Inhibitory activity of stilbenes against filamentous fungi

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Abstract

Stilbenoids (resveratrol and itsderivatives) are secondary metabolites produced by plants as defence mechanism to microbial infection. These compounds are known for their anti-inflammatory action and health benefits in preventing a wide range of disorders (e.g. cancer and cardiovascular diseases). However, their antimicrobial properties are less investigated. A series of 8 stilbenoid compounds were synthesized and their antifungal activity against 19 wild strains of filamentous fungi and yeasts (isolated from the environment and food) was tested in vitro. Using an agar diffusion assay, compounds were tested at the concentration of 100 μg/ml on filamentous fungi and yeasts at 106 CFU/ml. The results showed that tested derivatives possess moderate antifungal activity: in particular, monomeric stilbenoids 3’-hydroxy-petrosilbene and piceatannol, and dimeric stilbenoids (+)-trans-δ-viniferin and pallidol were active against mycotoxicogenic fungi.

Introduction

Stilbenoids are among the most important classes of phytoalexins produced by 72 plant species belonging to 31 genera, with a particular emphasis on stilbenes from the Vitaceae (Valletta et al., 2021; Jaillon et al., 2007). Over 400 different stilbenoids are currently known (El Khawand et al., 2018), mostly derived from trans-resveratrol (3,5,4'-trihydroxy-trans-stilbene), although different structures can be found in some plant families (Chong et al., 2009).

Stilbenoids are mainly involved in constitutive and inducible protection of the plant against biotic (phytopathogenic microorganisms and herbivores) and abiotic (e.g. UV radiation and tropospheric ozone) stresses (Chong et al., 2009; Jeandet et al., 2010), due to their antibiotic and antioxidant activities (Valletta et al., 2021). Among stilbenoids, resveratrol is the most investigated and its health benefits as anti-inflammatory, anticancer, estrogenic, neuroprotective, cardiac protective, anti-atherosclerotic, anti-aging, anti-diabetic, anti-osteoporosis and anti-obesity agent have been documented in several preclinical (in vitro/in vivo) studies (Valletta et al., 2021).

In the last decades, the interest in resveratrol has been amplified since its presence in wine was indicated as a possible explanation for the “French paradox”, i.e. the reduced risk of cardiovascular disease associated to moderate, regular consumption of red wine at main meals for people consuming a diet rich in saturated fats (Catalgol et al., 2012; Jeandet et al., 2021).

Currently, resveratrol has been exploited in pharmaceutical, cosmetic and food industries. In this latter area, possible applications as antimicrobial agent in the conservation of food are under evaluation (Oh et al., 2018; Ma et al., 2018). The in vitro antimicrobial properties of resveratrol are widely known (Albert et al., 2011; Chalal et al., 2014), although the mechanism of action on pathogenic and food-borne bacteria, fungi and yeasts is not yet fully understood (Lee et al., 2015).

Resveratrol exhibited inhibitory activity against yeasts and filamentous fungi (Seppanen et al., 2019) such as Botrytis cinerea (Adrian et al., 1993; Hoss et al., 1990; Paul et al., 1998; Sarig et al., 1997), Rhizopus stolonifer (Valletta et al., 2021), Phomopsis viticola (Hoss et al., 1990), Fusarium nivale (Bala et al., 1999), Saccharomyces cerevisiae, Penicillium expansum and Aspergillus niger (Seppanen et al., 2019; Valletta et al., 2021; Adrian et al., 1997). Conversely, Weber et al. (Weber et al., 2011), showed that resveratrol was not active on several Candida species (Weber et al., 2011; Shevelev et al., 2018), though recent reports suggested that resveratrol derivatives could inhibit Candida species as well (Lee et al., 2015; Shevelev et al., 2020).

Recently, other monomeric and oligomeric stilbenoid derivatives including pterostilbene, pinosylvin, piceatannol and viniferins have attracted the attention of researchers. Pterostilbene and trans-ε-viniferin have been found to be 5-fold more active than resveratrol as antifungal agents, indicating their high antimicrobial potential (Chalal et al., 2014; Houillé et al., 2014). These compounds were tested in vitro against B. cinerea, and pterostilbene, in particular, inhibited conidial germination and in vitro mycelium growth more effectively than resveratrol, indicating that methylation of -OH groups could have a role in the antifungal activity. Schouten et al. (Schouten et al., 2002) demonstrated that resveratrol, though not toxic to B. cinerea, is converted into a fungitoxic derivative by a specific fungal laccase (Caruso et al., 2011).

A possible mode of action of stilbenoids may involve membrane peroxidation (Lee et al., 2017). Pterostilbene caused destruction of the endoplasmic reticulum, and the nuclear and mitochondrial membranes in B. cinerea dormant conidia. A positive correlation between antifungal activity of natural and synthetic stilbenoids and their hydrophobicity was found, suggesting that pterostilbene is more active than the less hydrophobic resveratrol due to its increased diffusion through the cytoplasmic membrane (Caruso et al., 2011).

Owing to the need of alternative compounds to control food spoilage and contamination, the aim of this study was to assay in vitro the antimicrobial activity of a small collection of stilbenoid monomers and dimers against filamentous fungi and yeasts isolated from the environment and food of animal origin.

Materials and methods

Sixteen fungal strains, Alternaria alter-
nata, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Byssochlamys nivea, Botrytis cinerea, Fusarium graminearum

Fusarium verticillioides, Geotrichum candidum, Macor circinelloides, Penicillium expansum

Penicillium italicum, Penicillium roqueforti, Rhizopus nigricans and 3 yeast strains Candida albicans, Candida parapsilosis, Malassetia pachydermatis were grown to assess the inhibitory activity of 4 monomeric stilbenoids (i.e. resveratrol, piceatannol, pterostilbene, 3'-hydroxy-pterostilbene) and 4 dimers (i.e. (±)-trans-δ-viniferin, (±)-trans-ε-viniferin, pallidol (±)-pterostilbene-trans-dihydrodimer) (Figure 1). Stilbenoids were dissolved in DMSO to obtain a stock solution of 10 mg/ml. Wild strains were used instead of ATCC strain because more aggressive and less tamed to tested compounds.

The bioassay was carried out on Sabouraud agar medium, by paper disk diffusion assay. From microbanks, all the mycetes were first suspended into M2 broth and incubated for 5 days at 25°C. A spectrophotometer was used to adjust the final cell concentration at 10⁴ cfu/ml by reading the OD at 600 nm.

Then, 100 µl of the microbial suspensions were spread on Sabouraud agar medium. The 6-mm-diameter, sterile disks impregnated with 10 µl of stilbenoid compounds at the concentration of 100 µg/ml were placed on the inoculated agar. The inoculated plates were incubated at 25°C for 7 days. As positive controls, cyclopiroxolamine and tebuconazole (1 µg/disk) were used. Inhibitory activity was determined by measuring the zone of inhibition.

Results

The inhibitory activity of the tested stilbenoids varied according to the different strains. Piceatannol showed an antifungal activity against P. roqueforti, A. flavus, P. italicum, A. terreus, G. candidum, F. verticillioides and C. parapsilosis. 3'-Hydroxy-pterostilbene affected the growth of C. parapsilosis, P. roqueforti, A. ochraceus, A. flavus and C. albicans. Pallidol inhibited the growth of P. italicum and F. verticillioides. Finally, (±)-trans-δ-viniferin and (±)-pterostilbene-trans-dihydrodimer were effective only on one strain, A. flavus and F. verticillioides, respectively. Overall, A. flavus was sensitive to piceatannol, δ-viniferin and 3'-hydroxy-pterostilbene; F. verticillioides was sensitive to (±)-pterostilbene-trans-dihydrodimer, pallidol and piceatannol; P. roqueforti was sensitive to piceatannol and 3'-hydroxy-pterostilbene; P. italicum was sensitive to pallidol and piceatannol and C. parapsilosis was sensitive to 3'-hydroxy-pterostilbene and piceatannol; A. terreus and G. candidum were sensitive to piceatannol; A. ochraceus and C. albicans were sensitive to 3'-hydroxy-pterostilbene (Table 1). Values are mean inhibition zone (mm) ± S.D. of three replicates; positive controls (tebuconazole and cyclopiroxolamine) > 20 mm.

Discussion

Results indicated that the antifungal activity of selected stilbenoids was strictly related to the chemical structure of the
Table 1. Inhibitory activity of selected stilbenoids against filamentous fungi and yeasts.

| Fungi and yeasts                  | piceatannol | 3'-hydroxy-petrostilbene | (±)-trans-δ-viniferin | (±)-peterostilbene-trans-dihydromer | pallidol |
|----------------------------------|-------------|--------------------------|----------------------|-------------------------------------|---------|
| *Alternaria alternata*           | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Aspergillus flavus*             | 20 ± 0.0    | 3 ± 0.0                  | 5 ± 0.2              | n.i.                                | n.i.    |
| *Aspergillus niger*              | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Aspergillus ochraceus*          | n.i.        | 5 ± 0.1                  | n.i.                 | n.i.                                | n.i.    |
| *Aspergillus terreus*            | 5 ± 0.1     | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Byssoschlamys nivea*            | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Botrytis cinerea*               | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Fusarium graminearum*           | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Fusarium verticillioides*       | 2 ± 0.2     | n.i.                     | 10 ± 0.0             | 5 ± 0.1                             | n.i.    |
| *Geotrichum candidum*            | 5 ± 0.1     | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Mucor circinelloides*           | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Penicillium expansum*           | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Penicillium italicum*           | n.i.        | n.i.                     | n.i.                 | n.i.                                | 20 ± 0.2|
| *Penicillium roqueforti*         | 20 ± 0.1    | 6 ± 0.2                  | n.i.                 | n.i.                                | n.i.    |
| *Rhizopus nigricans*             | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |
| *Candida albicans*               | n.i.        | 2 ± 0.2                  | n.i.                 | n.i.                                | n.i.    |
| *Candida parapsilosis*           | 2 ± 0.0     | 10 ± 0.1                 | n.i.                 | n.i.                                | n.i.    |
| *Malassezia pachydermatis*       | n.i.        | n.i.                     | n.i.                 | n.i.                                | n.i.    |

n.i.: no inhibition zone formation.

Conclusions

In this study, some stilbene derivatives were assayed and exhibited a weak to moderate antifungal activity against a panel of fungal strains and yeasts. Monomeric stilbenoids, in particular 3'-hydroxy-petrostilbene and piceatannol, and dimeric stilbenoids (±)-trans-δ-viniferin and pallidol were active against mycotoxigenic fungi, thus showing a promising potential as food preservatives. In further studies, these compounds could be tested at higher concentrations, in combination with other natural compounds or low-dose conventional antimicrobials. In this view, stilbenoids could contribute to reduce the risk of selecting resistant fungal strains, a relevant issue due to the global burden of antimicrobial resistance. Finally, the efficacy of these compounds could be improved by formulation, including their functionalization with nanostructures or incorporation in active packaging.

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