., 2003).

Maintenance: All bacteria and yeasts were stored at a temperature of -20°C and the filamentous fungi were stored at a temperature of 4°C. Prior to the experiments, the bacteria were subcultured in tryptic soy agar (TSA), and the yeasts were subcultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 24 h. The filamentous fungi were transferred to SDA for 7 days.

Susceptibility testing

The disk diffusion method, described by NCCLS document M2-A8 (2003), was adjusted to determine antimicrobial activities from plant extracts. Filter paper discs (6 mm in diameter) were impregnated with the extracts in order to reach a final concentration of 1000 µg per disc. A suspension of the microorganism tested, adjusted to 0.5 McFarland turbidity standard [10^8 colony-forming units (CFU)/mL], was spread on solid media plates made with Mueller- Hinton agar for bacteria, and SDA for yeasts and fungi. Bacteria and yeasts were incubated in aerobic conditions at 37°C for 24 h, and fungi at room temperature for 7 days. The diameter of the inhibition zone was measured in millimeters, from the edge of the disk to the inner margin of the surrounding pathogen. Each assay of this experiment was repeated twice. Ampicillin, gentamicin and cetoconalzol were used individually as positive controls (100 µg/ disk).

The minimal inhibitory concentrations (MIC) of the CAE and HAE were determined by micro dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS (2002). Inoculates were prepared in the same medium at a density adjusted to 0.5 McFarland turbidity standard (10^8 CFU/mL), and were diluted 1:10 for the broth micro dilution procedure. 96-well plates were incubated at 37°C, and the MICs were recorded after 24 h incubation. The MICs of the methanol extract (MeOH) were determined by the micro dilution technique in Mueller-Hinton agar (Merck) according to Machado et al. (2003).

The extract was diluted in Muller-Hinton medium at 45 - 50°C, and then a 108 CFU/mL bacterial suspension was inoculated on the agar surface. The bacterial-growth control was grown on agar without extract, and the MIC was defined as the lowest concentration of the extract at which the microorganism showed no visible growth after a 24 h incubation period at 35°C.

RESULTS

As assayed by the disc diffusion method (Table 1), all the

Table 1. Zone of inhibition (mm diameter) and Minimal inhibitory concentration (μg/ml) of *A. cochliacarpos* stem bark extracts.

Microorganisms \ Extracts[b]	Zone of inhibition[a]				MIC		
	CAE	HAE	MeOH	RA[c]	CAE	HAE	MeOH
S. aureus ATCC 6835	12	12	10	38	7.81	7.81	5.0
M. luteus ATCC 9341	13	14	12	45	7.81	15.62	10.0
S. choleraesuis ATCC 10708	14	14	0	26	250	250	ND*
E. coli ATCC 10536	0	0	0	25	ND	ND	ND
P. aeruginosa ATCC 15442	0	0	0	25	ND	ND	ND
S. aureus MR01	12	10	10	7	7.81	15.62	>10
S. aureus MR02	11	10	10	7	15.62	15.62	>10
S. aureus MR03	11	12	12	12	15.62	15.62	>10
C. albicans ATCC 10231	0	0	0	27	ND	ND	ND
T. mentagrophytes ATCC 9533	0	0	0	40	ND	ND	ND
Aspergillus niger ATCC 16404	0	0	0	56	ND	ND	ND

[a] Between the edge of the filter paper and the edge of the inhibition area. [b] CAE – cold aqueous extract; HAE – hot aqueous extract; MeOH – methanol extract. [c] Reference antibiotics (ampicillin for gram- positive bacteria, gentamicin for gram-negative and cetoconazol for fungi and yeast). * ND: not determinate because the extract was not active by the disc diffusion test.

extracts showed antimicrobial activity, with formation of an inhibition zone, against gram-positive ATTC strain standard bacteria, ranging from 12 to 14 mm for *M. luteus,* from 10 to 12 mm for *S. aureus* and from 10 to 12 mm for the clinical multiresistant samples (*S. aureus* MR01, MR02, and MR03). The inhibition zones obtained for clinical multiresistant bacteria *S. aureus* MR01 and MR02 from all extracts were greater than the CLSI ampicillin standard (7 mm) while *S. aureus* MR03 responded similarly to the CLSI ampicillin standard (Table 1). Except for the MeOH extract, all others showed a positive result against the gram-negative bacterium *S. choleraesuis,* with the formation of a similar inhibition zone (14 mm). No antimicrobial activity was observed against the other microorganisms tested (Table 1).

The MICs of the active extracts ranged between 5.0 and 250 μg/ml (Table 1). The methanol extract gave the lowest MIC value (5.0 μg/ml) for *S. aureus* ATCC6835. The MICs in the cold aqueous extract were lower than in the hot aqueous extract for *M. luteus* and *S. aureus* MR01. For gram-positive bacteria, the MIC values were higher for multiresistant strains compared with ATCC strains, except for the cold aqueous extract, for which the MIC values were the same for *S. aureus* MR01 and ATCC. For the gram-negative bacterium *S. choleraesuis,* the MIC value was much higher than those obtained for the gram-positive bacteria in general.

DISCUSSION

Several studies on the selection of medicinal plants with proven antimicrobial activity have been recently published. Although, the family Mimosaceae comprises of 40 genera and 2500 species (Judd et al., 2009) few studies have assessed the members of this plant family for bio-logical activities, especially antimicrobial. Testing crude extracts from several plants in French Guiana, Rovira et al. (1999) observed antimicrobial activity against *S. aureus* in 72% of the plants, mostly from the family Mimosaceae. Palombo and Semple (2002) detected antimicrobial activity in alcohol extracts from Australian plants against *S. aureus* MRSA and *Enterococcus faecalis* VRE clinical isolates. Lopes et al. (2005) demonstrated the activity of aqueous and methanol extracts from stem bark of *Stryphnodendron polyphyllum* and *Stryphnodendron obovatum* against gram positive bacteria.

The methanol extract of *Acacia auriculoformis* demonstrated activity against various gram-positive and gram-negative bacteria (Pennachio, 2005). Kouitcheu et al. (2007) demonstrated antimicrobial activity of the ethyl acetate extract of stem bark of *Cylicodiscus gabunensis* against *S. aureus*, *Proteus vulgaris* and *Bacillus cereus*. Millogo-Kone et al. (2008) showed antibacterial activity of hydroalcoholic and aqueous extracts of leaf and stem bark of *Parkia biglobosa* against enterobacteriaceae, and reported that the hydroalcoholic extract of the bark is more active than the aqueous extract of the leaf. The results here described for extracts of the stem bark of *A. cochliocarpos* concord with previously reported observations on various plants of the family Mimosaceae, which have a narrow spectrum of antibacterial activity, effective mainly against *S. aureus*. The aqueous extract that showed antibacterial activity against *S. aureus, M. luteus*, and *S. choleraesuis,* showed no difference in activity profile whether it was extracted hot or cold. The methanol

extract showed a narrower spectrum of activity than the aqueous extract, with antibacterial activity only against gram-positive strains. These results are similar to those of Santos et al. (2007), who reported that the hydro-alcoholic extract of the bark of *A. cochliocarpos*, assayed *in vitro*, showed antibacterial potential only against *S. aureus* and *M. luteus.*

The present study showed that the aqueous and methanol extract had no antifungal activity against the fungi tested. No antifungal activity has been described for other extracts of *A. cochliacarpos*. Considering other plants of the family Mimosidaceae, antifungal activity has been described only for the ethyl acetate extract of stem bark of *Cylicodiscus gabunensis*, against the yeasts *C. albicans* and *C. glabrata (*Kouitcheu et al., 2007).

Using the most stringent endpoint criteria as a rule of thumb, extracts or compounds with a selective activity and IC50 or MIC values below 1 - 10 µM (pure compounds) or 1 - 50 µg/ml (extracts) can be considered as active 'hits' for most organisms; for the gram-negative bacteria, mycobacteria, and fungi, 10 - 100 µM (pure compounds) or 1 - 50 µg/ml (extracts) may be more appropriate as endpoint criteria (Cos et al., 2006). In this regard, all extracts from *A. cochliacarpos* can be considered to be useful antimicrobial agents, because they showed MICs between 5.0 and 15.62 µg/ml for most microorganisms tested.

Demonstration of antimicrobial activity against both gram-positive and gram-negative bacteria may indicate the presence of broad-spectrum antibiotic compounds (Salama and Marraiki, 2010). Previous phytochemical analysis with the same extracts from *A. cochliacarpos* that were used for the antimicrobial test described here, revealed the presence of saponins, catechins, tannins, phenols and anthraquinones for all three extracts analyzed (Silva et al., 2009). The amphiphilic behavior of saponins and their capacity to form complexes with steroids, proteins and membrane phospholipids determine several biological properties for saponins, including antimicrobial action (Wallace, 2004).

The antimicrobial property of tannins has been described by many investigators (Scalbert, 1991; Djipa et al., 2000). Tannins can be toxic to filamentous fungi, yeasts, and bacteria. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins and they can also complex with polysaccharides (Cowan, 1999). Catechins have been extensively researched because of their occurrence in green teas and they inhibit *in vitro* a large number of bacteria and other microorganisms (Romani et al., 2006).

The results of this investigation support the claims by local practitioners of ethnomedicine regarding the therapeutic efficacy of this plant. The antimicrobial action of the medicinal plant used in this study demonstrates that plant extracts can be a potential source of antimicrobial agents, and that further research to isolate, purify, and test these compounds should be performed.

ACKNOWLEDGEMENTS

The authors are grateful to CNPq for financial support. The authors also thank all members of the community of Barra II, Morro do Chapéu, Bahia, for their permission to carry out the ethnobotanical study. Thanks to Erika von Sohsten de Souza Medeiros of the Rio de Janeiro Botanical Garden Herbarium, for the botanical identification.

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