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* Show (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 Organisation[1] 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.[2-5] 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.[6,7]

The genus *Xanthium* L of the Asteraceae family, (tribe Heliantheae) comprises 30 species of cosmopolitan distribution, many of which, as *X. spines* and *X. strumarium* are used as medicinal plants.[8,9] 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

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in Uruguay is used as antiseptic in ethnomedicine.[18] In previous works we study its antimicrobial activities and toxicity and isolated a new sesquiterpene lactone, named xanchristin.[19]

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 CH_2Cl_2/acetone (6:1) as solvent and H_2SO_4/heating or *p*-hydroxylbenzaldehyde as detection reagents.[13]

Infrared (IR) analysis was performed in a Nicolet 8700 Fourier transform (FT) - IR. Nuclear magnetic resonance (^{1}H NMR) spectra were obtained at 400 MHz and ^{13}C NMR spectra at 100 MHz, on a Brucker Advance DPX 400 spectrometer, using CD3OD or $CDCl_3$ as solvents and tetramethylsilane (δ_H 0.00) and acetone (δ_C 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 Jardin Botánico "Atilio Lombardo," Montevideo. Voucher specimens are kept in the MVFQ Herbarium, Jardin Botánico, Montevideo.

Extraction and isolation

X. cavanillesii leaves (240 g) were extracted exhaustively with CH_2Cl_2 (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 (Merck, Darmstad). The extract was separated into eight fractions (hexane, hexane-CH_2Cl_2 4:1, hexane-CH_2Cl_2 1:1, CH_2Cl_2, CH_2Cl_2-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.[19]

Microbiological analysis

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and Laboratory Standards Institute[20] 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[21] using *S. aureus* (ATCC 6538p).

4-*epi*-Xanthanol. Colourless oil, IR ν_{max} (cm^{-1}, thin film) 3350, 1762, 1735.

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

^{1}H and ^{13}C NMR spectroscopy (Table 1).

4-*epi*-Isoxanthanol. Light yellow oil, IR ν_{max} (cm^{-1}, thin film) 3400, 1765, 1740.

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

^{1}H and ^{13}C 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

Table 1: NMR spectroscopic data of compounds 2-3

Carbon nº	2		3	
	δC	δH (m, J)	δC	δH (m, J)
1	149.7	-	146.0	-
2	74.8	4.03 (dd, 7.9, 5.3)	78.0	5.31 (dd, 9.8,4.3)
3	41.5	1.73 (m)	42.7	1.83 (m)
4	68.5	4.98 (m)	63.6	1.64 (m)
5	123.3	5.85 (dd, 6.3,3.4)	126.1	3.76 (m)
6	24.6	2.60 (ddd, 15.7, 9.4, 2.3)	24.6	5.96 (dd, 9.4, 3.6)
7	48.1	2.16 (ddd, 15.8, 11.3, 3.4)	47.3	2.63 (ddd, 16.2, 9.7, 2.3)
8	82.9	2.51 (m)	82.6	2.15 (ddd, 16.1, 11.5, 3.7)
9	36.4	4.41 (ddd, 11.0, 2.9, 2.3)	36.5	2.49 (m)
10	29.1	1.80 (m)	29.7	4.39 (ddd, 11.2, 2.8, 2.3)
11	140.0	2.30 (ddd, 12.0, 2.9, 1.5)	139.9	2.28 (ddd, 12.6, 3.1, 1.6)
12	170.7	2.81 (m)	170.8	2.84 (m)
13	117.6	-	117.6	-
14	19.1	-	18.3	-
15	19.8	5.59 (d, 3.2)	22.6	6.11 (d, 3.3)
CH_3CO	20.5	6.11 (d, 3.4) 1.22 (d, 7.4) 1.25 (d, 6.2) 2.04(s)	19.8	5.59 (d, 3.2) 1.16 (d, 7.4) 1.21 (d, 6.2) 2.06 (s)
CH_3CO	171.1	-	170.8	-

NMR: Nuclear magnetic resonance

comparison with bibliographic data these compounds could be identified as xanchristin (1),[19] 4-*epi*-isoxanthanol (2)[22] and 4-*epi*-xanthanol (3) (Figure 1).[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.[25-28]

Table 2: MICs of samples against S. *aureus strains*

Sample/strain	MIC (μg/mL)		
	ATCC6538p	ATCC 700699	ATCC 43300
Xanthium extract	67	135	269
VLC 5	116	233	233
VLC 6	67	116	233
1	15	31	62.5
2	15	31	62.5
3	15	31	62.5
Gentamicin sulfate	4	236	15
Oxacillin	0.15	356	45

MIC: Minimum inhibitory concentration, VLC: Vacuum liquid chromatography

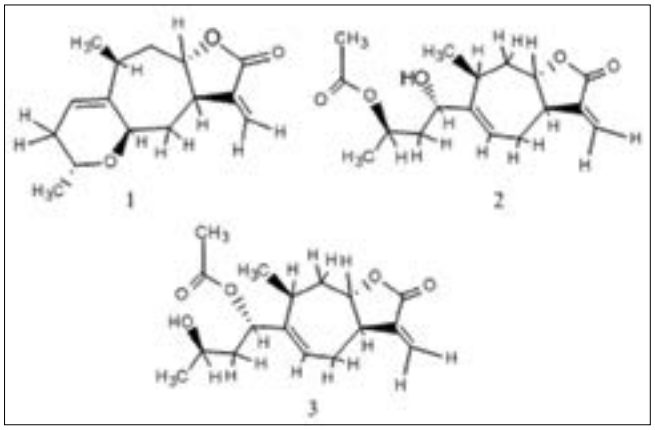

Figure 1: Structure of studied compounds, (1) Xanchristin, (2) 4-epi-Isoxanthanol: R1OH/R2AcO, (3) 4-epi-Xanthanol: R1AcO/R2 OH.

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REFERENCES

1. WHO. The World Health Report 2007 - A Safer Future: Global Public Health Security in the 21st Century. Geneva: World Health Organization; 2007.
2. Cassady JM, Baird WM, Chang CJ. Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. J Nat Prod. 1990;53:23-41.
3. Clark AM. Natural products as a resource for new drugs. Pharm Res. 1996;13:1133-44.
4. Lewis WH, Elvin-Lewis M. Medicinal plants as sources of new therapeutics. Ann Mo Bot Gard. 1995;82:16-24.
5. Strohl WR. The role of natural products in a modern drug discovery program. Drug Discov Today. 2000;5:39-41.
6. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564-82.
7. Nascimento GF, Juliana L, Paulo CF, Giuliana LS. Antibacterial

activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz J Microbiol. 2000;31:247-56.
8. Tsankova ET, Trendafilova AB, Kujumgiev AI, Galabov AS, Robeva PR. Xanthanolides of *Xanthium italicum* Moretti and their biological activity. Z Naturforsch C. 1994;49:154-5.
9. Hsu FL, Chen YC, Cheng JT. Caffeic acid as active principle from the fruit of *Xanthium strumarium* to lower plasma glucose in diabetic rats. Planta Med. 2000;66:228-30.
10. Omar AA, Ghazy NA, Metwally A, Ziesche J, Bohlman F. Xanthanolides from *Xanthium spinosum*. Phytochemistry. 1984;23:915-6.
11. Bohlman F, Zdero C. An isomer of xanthanol from *Xanthium orientale*. Phytochemistry. 1981;20:1891-3.
12. Ahmed AA, Jakupovic J, Bohlman F, Ahmed AM. Sesquiterpene lactones from *Xanthium pungens*. Phytochemistry. 1990;29:2211-5.
13. Mangel SM, Sangwan NK, Dhindsa K**.** Xanthanolides from *Xanthium strumarium*. Phytochemistry. 1992;32:206-7.
14. de Riscala EC, Fortuna MA, Catalan CA, Diaz JG, Herz W. Xanthanolides and a bis-norxanthanolide from *Xanthium cavanillesii*. Phytochemistry. 1994;35:1588-9.
15. Sato Y, Oketani H, Yamada T, Singyouchi K, Ohtsubo M, Kihara T. A xanthanolide with potent antibacterial activity against methicillin-resistant Staphylococcus aureus. J Pharm Pharmacol. 1997;49:1042-4.
16. Cerdeiras MP, Alborés S, Etcheverry S, Lucian V, Soubes M, Vázquez A. The antimicrobial activity of *Xanthium cavanillesii* extracts. Pharm Biol. 2007;45:251-3.
17. Ginesta-Peris E, Garcıa-Breijo FJ, Primo Yùtera E. Antimicrobial activity of xanthatin from *Xanthium spinosum*. Lett App Microbiol. 1994; 18(4): 206-8.
18. Lombardo A. Flora Montevidensis. Montevideo: ImdeM; 1983.
19. Olivaro C, Vazquez A. A new bioactive xanthanolide from *Xanthium cavanillesii*. Nat Prod Res. 2009;23:388-92.
20. CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard. 9th ed. Wayne, PA: CLSI; 2012.
21. Rahalison L, Hamburger M, Hostettman K, Monod M, Frenck E. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants**.** Phytochem Anal. 1991;2:199-203.
22. Winters TE, Geissman TA, Safir D. Sesquiterpene lactones of *Xanthium* species. Xanthanol and isoxanthanol, and correlation of xanthinin with ivalbin. J Org Chem. 1969;34:153-5.
23. Marco JA, Sanz-Cerver JF, Corral J, Carda M, Jakupovic J. Xanthanolides from *Xanthium*: Absolute configuration of xanthanol, isoxanthanol and their C-4 epimers. Phytochemistry. 1993;34:1569-76.
24. Bohlmann F, Zdero C. An isomer of xanthanol from *Xanthium orientale*. Phytochemistry. 1981;20:2429-30.
25. Vasas A, Hohmann J. Xanthane sesquiterpenoids: Structure, synthesis and biological activity. Nat Prod Rep. 2011;28:824-42.
26. Ordóñez PE, Quave CL, Reynolds WF, Varughese KI, Berry B, Breen PJ, *et al*. Sesquiterpene lactones from gynoxys verrucosa and their anti-MRSA activity. J Ethnopharmacol. 2011;137: 1055-9.
27. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Rep. 2004;21:263-77.
28. Schmidt TJ. Toxic activities of sesquiterpene lactones: Structural and biochemical aspects. Curr Org Chem. 1999;3:599-600.

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