Anti-Staphylococcus Activity of Uruguayan Riverside Forest Plants.

Stephanie Barneche; María P. Cerdeiras; Rodrigo Lucarini; Carlos H.G. Martins; Cristina Olivar; Alvaro Vazquez*

*Catedra de Farmacognosia, Facultad de Quimica, UDELAR. CC 1157, Montevideo, Uruguay.
*Catedra de Microbiología, Facultad de Quimica, UDELAR. CC 1157, Montevideo, Uruguay.
*Universidade de Franca, Laboratório de Pesquisa em Microbiologia Aplicada, LaPeMA, Franca-SP, Brasil.

ABSTRACT

Methicillin resistant *Staphylococcus aureus* is a major nosocomial pathogen which causes increased morbidity and mortality worldwide. Interest in plants with antimicrobial properties has thus revived as a consequence of current problems associated with the use of antibiotics. In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin. Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization. Extracts that were positive in the qualitative diffusion tests against *S. aureus* were selected for a more detailed study. The objective of the present work is to study the anti MRSA activity of 37 plant extracts that have previously shown activity against *S. aureus* in a qualitative diffusion assay. The plant material were separately twice extracted by maceration with EtOH/H2O 70:30, acetone and CHCl3. Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and Laboratory Standards Institute using sensitive (ATCC 6538p) and resistant (ATCC 43300, ATCC 700699 and USA 100) strains. Some of the extracts showed good activity specially species of the Myrtaceae family with MICs as low s 7.8 μg/mL. These promissory results were obtained encourage further work to be done regarding the isolation and structural elucidation of the active compound(s).

Key words: Antibacterial activity Methicillin resistant *S. aureus*, Myrtaceae

INTRODUCTION

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organization[1] states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2003. Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms makes them sometimes ineffective or lead to recurrence.[2,3] Amongst some of the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat.[4,5]

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases.[6-8] Interest in plants with antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics.[9-13]

Although Uruguay is usually considered a grassland country it has more than 30 woody families and a considerable amount of native forests, especially along river banks (gallery forests) and “quebradas” (gulch forests), with a subtropical and tropical vegetation intrusion. These forests comprise more than 810,000 has with a varied and distinctive flora, around 250 comprising woody and arbustive species.[10] The riverside forest along the Uruguay River banks is especially interesting as a great number of tropical species original from the Chaco and Espinal floristic provinces are also present.[17]

In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin.[18] Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization.[19] Extracts that were positive in the

*Address for correspondence:
Phone +598 2 9244068; Fax +598 2 9241906
E-mail: avazquez@fq.edu.uy

Qualitative diffusion tests against *S. aureus* were selected for a more detailed study.

In this work, we present the results of the antimicrobial activity of these extracts against methicillin sensitive and resistant *S. aureus* strains.

**MATERIAL AND METHODS**

**Plant Material**

Plants were collected during the 2006, 2007 and 2008 spring-summer season in different locations along the Uruguay River shore between the Chapicuy stream confluence and river Guaviyu confluence, near Paysandú and along the banks of Queguay river between the confluence of Queguay Chico y Queguay Grande and it’s opening in the Uruguay river. Plants were identified by Lic. F. Haretche, Museo y Jardin Botanico “Atilio Lombardo”, Montevideo. Voucher specimens are kept in the MVJB Herbarium, Jardin Botanico, Montevideo.

**Extraction**

The plant material was air dried in the dark and milled to a coarse powder. Samples (20 g) were separately twice extracted by maceration with EtOH/H₂O 70:30, acetone and CHCl₃ (100 mL) for 48 h. Combined extracts were evaporated under vacuum and lyophilised when necessary.

**Microbiological Assay**

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and qualitative diffusion tests against *S. aureus* were selected for a more detailed study.

In this work, we present the results of the antimicrobial activity of these extracts against methicillin sensitive and resistant *S. aureus* strains.

**MATERIAL AND METHODS**

**Plant Material**

Plants were collected during the 2006, 2007 and 2008 spring-summer season in different locations along the Uruguay River shore between the Chapicuy stream confluence and river Guaviyu confluence, near Paysandú and along the banks of Queguay river between the confluence of Queguay Chico and Queguay Grande and its opening in the Uruguay River. Plants were identified by Lic. F. Haretche, Museo y Jardin Botanico “Atilio Lombardo”, Montevideo. Voucher specimens are kept in the MVJB Herbarium, Jardin Botanico, Montevideo.

**Extraction**

The plant material was air dried in the dark and milled to a coarse powder. Samples (20 g) were separately twice extracted by maceration with EtOH/H₂O 70:30, acetone and CHCl₃ (100 mL) for 48 h. Combined extracts were evaporated under vacuum and lyophilised when necessary.

**Microbiological Assay**

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and qualitative diffusion tests against *S. aureus* were selected for a more detailed study.

In this work, we present the results of the antimicrobial activity of these extracts against methicillin sensitive and resistant *S. aureus* strains.

**Table 1: Antimicrobial activity of plant extracts**

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Used partᵃ</th>
<th>Extractᵇ</th>
<th>ATCC 6538p</th>
<th>ATCC 700699</th>
<th>ATCC 43300</th>
<th>USA 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allophylus edulis</td>
<td>L</td>
<td>3</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Combretum fruticosum</td>
<td>2</td>
<td>500</td>
<td>250</td>
<td>500</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Croton tenuissimus</td>
<td>2</td>
<td>&gt;500</td>
<td>500</td>
<td>500</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Dolichandra cynanchoides</td>
<td>1</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Eugenia mansonii</td>
<td>1</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Eugenia uruguayensis</td>
<td>2</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Gleditsia amorphoides m</td>
<td>1</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Gouania ulmilofila</td>
<td>2</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Guettarda uruguayensis</td>
<td>2</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Hexachlamys edulis</td>
<td>L</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Maytenus ilicifolia</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>125</td>
</tr>
<tr>
<td>Mimosa uruguayensis</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td></td>
</tr>
<tr>
<td>Myrcianthes cisplatensis</td>
<td>L</td>
<td>62.5</td>
<td>31.3</td>
<td>31.3</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Myrrhinium atropurpureum</td>
<td>L</td>
<td>125</td>
<td>125</td>
<td>250</td>
<td>125</td>
<td>250</td>
</tr>
<tr>
<td>Myrsine venosa</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Ocotea acutifolia</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Phyllanthus sellowians</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Polygonum punctatum</td>
<td>AP</td>
<td>3</td>
<td>500</td>
<td>500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Pouteria salicina</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Psychotria carthaginosis</td>
<td>L</td>
<td>500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Ruprechtia laxiflora</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Scutia buxifolia</td>
<td>L</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Terminalia australis</td>
<td>L</td>
<td>&gt;500</td>
<td>250</td>
<td>MIC</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Urvillea uniloba</td>
<td>AP</td>
<td>2</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

ᵃUsed Part: L leaf, F: fruit, AP: aerial parts
ᵇExtraction solvent: 1 EtOH-H₂O 70:30, 2 CH₃COCH₃, 3 CH₂Cl₂
ᶜStrains: ATCC 6538p (Staphylococcus aureus ATCC 6538p MSSA), ATCC 700699 (Staphylococcus aureus ATCC 700699 MRSA), ATCC 43300 (Staphylococcus aureus ATCC 43300 MRSA), USA 100 (Staphylococcus aureus USA 100 New York-Tokio clone).
Laboratory Standards Institute [24] using sensitive (ATCC 6538p) and resistant (ATCC 43300, ATCC 700699 and USA 100) strains.

RESULTS AND CONCLUSION

The results of the antibacterial assays are depicted in Table 1. As can be seen 19 out of 37 (50%) of the extracts showed measurable activity with MICs below 500 μg/mL. This is an expected result as the samples were carefully selected in an ethnobotanical survey and went through a pre-screening process [18, 19].

In particular extracts of *Eugenia mansonii*, *Eugenia uruguayensis*, *Myricanthes cisplatensis* and *Myrrhinium altopurpureum*, all belonging to the Myrtaceae Family, showed MICs as low as 7.8 μg/mL with several of the resistant strains.

The MRSA chosen were not only resistant to methicilin, but also to oxacillin (ATCC 43300), to vancomycine (ATCC 700699) and multiresistant (spectinomycine, clindamycine, erythromycine, vancomycine reduced susceptibility) the USA 100.[21–23]

Results obtained for *Myricanthes cisplatensis* fruit extracts EtOH-H₂O and CH₃COCH₃ are very interesting as they show higher activity for the multiresistant S. aureus USA 100 than against all other *S. aureus* strains assayed. It had shown a broad spectrum of antimicrobial activity for all its extracts, even including fungi and *Mycobacterium*, in this case with MIC values of 100-200 μg/mL. Results suggest that the compounds responsible for these antimicrobial activities most probably belong to at least two different groups with different solvent solubilities, as the extracts with antimycobacterial activity are those obtained with chlorophorm and acetone, whilst the most polar extract showed activity against *S. aureus* USA 100.

ACKNOWLEDGEMENTS

This work was supported by the State of São Paulo Research Foundation (FAPESP) – 2009/18278-0 and 2010/02066-0 and University of Franca (Br) and PEDECIBA program and the national Agency for Research (ANII) (Uy). The authors gratefully acknowledge Prof. Agnes S. Figueiredo for technical advice.

REFERENCES