Antistaphylococcal Activity of Xanthium cavanillesii Lactones

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ABSTRACT

Objective: The genus Xanthium L., of the Asteraceae Dum. family, (tribe Heliantheae) comprises 30 species of cosmopolitan distribution, many of which, as X. spinosum and X. strumarium are used as medicinal plants. This genus has been the object of numerous phytochemical investigations being sesquiterpene lactones with guaiane or secoguaiane frameworks the main secondary metabolites. Several sesquiterpene lactones have been demonstrated to have antimicrobial activity, in particular against Gram+ bacteria and in Uruguay the infusion of Xanthium cavanillesii Schouw (common name “Abrojo” or “Abrojo grande”) which grows wild, is used as antiseptic in popular medicine. In this work we present the results of the antibacterial analysis of several extracts, fractions and pure compounds from X. cavanillesii against both sensitive and resistant strains of Staphylococcus aureus.

Materials and Methods: Compounds were isolated from X. cavanillesii aerial parts by several chromatographic and spectroscopic methods antimicrobial analysis were performed according to Clinical and Laboratory Standards Institute guidelines. Results: The minimum inhibitory concentration (MIC) found were high for the sensitive 6538p strain when compared with common antibiotics. For the resistant strains, the pure compounds activity clearly outperformed the antibiotics, especially in the case of the multiresistant 700,699 strain with MICs of 31, 236 and 356 µg/mL for the Xanthium compounds, gentamicin and oxacillin respectively.

Keywords: Antimicrobial, abrojo, methicillin-resistant Staphylococcus aureus, sesquiterpene lactones

INTRODUCTION

In spite of the great advance in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organisation¹ states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2006.

Although it appears 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.

Higher plants have shown to be an important source of new bioactive compounds, including antihypertensive, analgesics, cytotoxic compounds, amongst others.²³

Though no plant derived compound has been found to compete with clinically used antibiotics, to date, the great structural variety found in plants makes them attractive as a source of novel lead compounds. In fact, higher plants frequently exhibit significant potency against human bacterial and fungal pathogens.⁶⁷

The genus Xanthium L. of the Asteraceae family, (tribe Heliantheae) comprises 30 species of cosmopolitan distribution, many of which, as X. spinosus and X. strumarium are used as medicinal plants.⁸⁹ This genus has been the object of numerous phytochemical investigations being sesquiterpene lactones with guaiane or secoguaiane frameworks the main secondary metabolites.¹⁰⁻¹³ In particular, in X. cavanillesii, the main sesquiterpene lactone constituents are xanthumin and its dihydro derivative.¹⁴

Several sesquiterpene lactones have been demonstrated to have antimicrobial activity, in particular against Gram+ bacteria,¹⁵⁻¹⁷ and inhibitory activity on NF-κB activation.

The infusion of Xanthium cavanillesii Schouw (common name “Abrojo” or “Abrojogrande”) which grows wild
in Uruguay is used as antiseptic in ethnomedicine. In previous works we study its antimicrobial activities and toxicity and isolated a new sesquiterpene lactone, named xanchristin.

In this work we present the results of the antibacterial analysis of several extracts, fractions and pure compounds from X. cavanillesii against both sensitive and resistant strains of Staphylococcus aureus.

**MATERIALS AND METHODS**

**General experimental procedures**

Gas chromatography (GC) analysis was performed in a Shimadzu GC 14 apparatus with an SE-52 column using a temperature program from 100 to 280°C with a 5°C/min gradient.

A Brucker micrOTOF-Q-TOF with electrospray ionization source in positive mode was used for mass spectrometry (MS) spectra and a Shimadzu QP 5050 with a SE 52 column was used for the GC-MS analysis.

Thin-layer chromatography (TLC) was performed on silica gel plates (Macherey Nagel, Dürin, Germany) using CH2Cl2/acetone (6:1) as solvent and H2SO4/heating or p-hydroxylbenzaldehyde as detection reagents.

Infrared (IR) analysis was performed in a Nicolet 8700 Fourier transform (FT) - IR. Nuclear magnetic resonance (1H NMR) spectra were obtained at 400 MHz and 13C NMR spectra at 100 MHz, on a Brucker Advance DPX 400 spectrometer, using CD3OD or CDCl3 as solvents and tetramethylsilane (dH 0.00) and acetone (dC 31.00) as references. 2D (different H,H-COSY, H,C-COSY, HMBC, HMQC and NOESY) and 3D (HSQC-TOCSY) experiments were carried out with programs available in the Brucker software.

**Plant material**

X. cavanillesii leaves were collected in Solymar (Canelones) near Montevideo and identified by Lic. F. Haretche, Museo y Jardín Botánico “Atilio Lombardo,” Montevideo. Voucher specimens are kept in the MVFQ Herbarium, Jardín Botánico, Montevideo.

**Extraction and isolation**

X. cavanillesii leaves (240 g) were extracted exhaustively with CH2Cl2 (3 L) for 72 h in the dark at room temperature. After vacuum evaporation of the solvent the dichloromethane extract (14 g) was submitted to vacuum liquid chromatography on SilicaGel 40 (Merrick, Darmstadt). The extract was separated into eight fractions (hexane, hexane-CH2Cl2 4:1, hexane-CH2Cl2 1:1, CH2Cl2, CH2Cl2-ethyl acetate 1:1, ethyl acetate, acetone, MeOH). Fractions were analyzed by TLC and pooled.

A sample of the ethylacetate fraction was fractionated trough flash chromatography (Si 50 SF 15-24 g Varian) with dichloromethane/acetone 10:1 as eluent and fractions 1-6 pooled and further fractionated using normal phase (Si 50 SF 15-24 g Varian) and reverse phase (C18 SF 15-16 g Varian) flash chromatography. Finally, preparative TLC (Machery-Nagel) gave two compounds (2, 3).

Xanchristin (1) was already isolated as previously reported.

**Microbiological analysis**

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) strains. Gentamicin and oxacillin were used as control.

Bioautographies were made on developed and dried TLC plates according to the agaroverlay method of Rahalison using S. aureus (ATCC 6538p).

4-epi-Xanthanol. Colourless oil, IR νmax (cm⁻¹, thin film) 3350, 1762, 1735.

MS m/z (rel. int.): 248, 230, 204, 189, 176.

1H and 13C NMR spectroscopy (Table 1).

4-epi-Isoxanthanol. Light yellow oil, IR νmax (cm⁻¹, thin film) 3400, 1765, 1740.

MS: 248, 230, 204, 189, 176.

1H and 13C NMR spectroscopy (Table 1).

**RESULTS AND DISCUSSION**

The bioactivity guided fractionation of a chloroform extract of X. cavanillesii leaves yielded after repeated chromatography, among several others, three sesquiterpene lactones. From chromatographic and spectroscopic data (GC-MS, HR-ESIMS, 1D, 2D and 3D NMR) and
comparison with bibliographic data these compounds could be identified as xanchristin (1),\textsuperscript{19} 4-epi-isoxanthanol (2)\textsuperscript{22} and 4-epi-xanthanol (3) (Figure 1).\textsuperscript{23,24}

Compound 1, with a new xanthanolide skeleton, was firstly isolated by us and compounded 2 and 3, although common Xanthium metabolites were never isolated before in X. cavanillesii.

The antimicrobial activity of the extract, fractions and pure compounds against sensitive and resistant S. aureus are presented in Table 2.

The MICs found were high for the sensitive 6538p strain when compared with common antibiotics. For the resistant strains, the pure compounds activity clearly outperformed the antibiotics, especially in the case of the multi resistant 700,699 strain with MICs of 31, 236 and 356 µg/mL for the Xanthium compounds, gentamicine and oxacillin respectively.

All the isolated compounds showed very similar activity against all strains that are consistent with previous studies as the principal pharmacophore of these molecules is the a-methylen-γ-lactone moiety, with the rest of the molecule acting as modulator of the activity.\textsuperscript{25-28}

Acknowledgments

The authors gratefully acknowledge Mr. H. Pezarogglio (NMR facility) and Dr. A. Rodriguez (Unidad de Servicios Tecnologicos, PTP) for NMR and MS analysis. This work was supported by PEDECIBA Program and the Agencia Nacional de Investigacion e Innovacion.

References

7. Nascimento GF, Juliana L, Paulo CF, Giuliana LS. Antimicrobial

<p>| Table 1: NMR spectroscopic data of compounds 2-3 |</p>
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NMR: Nuclear magnetic resonance

<p>| Table 2: MICs of samples against S. aureus strains |</p>
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<td>62.5</td>
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<tr>
<td>3</td>
<td>15</td>
<td>31</td>
<td>62.5</td>
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<tr>
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<td>Oxacillin</td>
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</table>

MIC: Minimum inhibitory concentration, VLC: Vacuum liquid chromatography

Figure 1: Structure of studied compounds, (1) Xanchristin, (2) 4-epi-Isoxanthanol: R1OH/R2AcO, (3) 4-epi-Xanthanol: R1AcO/R2 OH.

Source of Support: None. Conflict of Interest: None declared.