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Antimicrobial Activity of *Xanthium cavanillesii* Extracts

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Abstract

Development of new antimicrobial compounds against different microorganisms is becoming critically important, as infectious diseases are still one of the leading causes of death in the world. The pharmaceutical industry is searching for new lead compounds with novel chemical structures to overcome the increasing resistance to known antibiotics. Plants can be a useful source of these lead compounds. *Xanthium cavanillesii* Schouw (Asteraceae) grows wild in Uruguay, and its infusion is used in popular medicine as skin antiseptic. In this work, we present the study of the antimicrobial activity of several extracts of *X. cavanillesii* against different microorganisms. In the agar diffusion assays, the plant extracts showed an interesting antimicrobial activity, including activity against *Mycobacterium smegmatis* and *Candida albicans*. The extracts showed low toxicity in the acute oral assay performed with no deaths at a 200 mg/kg dose.

Keywords: Abrojo, antibacterial activity, *Candida*, *Mycobacterium*.

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 Organization (Anonymous, 2004) 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 these drugs sometimes ineffective or leads to recurrence. Higher plants have been shown to be an important source of new bioactive compounds, including antihypertensive, analgesic, and cytotoxic compounds, among others (Cassady et al., 1990; Lewis & Elvin-Lewis, 1995; Clark, 1996). 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 (Cowan, 1999). In fact, higher plants frequently exhibit significant potency against human bacterial and fungal pathogens (Cos et al., 2006).

The genus *Xanthium* L. (Asteraceae) (tribe Heliantheae) comprises 30 species of cosmopolitan distribution, many of which, such as *X. spinosum* L. and *X. strumarium* L., are used as medicinal plants (Tsankova et al., 1994; Hsu et al., 2000). The genus *Xanthium* has been the object of numerous phytochemical investigations. Sesquiterpene lactones with guaiane or secoguaiane frameworks are the main secondary metabolites (Bohlman & Zdero, 1981; Omar et al., 1984; Ahmed et al., 1990; Mangel et al., 1992). In particular, in *X. cavanillesii* Schouw, the main sesquiterpene lactone constituent is xanthumin and its dihydro derivative, but no xanthanin was observed (de Riscala et al., 1994).

Several sesquiterpene lactones have been demonstrated to have antimicrobial activity, in particular against Gram-positive bacteria (de Riscala et al., 1994; Tsankova et al., 1994; Ginesta-Peris et al., 1994). Considering that infusions of *Xanthium cavanillesii* (common name "abrojo" or "abrojo grande"), which grows wild in Uruguay, are used as an antiseptic in ethnomedicine (Lombardo, 1983), we decided to study its antimicrobial activities in order to validate its popular use.

Materials and Methods

Plant material

Plants were collected in Solymar, near Montevideo, and were identified by Lic. E. Alonso Paz, Botany

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Department. Voucher specimens (leg. E. Alonso Paz 3510) are kept at the MVFQ Herbarium, Facultad de Quimica, Montevideo.

Extraction

The plant material was air-dried in the dark and milled to a coarse powder. Samples (50 g) were separately extracted two-times by maceration with H_2O, EtOH/H_2O 70:30, EtOH, acetone, and $CHCl_3$ (200 mL) for 48 h. Combined extracts were evaporated in vacuum and lyophilized when necessary.

Test microorganisms

The test organisms used were *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538p), *Klebsiella pneumoniae* (ATCC 10031), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), and *Saccharomyces cerevisiae* (ATCC 2601).

The microorganisms were cultured overnight at 35°C in blood agar base.

Microbiological assay

The antimicrobial activity of extracts was determined by an agar-diffusion method (Barry & Thornsberry, 1985). Colonies of the test organisms were suspended directly into a small volume of 0.9% saline and further diluted until the turbidity matched the MacFarland tube no. 1, and 2.5 mL of this suspension was added to 100 mL of molten Mueller-Hinton agar (Difco) for bacteria and to Sabouraud agar (Difco) for yeasts. Inoculated medium (20 mL) was poured into Petri dishes. Four stainless steel cylinders (i.d. 1 cm) were placed on the surfaces of the medium, and 200 μL of each extract solution (10 mg mL^{-1} in water) pipetted into each of three of them. Two-hundred microliters of gentamicin (20 μg mL^{-1}) or nystatin (50 U mL^{-1}) was placed into the fourth in order to perform a positive control.

The Petri dishes were incubated at 35°C for 24 h for bacteria and 25°C for 48 h for yeasts. The inhibition zones were measured with a caliper and recorded as mean diameter (in millimeters) of six replications. The inhibition is reported as: 0, dr < 0.5; +, dr 0.5–0.6; ++, dr 0.6–0.7; +++, dr > 0.7 (where dr = diameter of extract inhibition zone/diameter of control inhibition zone). Minimum inhibitory concentration (MIC) was determined for the extracts that inhibited growth by the microdilution technique according to Elloff (1998), using 100 μL of Mueller-Hinton broth (Difco), 100 μL of two-fold dilutions of the extracts (10 mg mL^{-1}), and 10 μL of a suspension (10^8 microorganisms mL^{-1}) of the microorganisms. The trays were incubated (24 h, 37°C) and developed with *p*-iodonitrotetrazolium violet (INT; Sigma) 0.1% solution. Nystatin and gentamicin were used as control for yeast and bacteria, respectively.

Toxicity assay

Nulliparous and nonpregnant CD-1 female mice, 8 weeks old (27 ± 3 g), were used in all experiments. All animals came from our own breeding colony, and they were kept in a standard controlled environment according to the guidelines of the National Research Council (Institute of Laboratory Animal Resources, 1996) (temperature, humidity, light-dark cycle, noises, etc.). The animals were marked to permit individual identification and kept in their cages for at least 5 days prior to dosing. They were allowed standard laboratory feed and purified water *ad libitum*.

The acute oral toxicity of *X. cavanillesii* extracts was evaluated by the methodology described in the OECD (2001) Guidelines for Testing of Chemicals. The experimental protocol was submitted and approved by the Ethic Committee for Animal Experimentation of the

Table 1. Antimicrobial activity of "abrojo" extracts.

		Microorganism						
	Plant extract	1	2	3	4	5	6	7
Leaves	Aqueous	+	+++	0	+++	0	+	0
	EtOH	++	+	0	0	0	+	0
	$CHCl_3$	++	+++	+	++	+	+	0
Fruit	Aqueous	+	+	0	+++	+	NT	0
	EtOH	0	++	0	+++	0	+	0
	$CHCl_3$	+++	+++	+	0	+	+	+
Root	Aqueous	+	+	0	+++	+	0	0
	EtOH	+	+	0	+	+	0	0
	$CHCl_3$	+	+++	+	+++	0	+	+

Microorganisms: 1, *B. subtilis*; 2, *S. aureus*; 3, *K. pneumoniae*; 4, *P. aeruginosa*; 5, *M. smegmatis*; 6, *C. albicans*; 7, *S. cerevisiae*.

Table 2. Minimum inhibitory concentrations ($mg\,mL^{-1}$) of *X. cavanillesii* extracts for selected microorganisms.

		Microorganism					
	Plant extract	1	2	3	4	5	6
Leaves	Aqueous	5	2.5		1.25		0.31
	EtOH	2.5	1.25		1.25		0.31
	$CHCl_3$	2.5	2.5	10	2.5	1.25	0.63
Fruit	Aqueous	10	5		5		
	EtOH	–	2.5		5		0.63
	$CHCl_3$	2.5	2.5	5	2.5		0.31
Root	Aqueous	10	10		10	5	
	EtOH	5	2.5		2.5	0.625	
	$CHCl_3$	5	5	5	5	1.25	1.25
Control (μg/mL)		0.78	2.5	1.25	5	5	5

Microorganisms: 1, *B. subtilis*; 2, *S. aureus*; 3, *K. pneumoniae*; 4, *P. aeruginosa*; 5, *M. smegmatis*; 6, *C. albicans*.

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The animals were fasted prior to dosing (4 h). The fasted body weight of each animal was determined and the dose was calculated according to the body weight. The EtOH and $CHCl_3$ extracts were prepared in saline with 5% Tween 80 and administered by gavage using a stomach tube in maximal volume of 0.2 mL at 2 mg/kg, 20 mg/kg, 100 mg/kg, and 200 mg/kg.

The experimental procedure was performed according to the Main Test of OECD. Animals were observed individually during the first 30 min after dosing, every 4 h during the first 12 h, and daily during 14 days. After that period, all animals were sacrificed and subjected to gross necropsy.

Results and Discussion

The antimicrobial activity of different plant extracts measured by the agar diffusion method is depicted in Table 1. The activity of the aqueous extract could explain the traditional use of "abrojo" infusions for the treatment of skin infections (Lombardo, 1983).

The *X. cavanillesii* extracts under study showed a broad spectrum of activity, and the MIC values (Table 2) below $10\,mg\,mL^{-1}$ were very low for plant whole extracts, especially those for *M. smegmatis* and *C. albicans*. These results make *X. cavanillesii* an interesting source of antimicrobial compounds.

The EtOH and $CHCl_3$ extracts were submitted to the Acute Oral Toxicity Test. There were no deaths during the observation period. The animals looked healthy, and there were no changes in their normal behavior. No pathologic changes were observed in the necropsy in any of the animals.

X. cavanillesii extracts showed a very interesting antimicrobial activity, especially against *C. albicans* and *M. smegmatis*, and very low oral toxicity in rats. Further studies have to be performed to assign the antimicrobial activity determined to the sesquiterpene lactones or to some other class of compounds. These data provide some confirmation for the traditional use of *X. cavanillesii* infusions.

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