Italian Journal of Food Safety 2021; volume 10:8461

Inhibitory activity of stilbenes against filamentous fungi

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Abstract

Stilbenoids (resveratrol and its derivatives) 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 10^4 CFU/ml. The results showed that tested derivatives possess moderate antifungal activity: in particular, monomeric stilbenoids 3'-hydroxy-pterostilbene and piceatannol, and dimeric stilbenoids (±)-trans-δ-viniferin and pallidol were active against mycotoxigenic 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, cardio 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.*, 1997; 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-*

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Key words: Stilbenes, Resveratrol, Antimicrobial activity, Food-borne fungi.

Acknowledgments: The work of Giorgia Catinella has been partially funded by Fondazione F. lli Confalonieri (PhD Scholarship).

Contributions: The authors contributed equally.

Conflict of interest: The authors declared no potential conflict of interest.

Received for publication: 31 July 2020.
Revision received: 15 November 2020.
Accepted for publication: 18 November 2020.

Italian Journal of Food Safety 2021; 10:8461
doi:10.4081/ijfs.2021.8461

nata, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Byssochlamys nivea, Botrytis cinerea, Fusarium graminearum

Fusarium verticillioides, Geotrichum candidum, Mucor 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 M_2 broth and incubated for 5 days at 25°C. A spectrophotometer was used to adjust the final cell concentration at 10^4 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. verticillioide*. 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

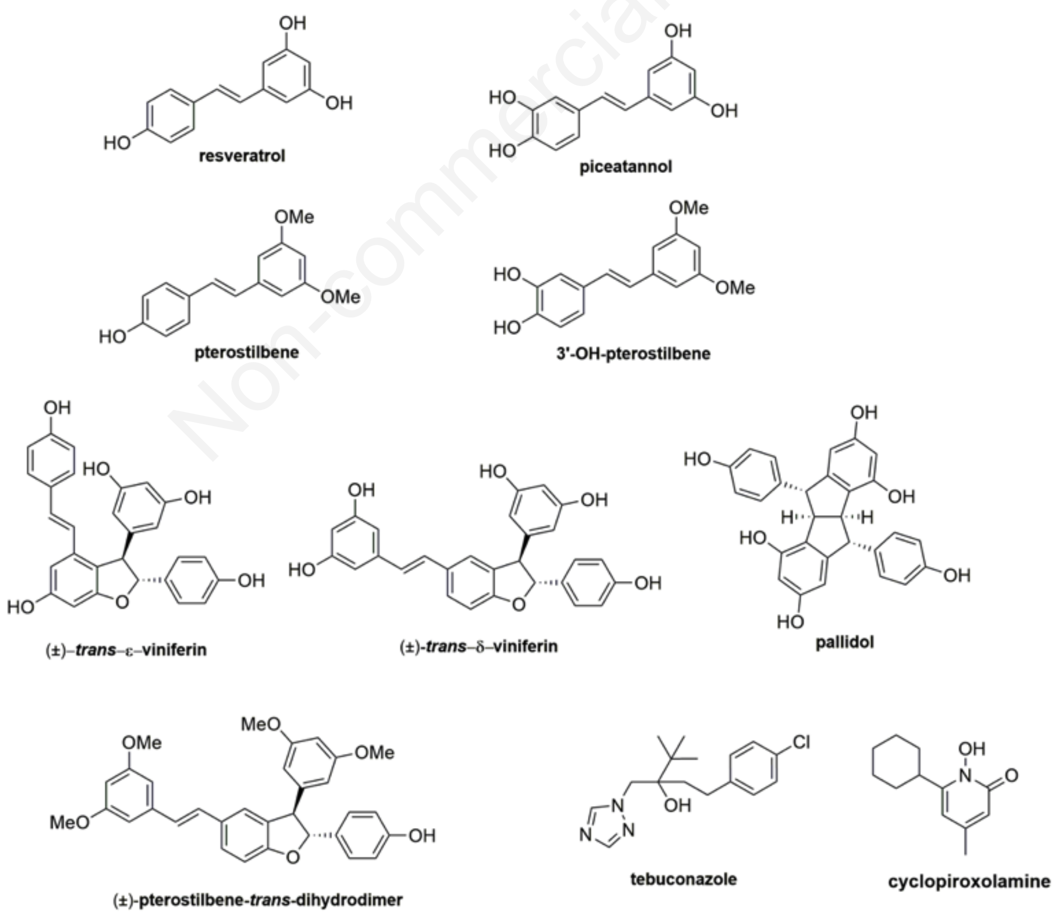

Figure 1. Structure of selected stilbenoids, tebuconazole and cyclopiroxolamine.

Table 1. Inhibitory activity of selected stilbenoids against filamentous fungi and yeasts.

Fungi and yeasts	piceatannol	3'-hydroxy-pterostilbene	(±)-trans-δ-viniferin	(±)-pterostilbene-trans-dihydrodimer	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.
Byssochlamys 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.	n.i.	10 ± 0.0	5 ± 0.1
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.
Malassetia pachydermatis	n.i.	n.i.	n.i.	n.i.	n.i.

n.i.: no inhibition zone formation.

molecule, although it is not possible to define a relationship between the structure of a given compound (number/position of -OH/-OMe groups) and its antimicrobial activity (Chalal *et al.*, 2014). In general, the monomeric species (in particular piceatannol) were more active against the tested fungi compared to the dimeric derivatives. Interestingly, the tested stilbenoids were more active against phytopathogenic fungi (i.e., *Aspergillus* spp., *Penicillium* spp. and *F. verticillioides*). We can speculate that this behaviour could be related to the coevolution between pathogen and host plant (and its phytoalexins). In this sense, phytopathogenic fungi would be more sensitive to the fungitoxic effects of stilbenoids.

Interestingly, on dermatophyte fungi, inhibitory activity has been demonstrated at concentrations of 25-50 μg/ml, while on *Candida albicans* it appeared evident at lower concentrations (10-20 μg/ml) (Vestergaard *et al.*, 2019).

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-pterostilbene 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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