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Phytotoxic metabolites from *Stilbocrea macrostoma,* a fungal pathogen of *Quercus brantii* in Iran

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ABSTRACT: Two phytotoxic furan derivatives were isolated, together with the well-known fungal and plant phytotoxin tyrosol, from the culture filtrates of *Stilbocrea macrostoma*. This fungal pathogen isolated from *Quercus brantii* trees induced wood necrosis and decline symptoms on the host plant in Iran. The two furan derivatives, isolated for the first time from *Stilbocrea macrostoma*, were identified by spectroscopic methods (essentially 1D and 2D ^{1}H and ^{13}C NMR and ESIMS spectroscopy) as 5-hydroxymethyl-2-furanldehyde and 2,5-dihydroxymethylfuran. The phytotoxic activity of the three metabolites was evaluated by leaf puncture assay on holm oak (*Quercus ilex* L.) and tomato (*Lycopersicon esculentum* L.) leaves. All compounds induced necrosis on holm oak leaves while very low toxicity was showed against tomato leaves. The two furan derivatives were more toxic than tyrosol and particularly 5-hydroxymethyl-2-furanldehyde was the most phytotoxic compound.

KEYWORDS: *Stilbocrea macrostoma*; oak trees, *Quercus brantii*, phytotoxins, furan derivatives, 5-hydroxymethyl-2-furaldehyde and 2,5-dihyroxymetylfuran

1. Introduction

Quercus is affected by many fungal pathogens which can cause serious diseases such as canker, die-back and decline. Most of these fungi belong to *Diplodia* species and showed to produce a plethora of phytotoxic metabolites probably involved in plant-pathogen interactions (Masi et al., 2018).

Recently, some oak decline-associated fungi were isolated from oak trees (*Quercus brantii*), showing decline and wood necrosis symptoms in the Zagros forest, Iran. These fungi were evaluated for their pathogenicity ability in greenhouse and grown *in vitro* to evaluate their ability to produce phytotoxic secondary metabolites. In a recent study, from the culture filtrates of *Fimetariella rabenhorstii* four phytotoxic metabolites have been isolated and chemically and biologically characterized (Bashiri et al., 2020).

Stilbocrea macrostoma, another virulent fungus was isolated from branches of oak trees (*Quercus brantii*) showing wood necrosis (Figure 1). So far *S. macrostoma* has been reported from 12 different woody plants in New Zealand (https://nt.ars-grin.gov/fungaldatabases) and an unknown host plant in Sri Lanka (Voglmayr and Jaklitsch, 2018). This is the first report of its isolation from *Quercus* trees from Zagros forest, in Paveh, Kermanshah Province.

This manuscript describes the isolation of three metabolites from the culture filtrates of *Stilbocrea macrostoma* and their chemical identification and biological characterization.

2. Results and discussion

The EtOAc extract obtained from the culture filtrates of *S. macrostoma*, showing strong phytotoxicity on host plant leaves, was purified as reported in detail in SI yielding three homogeneous compounds that were identified as 2,5-dihydroxymethylfuran, 5-hydroxymethyl-2-furaldehyde and tyrosol (**1-3**,

Figure 2). They were isolated for the first time from *S. macrostoma* and were identified by comparison of their spectroscopic data (1D ^{1}H and ^{13}C NMR and 2D COSY, HSQC and HMBC NMR and ESI MS analyses for **1** and **2**, ^{1}H and ^{13}C NMR and ESI MS for **3**) with those reported in literature for **1** by Schneider et al. 1996 and Mancilla et al. 2009, for **2** by Dohnal and Kisiel, 1993, Li et al., 2007 and Guo et al. 2016, and for **3** by Cimmino et al. 2017, and Masi et al. 2020).

The structure assigned to 2,5-dihydroxymethylfuran (**1**) was supported by its X-ray crystallographic analysis. One crystalline phase was found, with two different crystal habitus: a rare snow-like habitus and a common rhombe-plate habitus (Figure 3). ORTEP view of 2,5-dihydroxymethylfuran (**1**) molecular structure is reported in Figure 1. The crystal structure, previously reported by Glidewell et al. 1996, was redetermined by us at 173K and details are reported in the SI (Figures S3-S4 and Tables S1-S3).

2,5-Dihydroxymethylfuran (**1**) was isolated for the first time from *Xylaria longipes*, an ascomycete studied for its biotechnological application (Schneider et al. 1996), and it showed antifungal activity against *Nematospora coryli*. Then, it was isolated from *Colletotrichum acutatum* a phytopathogenic fungus inducting anthracnose on a wide range of plants (Mancilla et al. 2009) and *Lacrymaria velutina*, a fungus exhibiting inhibitory activity against nitric oxide synthase (Ju et al. 2010). More recently it was also isolated from *Paecilimyces* sp., a marine filamentous fungus and showed antimicrobial activity (Mosadeghzad et al. 2013).

5-Hydroxymethyl-2-furaldehyde (**2**) was isolated for the first time from the cultures of *Tylophilus felleus*, whose aqueous medium extract stimulates callus growth of *Holarrhena antidysenterica* (Dohnal and Kisiel 2014). Successively, it was also isolated from *Pleurotus ferulae*, an edible fungus and showed nematocidal activity against *Bursaphelenchus xylophilus* and *Pangrellus redivivus* (Li et al. 2007). **2** showed cytotoxic activity against BGC823 when was isolated from *Penicillium chrysogenum* HGQ6, a marine derived fungus (Guo et al. 2016). Recently, it was identified as one of the metabolites produced by *Bacillus subtilis* and showed *Candidida albicans* antibiofilm growth and this effect was concentration dependent (Subramenium et al. 2018).

Tyrosol (**3**) is a well-known fungal phytotoxic metabolite produced by several plant pathogens (Cimmino et al., 2017; 2018). Recently it was isolated from the culture filtrates of *Colletotrichum lupini*, the causal agent of lupin (*Lupinus albus* L.) anthracnose, a destructive seed-borne disease affecting stems and pods (Masi et al., 2020).

Compounds **1**-**3** were screened for phytotoxic activity using the leaf puncture assay on holm oak (*Quercus ilex* L.) and tomato (*Lycopersicon esculentum* L.) leaves, at a concentration of 5.0, 1.0 and 0.1 mM. All of them showed stronger phytotoxicity on oak at the three concentrations used while on tomato leaves were weakly active (Table S4). In particular, on holm oak leaves compounds **1** and

2 caused significant necrosis at two concentrations (5.0 and 1.0 mM) (Figure S5). However, the phytotoxic effects were reduced for both compounds at the lower concentration and a significant reduction was observed for **1**. The toxic activity of **3** was lower than those of the other two compounds, even independent from concentrations.

3. Conclusion

Two furan derivatives identified as 2,5-dihydroxymethylfuran and 5-hydroxymethyl-2-furaldehyde, were isolated for the first time together with tyrosol as phytotoxic metabolites from the culture filtrates of *S. macrostoma* an oak decline-associated fungus of *Q. brantii* in Iran. They showed strong phytotoxicity on holm oak leaves. This is an important contribution for the corrected taxonomic classification of the fungal producer and to better understand the pathogenic process and the disease symptoms induced on the host plant.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Bashiri S, Abdollahzadeh J, Di Lecce R, Alioto D, Górecki M, Pescitelli G, Masi M, Evidente A. 2020. Rabenchromenone and rabenzophenone, phytotoxic tetrasubstituted chromenone and hexasubstituted benzophenone constituents produced by the oak-decline-associated fungus *Fimetariella rabenhorstii*. J Nat Prod. 83: 447−452.

Cimmino A, Cinelli T, Masi M, Reveglia P, da Silva MA, Mugnai L, Michereff SJ, Surico G, Evidente A. 2017. Phytotoxic lipophilic metabolites produced by grapevine strains of *Lasiodiplodia* species in Brazil. J Agric Food Chem. 65:1102-1107.

Cimmino A, Nocera P, Linaldeddu BT, Masi M, Gorecki M, Pescitelli G, Montecchio L, Maddau L, Evidente A. 2018. Phytotoxic metabolites produced by *Diaporthella cryptica*, the causal agent of hazelnut branch canker. J Agric Food Chem. 66:3435-3442.

Dohnal B, Kisiel W. 1993. The effect of a fungal preparation on the tissue cultures of *Holarrhena anfidysenterica* (Roxb.) Wall. Acta Soc Bot Pol. 62:43-45.

Glidewell C, Zakaria CM, Ferguson G. 1996. Multiple hydrogen-bonding modes in furan-2,5-dimethanol and furan-2, 5-diylbis (diphenylmethanol). Acta Crystallogr C. 52:1305-1309.

Guo L, Huang T, Hu B, Wang C. 2016. Bioactivity-guided isolation and characterization of antitumor active compound from marine-derived fungus *Penicillium chrysogenum* HGQ6. Adv J Food Sci Technol. 10:353-355.

Ju Y-M, Wang G-J, Chen C-Y, Tsau Y-J, Chou C-H, Lee T-H. 2010. Chemical constituents from fermented broth and mycelium of the basidiomycete Lacrymaria velutina. Bot Stud. 51:311–315.

Li G, Wang X, Zheng L, Li L, Huang R, Zhang, K. 2007. Nematicidal metabolites from the fungus *Pleurotus ferulae* Lenzi. Ann Microbiol. 57:527.

Mancilla G, Jiménez-Teja D, Femenía-Ríos M, Macías-Sánchez AJ, Collado IG, Hernández-Galán, R. 2009. Novel macrolide from wild strains of the phytopathogen fungus *Colletotrichum acutatum*. Nat Prod Comm. 4:1934578X0900400316.

Masi M, Maddau L, Linaldeddu BT, Scanu B, Evidente A, Cimmino A. 2018. Bioactive metabolites from pathogenic and endophytic fungi of forest trees. Curr Med Chem. 25:208-252.

Masi M, Nocera P, Boari A, Zonno MC, Pescitelli G, Sarrocco S, Baroncelli R, Vannacci G, Vurro M, Evidente A. 2020. Secondary metabolites produced by *Colletotrichum lupini*, the causal agent of anthachnose of lupin (*Lupinus* spp.). Mycologia DOI:10.1080/00275514.2020.1732148.

Mosadeghzad Z, Zuriati Z, Asmat A, Gires U, Wickneswari R, Pittayakhajonwut P, Farahani GHN. 2013. Chemical components and bioactivity of the marine-derived fungus *Paecilomyces* sp. collected from Tinggi Island, Malaysia. Chem Nat Comp. 49:621-625.

Schneider G, Anke H, Sterner O. 1996. Xylaramide, a new antifungal compound, and other secondary metabolites from *Xylaria longipes*. Z Naturforsch C. 51:802-806.

Subramenium GA, Swetha TK, Iyer PM, Balamurugan K, Pandian SK. 2018. 5-hydroxymethyl-2-furaldehyde from marine bacterium *Bacillus subtilis* inhibits biofilm and virulence of *Candida albicans*. Microbiol Res. 207:19-32.

Voglmayr H, Jaklitsch WM. 2019. *Stilbocrea walteri* sp. nov., an unusual species of *Bionectriaceae*. Mycol. Prog. 18:91-105.

Figure Caption

Figure 1. Cross-section of *Q. brantii* branches showing wood necrosis induced by *S. macrostoma* (4B-212) (left) was isolated from (left). Die-back symptoms showed on the 2-year old *Q. brantii* trees inoculated by *S. macrostoma* in greenhouse experiments after 2 months (right).

Figure 2. A: Structure of 2,5-dihydroxymethylfuran, 5-hydroxymethyl-2-furaldehyde and tyrosol (**1-3**); **B**: ORTEP view of **1** crystal obtained from a slow evaporation of $CDCl_3$ solution. Thermal ellipsoids were drawn at 30% probability level.

Figure 3. Different crystal habitus of 2,5-dihydroxymethylfuran: A) Snow-like habitus; B) rhombeplate habitus (observation under polarized light at optical microscope).

Fig. 1.

Fig. 2.

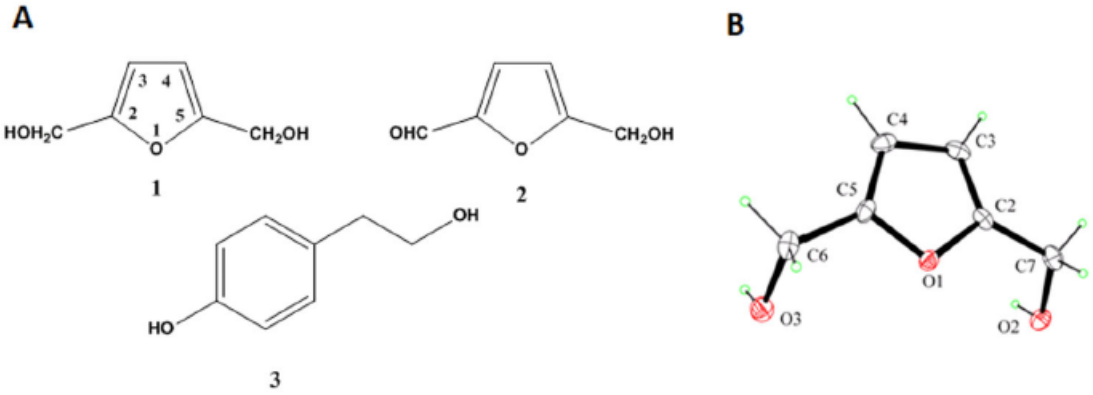

Fig. 3.