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Preliminary Evaluation of Antifungal Activity of Xanthoxyline

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SUMMARY. Xanthoxyline, an active constituent isolated from leaves and stems of *Sebastiania schottiana* and *Phyllanthus sellowianus* was found to possess antifungal activity. The disk diffusion method was used for the test. The results showed that xanthoxyline inhibited 69 % of the fungi species like *Candida, Microsporum, Trichophyton, Aspergillus* and *Penicillium,* producing inhibition zones more than 10 mm in diameter. These effects support the popular use of these plants in folk medicine.

RESUMEN. "Evaluación Preliminar de Actividad Antifúngica de la Xantolina". Se encontró que la Xantolina, un principio activo aislado de hojas y tallos de *Sebastiania schottiana* y de *Phyllantus sellowianus*, posee actividad antifúngica. Los resultados demostraron que la Xantoxilina inhibió el 69% de las especies de hongos de los géneros *Candida, Microsporum, Tricophyton, Aspergillus* y *Penicillium,* produciendo zonas de inhibición superiores a los 10 mm de diámetro. Los efectos observados apoyan el uso popular de estas plantas en medicina popular.

INTRODUCTION

2-hydroxy-4,6 dimethoxyacetophenone (xanthoxyline) is an active constituent present in leaves and stems of *Sebastiania schottiana* and *Phyllanthus sellowianus* (Euphorbiaceae), two abundant plants of the southern region of Brazil, used in folk medicine for the treatment of kidney disease and intestinal infections, hepatitis, dysentery, etc. [1-3]

In previous studies, we have demonstrated that xanthoxyline exhibits potent antispasmodic activity in several pharmacological *in vitro* models [4] and antibacterial effect against some bacteria frequently found in the urinary tract [5].

Hertmann and Nienhaus [6] reported the isolation of this compound from *Citrus limon* and its action against two plant pathogenic fungi: *Phytophthora citrophthora* and *Hendersonula toruloidea.*

KEY WORDS: *Candida, Microsporum, Trichophyton, Aspergillus*, Xanthoxyline, Antifungal activity.
PALABRAS CLAVE: Candida, Microsporum, Trichophyton, Aspergillus, Xantonina, Actividad antifúngica.

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In the present work, we have investigated the preliminary action of xanthoxyline against several yeasts and mycelial fungi that cause human infections[7,8] using the diffusion method on solid media as previously described [9].

EXPERIMENTAL

Plant material

Leaves and stems of *S. schottiana* Muell. Arg. and *Phyllanthus sellowianus* were collected from the course of the Itajaí river near Apiuna in the State of Santa Catarina, Brazil and classified by Dr. Ademir A. Reis (Departamento de Botânica, Universidade Federal de Santa Catarina). The vouchers were deposited in the Herbarium FLOR (S.s - N° 5397, P.s- N° 2757).

Isolation of xanthoxyline

Xanthoxyline was isolated from leaves and stems of extracts of *S. schottiana* and *P. sellowianus* according to the previously described method [3,10].

Assay for antifungal activity

The microrganisms used included *R. rubra* (ICB-36), *Candida albicans* (FCF-243), *C. albicans* (ICB-12), *C. tropicalis* (FCF-163), *Microsporum canis* (LM-003), *M. canis* (72T), *Trichophyton rubrum* (54T), *T. rubrum* (45T), *T. mentagrophytes* (82T), *Epidermophyton floccosum* (27T), *Aspergillus parasiticus* (NRRL-2999), *A. flavus* (FCF-29) and *Penicillium* (FCP-281), cultivated on Sabourand dextrose agar (DIFCO). The assays were accomplished by diffusion method in solid media. Suspensions of the cultures in 0.85% physiological saline sterile solution standardized with number 0.5 of the Mc Farland scale (106 UFC) and with 90% T (530 nm) were made. From these suspensions, 1 ml was transferred to the sterile Petri plates and soon after 21 ml of the fused solid media were added at 45-50o C, carefully homogenized. After solidification, the compound solutions in dimethylsulfoxide (50 l) were introduced into preformed cavities. A control of the microrganisms and standard ketoconazole (500 g/ml) was made. The assays with yeasts and mycelial fungi were incubated, respectively, at 37 °C for 24-48 h and at room temperature (28-30oC) for 10-14 days [11].

RESULTS AND DISCUSSION

The results of antifungal activity of xanthoxyline are reported in Table 1. In the maximal concentration employed (500 ug/ml), xanthoxyline was active against fungi, especifically against *C. albicans* FCF-243, *C. albicans* ICB-12, *M. canis* LM-003, *M. canis* 72T, *T. rubrum* 45T, *A. parasiticus*, *A. flavus* and *Penicillium*. Inhibition potential as measured by the inhibition zone halo was respectively 12, 20, 15, 15, 12, 13, 10, 12 and 12 mm. Zero values for inhibition halos were obtained for xanthoxyline in the presence of *R. rubra, C. tropicalis, T. mentagrophytes* and *E. floccosum*. In the concentration of 125 µg/ml, this compound exhibited weak antifungal activity against *T. rubrum* 54T, *T. rubrum* 45T and *A. flavus*. Control experiments were carried out involving the same microrganisms and the same solvent.

Fungi	Inhibition Zone (mm) Concentration of xanthoxyline (µg/ml) 500	250	125	Growth control of test strains	Susceptibility control with ketoconazole
R. Rubra	00	00	00	+	20
C.albicans FCF-243	12	10	00	+	20
C. albicans IBC-12	20	16	12	+	22
C. tropicalis	00	00	00	+	21
M. canis LM-003	15	12	10	+	18
M. canis 72T	15	10	08	+	20
T. rubrum 54T	12	10	08	+	22
T. rubrum 45T	13	11	06	+	21
T. mentagrophytes	00	00	00	+	22
E. Floccosum	00	00	00	+	20
A. parasiticus	10	08	00	+	23
A. flavus	12	11	10	+	22
Penicillium	12	08	07	+	30

Table 1. Antifungal activity of xanthoxyline. The results are expressed as average inhibition zone diameter (mm) in diffusion assays.

These results indicate that xanthoxyline, an important active compound isolated from extracts of *S. schottiana and P. sellowianus,* besides its antibacterial activity [5], possesses antifungal activity against fungi isolated from human infections, in agreement with previous studies [6].

The results show that 69% of the tested fungi presented high sensibility to the xanthoxyline at 500 g/ml. They were equivalent to the results published in the literature using difusion method [12-14]. On the other hand, some xanthoxyline derivatives, such as xanthoxyline methylated, acetylated and benzylated, obtained as previously described [15,16] were inactive against all microrganisms tested, suggesting that the hydroxyl group contributes, at least in part, to the antifungal activity exhibited by xanthoxyline. The studies for determination of Minimal Inhibitory Concentration (MIC) of xanthoxyline and its derivatives are in progress in our laboratories.

In conclusion, the antifungal activity of xanthoxyline supports the popular use of these plants in traditional medicine against some infectious diseases caused by fungi.

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