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Antimicrobial activity of xanthatin from *Xanthium spinosum* L.

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E. GINESTA-PERIS, F.J. GARCIA-BREIJO AND E. PRIMO-YUFERA. 1994. Dichloromethane extracts from *Xanthium spinosum* L. were fractionated and the fractions tested for their bactericidal and fungicidal activity. From the active fraction, a compound was isolated and identified as xanthatin (I). Xanthatin was active against *Colletotrichum gloesporoides*, *Trichothecium roseum*, *Bacillus cereus* and *Staphylococcus aureus*.

INTRODUCTION

In an exploratory study of mediterranean flora, with the goal of isolating bioactive compounds, we analysed *Xanth-ium spinosum* L. (Asteraceae) (Cuñat *et al.* 1990). Extracts and fractions from *X. spinosum* showed bactericidal and fungicidal activity. In this paper, we report the isolation, identification and antibacterial activity of the active compound.

MATERIALS AND METHODS

Plant material

Xanthium spinosum was collected in Valencia and air-dried in shade. A voucher specimen was deposited in the Depart-ment of Botany (Valencia University).

Fractionation, purification and identification of the active compound

Air-dried *X. spinosum* leaves (3 kg) were extracted in soxhlet with dichloromethane for 5 h and the solvent evaporated in a rotavapor. The extract was chromato-graphed on a 60 mesh silica gel column, eluted with a gra-dient of hexane-dichloromethane-acetone, and fractions assayed. The fraction eluted with dichloromethane showed antimicrobial activity and was further refractionated with a silica gel Sep-Pak column (Waters, 10 μm), using dichloro-methane, ethyl acetate and acetone as eluents. The anti-microbial activity was found in the fraction eluted with dichloromethane.

From this fraction, the pure major compound was iso-lated by semipreparative HPLC (column: 8 × 30 cm, 10 μm particle μ-Porasil; eluents: dichloromethane : ethyl acetate, 95 : 5; flow: 2 ml min^{-1}; detector: Full Range u.v. Diode Array H.P. 85-B).

The structure was determined by infrared, mass spec-trometry and ^{1}H-nuclear magnetic resonance.

Xanthatin. mp: 114–115°C; $[\alpha]_D^{20} = -19°C$ (C = 1·0, $CHCl_3$); IR ν_{max} cm^{-1} (nujol): 1775, 1670, 1590; EIMS m/z: 246 (M^+, 8), 93 (13), 43 (100), 41 (23); ^{1}H-NMR (60 MHz, $CDCl_3$): δ 1·18 (d, 3H), 1·85–2·18 (m, 3H), 2·29 (s, 3H), 2·55 (m, 2H), 3·16 (m, 1H), 4·5 (m, 1H), 5·52 (m, 1H), 6·1–6·5 (m, 3H), 7·1 (d, 1H); *Anal.* Found: C, 73·15; H, 6·95; Calcd. for $C_{15}H_{18}O_3$: C, 73·17; H, 7·31%.

Microbial strains and growth conditions

The bacterial cultures used were: *Escherichia coli* and *Sal-monella typhi*, *Bacillus cereus* and *Staphylococcus aureus*. The bacterial strains were grown on Nutrient Broth Difco at 37°C and suspended in saline at A_{600}O.D. 0·5 concen-tration.

The fungal cultures used were: *Colletotrichum gloespo-roides*, *Fusarium oxysporum*, *Botrytis cinerea*, *Penicillium italicum*, *Aspergillus flavus* and *Trichothecium roseum*. The fungi were grown on potato dextrose agar for 6 d at 28°C.

Bactericidal activity

The inhibition of the bacterial growth, around a fil ter paper disk (diam. 0·5 cm) impregnated with the pre-established amounts of the assay compound, was measured. The medium was Mueller Hinton Agar (15 ml) in Petri dishes 9 cm in diameter with 1 ml per dish of the bacterial suspension.

The compounds to be tested were dissolved in acetone (1-10 /-lg /-ll-1). Ten /-ll of the dissolu tion were added to the paper disk, and the solvent evaporated. Parallel control

tests, with the pure solvents (10 μl) in the disks, were accomplished. The controls showed no inhibition.

Disks containing 10 μg of chloramphenicol were also used for comparison.

Fungicidal activity

The assay compounds to be tested were incorporated in PDA medium; 5 ml of medium were poured into 5 cm diameter Petri dishes.

The products to be tested were dissolved in acetone (50–500 μg per 100 μl) and added to the medium (100 μl per dish). All the tests were compared with their corre-sponding controls with the pure solvents in the dishes (100 μl per dish).

The content of one Petri dish, with the fungi grown over 6 d at 28°C, in PDA medium, was cut in cylinders (diam. 1 cm). Each cylinder was placed in the centre of another dish containing the same medium with the compound to be tested. The dish was incubated at 28° ; after 6 d it was tested for fungi growth.

Results are given in % of growth (diameter) in compari-son with the control.

As comparative value, activity of imazalil sulphate (500 μg per dish) was included.

RESULTS

The purity of the isolated product was confirmed by ana-lytical HPLC and u.v. spectra at three points on the peak, registered by the Diode Array detector.

By the spectral data, the product was identified as xanth-atin (Fig. 1).

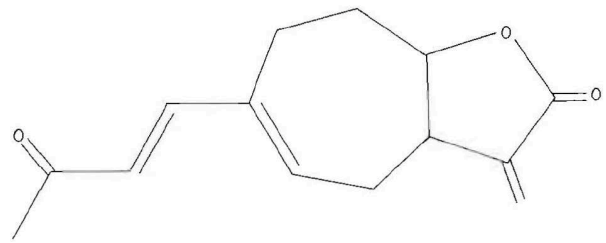

Fig. 1 Xanthatin structure

Xanthatin showed activity against *B. cereus* and *Staph. aureus* but not against *E. coli* and *Salm. typhi* in the bacteri-cidal assay. It also showed high activity against *Colletotrichum gloesporoides and Trichothecium roseum, very low activity against F. oxysporum and Botrytis cinerea but none against Penicillium italicum and Aspergillus flavus, in the fungicidal assay.*

Table 1 Bactericidal and fungicidal activities of xanthatin

	Bactericidal activity*			Fungicidal activity†		
Compound	Dose (μg per disk)	*Bacillus cereus*	*Staphylococcus aureus*	Dose (μg per ml)	*Colletotrichum gloesporoides*	*Trichothecium roseum*
Xanthatin	100	18	18	100	25	20
	50	16	14	50	40	30
	25	15	13	25	60	60
	10	12	10	10	85	75
Chloramphenicol	10	17	14			
Imazalil sulphate				100	5	20

* Bactericidal activities are given in mm of growth inhibition around the disks. Mean of three tests; maximum range ± 2 mm.

† Fungicidal activities are given in % of growth in comparison with the blank. Mean of three tests; maximum range ± 4.

Blank growth: *C. gloesporoides*, 3·9 cm; *T. roseum*, 2·0 cm.

Table 1 shows the fungicidal and bactericidal activities of the compound. Chloramphenicol and imazalil sulphate values are also included.

DISCUSSION

Xanthatin was previously isolated from *Xanthium stru-marium* L. (Pashchenko and Pivnenko 1964), *Pul icaria crispa* (Bohlmann *et al.* 1979), *X. orientale* (Bohlmann and Zdero 1981), *X. indicum* (Bohlmann *et al.* 1982), *X. spinosum* (Omar *et al.* 1984) and *X. pungens* (Ahmed *et al.* 1990).

The structure of xanthatin was identified by Deuel and Geissmann (1957) as a product obtained from xanthinin.

Xanthatin was active against the two Gram-positive bac-teria tested but not against the Gram-negative; both bac-teria are common food contaminants.

Xanthatin was less active than chloramphenicol against *Staph. aureus* and *B. subtilis*. At 50 μg per disk it was as active as chloramphenicol at 10 μg per disk, against *Staph. aureus*.

Xanthatin showed lower activity than imazalil sulphate against *C. gloesporoides*, but similar activity against *T. roseum*, at doses of 100 μg ml^{-1}. *Colletotrichum gloespo-roides* and *T. roseum* are phytopathogenic fungi.

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