Antifungal Activity of (+)-Curcuphenol, a Metabolite from the Marine Sponge *Didiscus oxeata*

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Abstract: The antifungal activity of the sesquiterpenoids (+)-curcuphenol and (+)-curcudiol isolated from the Caribbean sponge *Didiscus oxeata* was evaluated against several filamentous fungi.

Keywords: Porifera, *Didiscus oxeata*, antifungal, curcuphenol, curcudiol.

Introduction

In the marine environment, sponges (Porifera) are one of the richest sources of biological active secondary metabolites [1]. As part of our general interest in the isolation and characterization of bioactive metabolites from sponges with potential pharmaceutical and agrochemical applications the sesquiterpenoids (+)-curcuphenol (1) and (+)-curcudiol (2) were isolated from the antifungal extract of the Curacao marine sponge *Didiscus oxeata*. 
(+)-Curcuphenol and (+)-curcudiol were previously isolated from several marine sponges [2-8] and were described as ichthyotoxic and antifouling metabolites [3,6]. (+)-Curcuphenol also exhibited antimicrobial activity, antiyeast activity against Candida albicans, cytotoxicity against P-388 murine leukaemia, A-549 lung, HCT-8 colon, and MDAMB mammary cancer cell lines and strongly inhibited the activity of gastric H, K-ATPase [2,4,8].

Although several biological activities were reported for both (+)-curcuphenol and (+)-curcudiol, there are no studies concerning the antifungal activity against filamentous fungi. So the main aim of this study is to evaluate the antifungal activity of both sesquiterpenes against several filamentous fungi.

**Results and Discussion**

The antifungal activity of the sesquiterpenoids (+)-curcuphenol and (+)-curcudiol isolated from the Caribbean sponge Discus oxeata was evaluated against the filamentous fungi Absidia ramosa, Aspergillus niger, Botrytis cinerea, Cladosporium cucumerinum, Fusarium oxysporum, Penicillium expansum, Rhizopus oryzae, and Trichoderma harzianum at a concentration of 200 µg/disc. (+)-Curcuphenol was also tested, at the same concentration, against the fungi Fusarium solani, Nodulosporium sp., Phytophthora sp., Trichoderma sp., T. koningii, T. lignorum, T. virgatum and Trichophyton mentagrophytes.

The antifungal activity towards several wood surface contaminant fungi was evaluated due the interest of the search for natural compounds for wood protection. Sapstain and mould growth on lumber are serious problems in the forest, pulp and paper industry; although structure damage is minimal to timber, the discolorations are objectionable to buyers. Moreover, the conventional protective solutions are toxic and accumulate in animal tissues [9].

The antidermatophytic effect of (+)-curcuphenol against the human pathogenic fungus Trichophyton mentagrophytes was also evaluated, as dermatophytoes are the most common form of fungal infections found in most countries, affecting skin, hair and nails [10].

While (+)-curcuphenol inhibited the growth of the fungi A. ramosa, A. niger, B. cinerea, C. cucumerinum, F. oxysporum, P. expansum, R. oryzae, T. Harzianum, T. Koningii and T. mentagrophytes, (+)-curcudiol only partially inhibited the growth of A. ramosa (table 1). None of
the tested compounds exhibited activity towards *Fusarium solani*, *Nodulosporium* sp., *Phytophthora* sp., *Trichoderma* sp., *T. lignorum* and *T. virgatum*.

Light microscopy observations of the inhibition zone of fungal growth treated with (+)-curcuphenol showed the presence of non-germinated cells and spores presenting morphological alterations, such as reduced germ-tube and multiple germ-tube formation.

**Table 1.** Antifungal test results.

| Organism                  | (+)-Curcuphenol | Carbendazim® | Amphotericin B |
|---------------------------|-----------------|--------------|----------------|
| **Filamentous Fungi**     |                 |              |                |
| *Absidia ramosa*          | 4.5 cm          | 6.0 cm       | -              |
| *Aspergillus niger*       | 1.0 cm          | 3.5 cm       | -              |
| *Botrytis cinera*         | ±               | 2.0 cm       | -              |
| *Cladosporium cucumerinum*| 2.0 cm          | 3.7 cm       | -              |
| *Fusarium oxysporum*      | ±               | 2.0 cm       | -              |
| *Penicillium expansum*    | ±               | 4.0 cm       | -              |
| *Rhizopus oryzae*         | 1.6 cm          | 3.5 cm       | -              |
| *Trichoderma harzianum*   | ±               | 4.0 cm       | -              |
| *Trichoderma koningii*    | 1.0 cm          | 4.2 cm       | -              |
| *Trichophyton mentagrophytes* | 1.0 cm      | -            | 5.0 cm         |

± partial inhibition of fungal growth, - not tested

**Conclusion**

(+)-Curcuphenol inhibited the growth of several phytopathogenic and wood surface contaminant fungi as well as the human pathogenic fungus *T. mentagrophytes*.

Although (+)-curcuphenol showed less efficacy than the commercial products (Amphotericin B, Carbendazim®) it should be worthily studied as these fungicides are toxic and several resistant strains were detected for Carbendazim®.

The results obtained show that (+)-curcuphenol possesses a broad antifungal spectrum when compared with (+)-curculiol that only partially inhibited the growth of *A. ramosa*, suggesting that the double bond in the aliphatic chain is required for antifungal activity. Thus, chemical modifications of this group could be a tool in the search for new and safer antifungal agents.
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Experimental

Biological material

The marine sponge Didiscus oxeata was collected in June 1998 at a depth of 30 m on the reef of Curacao (Netherlands Antilles) by M.J. De Kluijver. The specimen was massive-amorphous, with characteristic sinuous grooves at the surface. The surface skeleton is a detachable crust of partly tangentially arranged megasccleres. The skeleton of the interior is largely confused, with many loose megasccleres and microsccleres randomly arranged, but thick ascending spicule tracts may be distinguished. The spicules are oxeas in a large size range, divisible in at least two size categories: 546-1220 x 11-30 μm and 228-366 x 4-7 μm. Microscleres are characteristic didiscorhabads, 60-74 μm long. The specimen conformed in all aspects to previous descriptions. A voucher is kept in the collections of the Zoological Museum Amsterdam under reg. nr. POR.14326.

Isolation of sesquiterpenes

The sponge was immersed in MeOH immediately after collection and kept at −20°C until extraction. After filtration the sponge was cut into small pieces and successive washed (3x 0.5 L to 1.5 L) with methanol and dichloromethane at room temperature. Evaporation of the organic solvents and the residual sea water yielded the crude extract (18 g). The dichloromethane soluble fraction (4 g) of the crude methanolic extract was subjected to flash column chromatography on Si gel (MN Kieselgel 60 mesh) using a step gradient of n-hexane/EtOAc. The fractions eluting with 10% EtOAc in n-hexane gave (+)-curcuphenol (2 g, 11% methanolic extract) while 30% EtOAc gave (+)-curcudiol (0.2 g, 0.1% methanolic extract). These sesquiterpenes were identified by comparison of their spectroscopic data (NMR, IV, MS, [α]D) with those reported for natural and synthetic compounds [2,11,12] and by HMBC and HMQC.

Biological activity

The wood surface contaminant fungi tested (Fusarium solani, Nodulosporium spp., Phytophthora spp., Trichoderma sp. and Trichoderma virgatum) were isolated from cork. All the other fungi tested were obtained from the Culture Collection of Industrial Microorganisms (CCMI), INETI. The disc-diffusion assay according to Amade et al. [13] was used as a screening test for antifungal activity. The products dissolved in dimethyl sulphoxide (DMSO) were applied on the
discs at 200 µg/disc, and set on the plates. Amphotericin B (Sigma) was used as positive control for *T. mentagrophytes* whereas Carbendazim® was used for all the other fungi. These standards were tested at 100 µg/disc. The fungal inhibition zone around the disc was observed by light microscopy (x 320).

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Sample Availability: Samples are available from the authors.

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