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Antifungal effects of secondary metabolites isolated from marine organisms collected from the Tunisian coast

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ABSTRACT

Phallusides 1,2,3 *(1)*, Fasciculatin *(2)*, Acanthelline *(3)*, Axisonitrile *(4)*, Oroïdin *(5)* and the Novel bromopyrolimidazolic compound Axinellizine *(6)* were evaluated for their antifungal effects against several phytopatogenic fungi and were found to possess considerable activities. Insecticidal effect of only Acanthellin *(3)* against the major pest of stored products *Tribolium confusum Duv* has been carried out using direct contact application method showing a significant inhibitory effect of the test material on the *T. confusum Duv* larvae growth. Forty-five percent mortality of the adults was achieved 8 days after treatment.

RÉSUMÉ

Phallusides 1,2,3 *(1)*, Fasciculatin *(2)*, Acanthelline *(3)*, Axisonitrile *(4)*, Oroïdin *(5)* et le nouveau sel d'alkaloïde Axinellizine *(6)* ont été isolés et valorisés par leurs effets antifongiques contre plusieurs souches de champignons phytopatogènes. L'effet anti-insecte de l'acanthelline *(3)* contre le parasite majeur de produits stockés *Tribolium confusum Duv* a été testé en se basant sur la méthode de contact direct montrant un effet inhibiteur significatif de la croissance des larves du *T. confusum Duv*. Une mortalité de 45 % des adultes a été observée, huit jours après traitement.

1. Introduction

Marine sponges and tunicates are known as rich sources of novel microorganisms showing a vast array of biological activities [1,2], many of which can be used for drug development. Continuing our search of new bioactive compounds from plants and marine organisms collected from the Tunisian coast [3–11], we have isolated from ethyl ether extracts four marine organisms: the glycosphingolipids phallusides 1-3 *(1)*, the furanosesquiterpene Fasciculatin *(2)* Acanthellin *(3)*, Axisonitrile *(4)*, Oroïdin *(5)* and a new bromopyrrole derivative named Axinellizine *(6)* (Fig. 1). Fasciculatin, previously isolated from *Ircinia fasciculata*, was reported to have a moderate cytotoxicity and inhibition of lymphocyte proliferation [12]. Preliminary work on Acanthellin and Axisonitrile, indicated their utility as antimalarial drugs [13]. In this study, we report the investigation of the effects of the bioactive secondary metabolites indicated above towards five pathogenic fungi as well as insecticidal activity of Acanthellin against *Tribolium confusum Duv* larvae.

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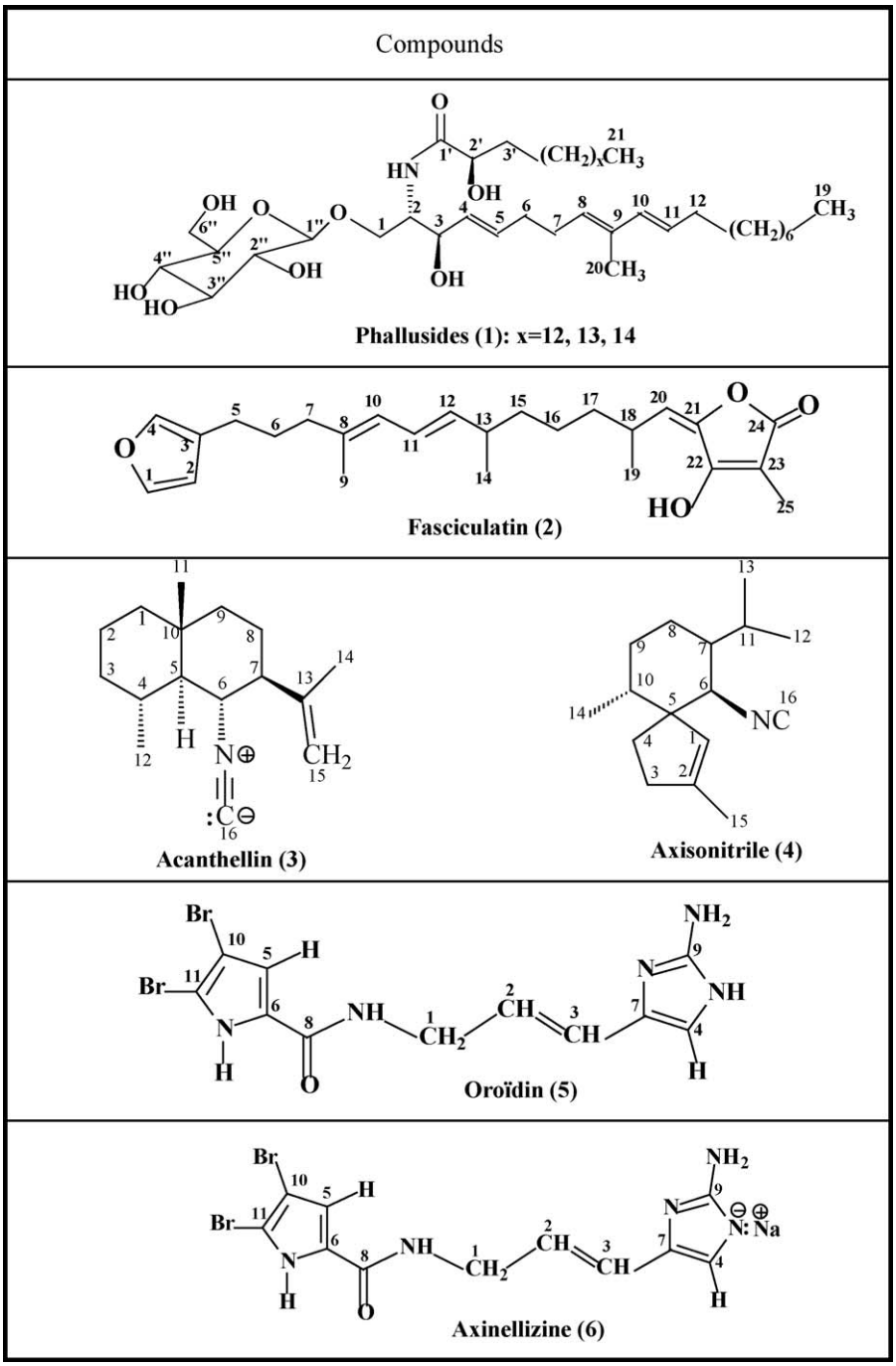

Fig. 1. Natural substances evaluated for their antifungal effects.

2. Material and methods

2.1. Animal material

The tunicate *Sidnyum turbinatum* (polyclinidae family, ascidiaceae order) and the sponges *Ircinia variabilis*, *Acanthella acuta* and *Axinella damicornis* (Axinellidae) were collected by hand at depths of 10 m, 18 m and 25 m, from sidi Elghdamssi island in Monastir region (Center East coast of Tunisia) in August 2003 and were stored in a freezer (−20 °C) until extraction. Voucher specimens were deposited in the Laboratorio de Sostanze Naturali Consiglio Nazionale delle Ricerche, Instituto di Chimica Biomolecolare Pozzuoli, Napolie, Italy.

2.2. Extraction and isolation

2.2.1. Sidnyum turbinatum

The frozen organism (33.9 g dry weight after extraction) was exhaustively extracted with acetone in an ultrasound apparatus at room temperature and the extract was filtered and concentrated by rotary evaporation. The resulting water residue was extracted subsequently with diethyl ether and butanol yielding 560 and 60 mg, respectively.

2.2.2. Ircinia variabilis, Acanthella acuta and Axinella damicornis

Ethyl ether extracts from three sponges were prepared in the same way as previously described for the tunicate.

2.3. Fungitoxicity assay

2.3.1. Fungal isolate

Five phytopatogenic fungal species were used for the antifungal testing, namely: *Fusarium oxysporum f. sp. Niveum* (Boughalleb and El Mahjoub 2005) [14], *Fusarium solani f. sp.* Cucurbitae (Boughalleb et al. 2005) [15], *Pythium ultimum* and *Alternaria solani*. Samples of each isolated and identified fungi were deposited in the collection bank at the plant pathology laboratory *institut supérieur agronomique de Chott Mériem, université du centre*, Sousse, Tunisia.

2.3.2. Effect on mycelial growth of fungi

Fungitoxicity of the indicated pure natural products was assessed using the disk diffusion method [16,17]. Fungal broth culture aliquots were added to potato dextrose agar medium (PDA) and distributed uniformly in 9 cm Petri dishes. Once the substrate solidified, four Wattman disks were placed in Petri dishes. Each one was moistened with 20 μg of pure compound dissolved in the appropriate solvent at a concentration of 1 mg mL^{-1}.

A control was prepared by moistening a small disk with the same volume of SDW + Tween 80 (10%). Inhibition zone diameters around the disks were measured after cultivation at 25 °C for eight days. Each experiment was performed in triplicate.

2.4. Insecticidal assay

2.4.1. Insecticidal activity

Three millimeter long larvae of the *T. confusum* insect were obtained from same-age cultures. All insects were fed with white wheat flour and beer yeast (95:5) and incubated at a constant temperature of 32 °C, in darkness. Parent adults were provided by the laboratory of entomology reserve, *école supérieure d'horticulture et d'élevage*, Chott Mériem, Sousse, Tunisia.

2.4.2. Bioassays

Acanthellin was tested for its toxic and larval growth inhibition effects. In fact, 5 μL of the compound (solution of 10 mg/mL) were mixed with discs weighing about 20 mg and having 1 cm diameter, dried at 32 °C during 24 h and then weighed before being afforded to larvae inside 4 cm diameter glass Petri dishes. Each test is done in three replications for 10 insects. A control was prepared in the same way using only the dissolving solvent. Larval growth inhibition was obtained by measuring length growth, recorded 16 days after treatment compared with the control. Percentage of alive larvae (CI) was calculated using the following formula:

$$\mathrm{CI} = \frac{\text{Larvae length 16 days after treatment} - \text{control larvae length}}{\text{Larvae length 16 days after treatment}}$$

In the precedent described Petri dishes, mortality was determined every four days during the essay (16 days) [10].

3. Results and discussion

Phallusides *(1),* Fasciculatin *(2),* Acanthellin *(3),* Axisonitrile 3 *(4),* Oroïdin *(5)* and the Novel Alcaloïd *(6)* were isolated from diethylether extracts of marine organisms collected from the Tunisian coast. Structural elucidation of all the compounds was established using 1D and 2D NMR spectra [9]. In the present work, we report the evaluation of their antifungal effects against the filamentous fungi: *F. oxysporum f. sp. Niveum*, *F. solani f. sp.* Cucurbitae, *P. ultimum* and *A. solani* at a concentration of 20 μg/disc as well as effects of Acanthellin on larvae of the stored product pest *T. confusum Duv* (Table 1).

3.1. Antifungal activity of six pure compounds

Antifungal evaluation showed some interesting results and the inhibition zones of fungal growth are presented in Table 2. Thus, Oroïdin, Phallusides and the new Axinellizine have shown antifungal activities against *A. solani*, Axisonitrile was found to be the most active compound inhibiting the growth of *Fusarium oxysporom*, *Fusarium solani* and *A. solani* fungi. None of the tested compounds exhibited activity towards *Pythium ultimum*.

Table 1
Fungi used to evaluate activity of the pure indicated compounds.

Fungus	Origin	Plant part sampled	Location	Collection date
F. Oxysporum f. sp. niveum	Watermelon	Roots	Skhira	23/05/2001
F. Solani f. sp. cucurbitae	Watermelon	Roots	Skhira	23/05/2001
Pythium ultimum	Watermelon	Roots and stems	Regueb	23/05/2001
Alternaria solani	Tomato	Leaves	Chott Mariem	12/11/2002

Table 2
Antifungal test results.

Organism	Phallusides	Fasciculatine	Acanthelline	Axisonitrile	Oroïdin	Axinellizine
Filamentous fungi						
Fusarium oxysporum f. sp. Niveum	–	*(22.5)*	*(23)*	*(13.5)*	–	–
Fusarium solani f. sp	–	–	–	*(15)*	–	–
Pythium ultimum	–	–	–	–	–	–
Alternaria solani	*(25)*	–	–	*(16)*	*(16)*	*(11.5)*

(): Fungal growth inhibition zones in mm.

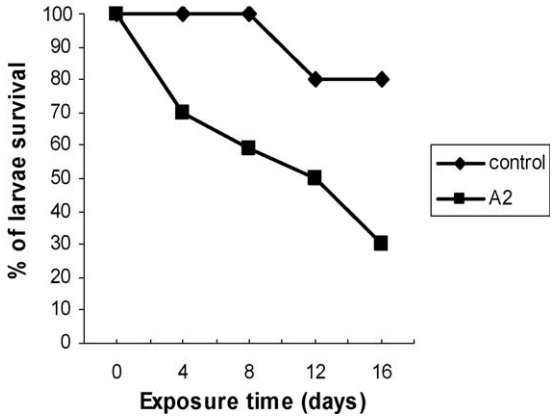

Fig. 2. Toxicity effect of Acanthellin on *Tribolium confusum* larvae.

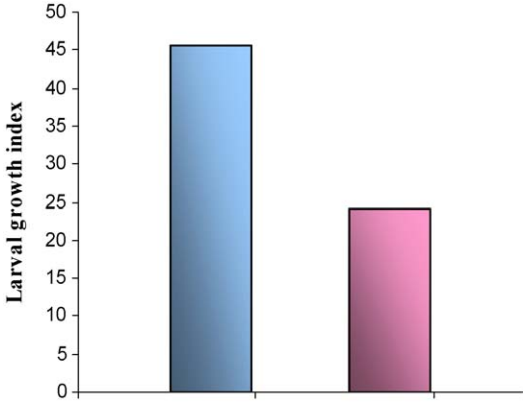

Fig. 3. Effect of Acanthellin on *Tribolium confusum* larval growth.

3.2. Insecticidal activity of Acanthellin (3)

3.2.1. Grain contact toxicity

The percentage of alive larvae were determined each 4 days of insect exposure, indicated that a dose of 0.025 mg/grain of the oil was able to induce 35 and 45% mortality of insects within 4 days and 8 days of exposure, respectively (Fig. 2).

3.2.2. Insect growth inhibition bioassay

The evolution comparison of *T. confusum* larval growth index of treated discs with that of the control shows a larval growth inhibition of Acanthellin on *T. confusum* larvae (Fig. 3), the pure compound was tested at a concentration of 10 mg mL^{-1}.

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