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Bioprospecting of *Diaporthe terebinthifolii* LGMF907 for antimicrobial compounds

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Abstract

Antibiotic-resistant bacteria have been observed with increasing frequency over the past decades, driving the search for new drugs and stimulating the interest in natural products sources. Endophytic fungi from medicinal plants represent a great source of novel bioactive compounds useful to pharmaceutical and agronomical purposes. *Diaporthe terebinthifolii* is an endophytic species isolated from *Schinus terebinthifolius*, a plant used in popular medicine for several health problems. The strain *D. terebinthifolii* LGMF907 was previously reported by our group to produce secondary metabolites with biological activity against phytopathogens. Based on these data, strain LGMF907 was chosen for bioprospecting against microorganisms of clinical importance and for characterization of major secondary metabolites. In this study, different culture conditions were evaluated and the biological activity of this strain was expanded. The crude extracts demonstrated high antibacterial activity against *Escherichia coli*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, methicillin-sensitive *Staphylococcus aureus*, and methicillin-resistant *S. aureus*. The compounds diaporthin and orthosporin were characterized and also showed activity against the clinical microorganisms evaluated. This study discloses the first isolation of diaporthin and orthosporin from *D. terebinthifolii*, and revealed the potential of this endophytic fungus to produce secondary metabolites with antimicrobial activity.

Keywords

Diaporthe terebinthifolii; *Schinus terebinthifolius* Raddi; Endophyte; Secondary metabolites; Diaporthin; Orthosporin

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Introduction

Fungal endophytes are hyper-diverse and abundant groups that can be found colonizing the tissues of all plants, regardless of their taxonomical affiliation or environmental preferences (Ismail et al. 2010). Throughout centuries, medicinal plants have been used as a source of molecules with therapeutic potential, and until today they represent an important pool for the identification of novel drug leads (Atanasov et al. 2015). Many of the clinically used drugs derived from natural products (NPs) and are originated from microbial species, and the current push to find new therapeutics to combat antibacterial drug resistance stimulates renewed interest in NP sources for new leads (Silver 2015). *Schinus terebinthifolius* Raddi, also known as Brazilian peppertree, rose pepper, or “aroeira-da-praia,” is used in folk medicine to combat diseases of the urogenital system and has antipyretic, analgesic, depurative, antimicrobial, anti-inflammatory, and antiulcerogenic activities (Carvalho et al. 2013).

Due to their ability to produce biologically active compounds, endophytes from medicinal plants are becoming more interesting to be explored (Alvin et al. 2014). Several new natural products useful for medicine purposes have been discovered from researches on fungal endophytes, and several recent reviews highlight their importance for bioprospecting (Aly et al. 2011).

Our research group has particular interest to explore endophytes with biotechnological potential. In this context, a previous study of endophytes from medicinal plants, *Diaporthe terebinthifolii* LGMF907 was isolated from *S. terebinthifolius* and described as a new species (Gomes et al. 2013). Strain LGMF907 showed the ability to produce secondary metabolites with biological activity against *Phyllosticta citricarpa*, the causal agent of citrus black spot (Santos et al. 2016), suggesting the possibility of this strain to produce secondary metabolites against other microorganisms. The genus *Diaporthe* comprises a widely studied group of fungi for its ability to generate metabolites for several applications (Flores et al. 2013). Since the existence of an ever increasing range of infections, due the antimicrobial resistance, it requires action across all government sectors and society (WHO 2017), and endophytes can be a promising strategy to find active compounds.

Pathogenic *Escherichia coli* is well known to be associated with gastrointestinal illness and diarrhea in humans (Torres et al. 2005), whereas the gram-positive organism *Micrococcus luteus* is recognized as an opportunistic pathogen and implicated in many human health problems (Yang et al. 2001), as well as *Saccharomyces cerevisiae*, that has recently been related to a wide variety of infections, which range from vaginitis to bloodstream infections and infections of essential organs in immunocompromised and critically ill patients (Pérez-Torrado and Querol 2015). *Staphylococcus aureus* is a major pathogen responsible for not only severe infections of the skin and skin structures but also for life-threatening diseases such as pneumonia, endocarditis, and bacteremia. From 1999 to 2005, the number of admissions to hospital due to MRSA infections increased by 119% in the USA (Abdelmajid et al. 2016), and these pathogens remain problematic because it is associated not only with growing antimicrobial resistance but also with high rates of hospitalization and mortality and a heavy burden of associated costs (Abdelmajid et al. 2016).

In order to access and expand the knowledge about the biological activity of strain *D. terebinthifolii* LGMF907 against the clinically important microorganisms, extracts originated from the large-scale production and major compounds were evaluated against pathogens associated with hospital infections, *E. coli*, *M. luteus*, *S. cerevisiae*, methicillin-sensitive *S. aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA).

Materials and methods

Fungal culture

The strain *D. terebinthifolii* LGMF907 was obtained from leaves of *S. terebinthifolius* Raddi collected in Curitiba, Paraná, Brazil (25°28'31.847" S, 49°17'5.629" W). The microorganism is stored in the biological culture collection of the Laboratory of Genetics of Microorganisms (LabGeM) from Federal University of Paraná (<http://www.labgem.ufpr.br/>). This species was described by Gomes et al. (2013) using multilocus sequence analyses of the rDNA internal transcribed spacer (ITS1, 5.8S, ITS2) region, and partial translation elongation factor 1-alpha (TEF1), beta-tubulin (TUB), histone H3 (HIS), and calmodulin (CAL) genes.

To maximize the metabolite yield, before the large-scale cultivation, we evaluated different nutritional and cultural conditions, and the condition with higher antibacterial activity and the higher extract amount was used for large-scale cultivation. Strain LGMF907 was grown on potato dextrose agar (PDA; IBI Scientific) plates at 28 °C for 15–20 days. Using a sterile cork borer, three agar plugs of 10 mm were taken from culture plates and inoculated into an Erlenmeyer flask of 250 mL containing 100 mL of the following media: (a) R5a (100 g/L sucrose, 0.25 g/L potassium sulfate, and 10.12 g/L magnesium chloride; 10 g/L glucose, 0.1 g/L casamino acids, 5 g/L yeast extract, 21 g/L (3-(*N*-morpholino) propanesulfonic acid), and 2 mL R5 trace elements solution); (b) SG (20 g/L glucose, 10 g/L soytone, 2 g/L calcium carbonate, and 1 mg/L cobalt chloride (III)); (c) M2 (4 g/L glucose, 10 g/L malt extract, 4 g/L yeast extract, and 1 g/L calcium carbonate); (d) Czapeck (30 g/L glucose, 2 g/L sodium nitrate, 2 g/L dipotassium phosphate, 0.5 g/L magnesium sulfate, 0.5 g/L potassium chloride, and 0.5 g/L ferrous sulfate); (e) malt extract broth (MEB; 20 g/L malt extract, 20 g/L glucose, and 1 g/L peptone). Three starting pHs were tested, 5.8, 6.5, and 7.8, and culture flasks were incubated at 28 °C using a rotary shaker at 120 rpm, for 10, 15, and 21 days.

Large-scale fermentation and extraction

The large-scale cultivation was performed by inoculation of 24 agar plugs into 2-L Erlenmeyer flasks containing 800mL of MEB, with a total of 20 L of culture. The flasks were incubated for 10 days, at 28 °C, and 120 rpm. The fermentation was filtered over Whatman paper grade 4 and then subjected to extraction with EtOAc (5 V × V). EtOAc was removed under reduced pressure on a rotary evaporator at 40 °C, yielding 5.44 g of crude extract. The crude extract yielded 13 homogenous fractions, after initial silica gel column chromatography eluting with a gradient of chloroform and methanol. After purification using HPLC and Sephadex LH-20 of the major fractions, afforded compounds **1** (2.0 mg)

and **2** (2.3 mg) were obtained in pure forms as pale-yellow solids (Supplementary file, Fig. S1). The remaining fractions and subfractions were excluded due to the limited quantities.

HPLC-UV analyses were performed on a Waters 2487 HPLC system, equipped with a photodiode diode array detector, using a reversed phase C18 column (10 × 250 mm, 5 µm; Waters, USA). For elution, a gradient of water and acetonitrile (solvent A, H₂O; solvent B, acetonitrile; flow rate 3.0 mL/min; 0–7 min 90% A and 10% B, 7–20 min 70% A and 30% B, 20–30 min 40% A and 60% B, 30–40 min 25% A and 75% B, 40–45 min 0% A and 100% B, 45–46 min 90% A and 10% B) was used with injection volume 1.0 mL and a flow rate of 3.0 mL/min. The injection volume was 1.0 mL and flow rate was 3.0 mL/min. Peaks were monitored at 254 and 340 nm wavelengths, respectively. Size exclusion chromatography was performed on a Sephadex LH-20 (25–100 µm; GE Healthcare, Piscataway, NJ). HR-MS analyses were recorded on an Amersham Biosystem ABSciex 4000 Q-Trap MS system. NMR data were acquired on the Agilent DD2 500 and DD2 600 NMR spectrometers, operating at 11.7 and 14.1 Tesla, observing ¹H and ¹³C at 500 and 600 and 125 and 150 MHz, respectively. The spectrometer was equipped with a 5-mm multinuclear direct detection probe with z-gradient.

Antibacterial tests

Preliminarily antimicrobial assay was carried out with the gram-negative bacteria *E. coli* NRRL B-3708, gram-positive bacteria *M. luteus* ATCC 9341, and the yeast *S. cerevisiae* ATCC 204508 to test the activity of the crude extracts and selection of the best culture conditions. The bioactivity of isolated compounds was evaluated against the previously mentioned strains and methicillin-sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) ATCC 33591.

An adapted disk diffusion assay protocol was used, standardized by the National Committee for Clinical Laboratory Standards (NCCLS) and followed by the Brazilian Health Surveillance Agency (ANVISA) (NCCLS 2003). Microorganisms were maintained in LB agar. Thus, one colony of each organism was inoculated into a 10-mL culture of LB broth and incubated in a 37 °C orbital shaker at 200 rpm for 15 h. Using a cotton swab, each test bacterium (10⁸ CFU/mL) was spread homogeneously on the surface of a sterile 90 mm polystyrene plate containing Mueller-Hinton agar (CRITERION™; Hardy Diagnostics). Sterile paper disks (6 mm diameter) were placed on the plates and loaded with 100 µg amount of each crude extract or compound dissolved in methanol. The plates were incubated at 37 °C for 24 h. Antibiotics ampicillin (100 µg/mL) and oxacillin (100 and 600 µg/mL) were used as positive controls, and methanol and saline solutions were used as negative controls. The experiment was carried out in triplicate with the inhibition zones measured in millimeters, and statistical parameters (normality test, ANOVA, and Tukey test) were calculated using R software (Redwan et al. 2016).

Results

Antibacterial activity of extracts produced in different culturing conditions

All media evaluated were able to stimulate the production of active secondary metabolites by LGMF907 strain. Considering the antibacterial activity involving the three clinical pathogens, the cultivation using MEB medium (malt extract and peptone as carbon and nitrogen sources) resulted in the best biological activity (Fig. 1). Based on these data, MEB medium pH 5.8 as initial condition and 10 days as fermentation period (Fig. 1) were selected for the large-scale cultivation.

Isolation and structure elucidation of the compounds

From 20 L of cultivation, we obtained 5.44 g of crude extract. After several chromatography techniques, compounds **1** and **2** were obtained in pure forms, 2.0 mg (retention time 23.8 min) and 2.3 mg (retention time 18.8 min), respectively. Compound **1** has a mass of 273.0739 and compound **2** has a mass of 237.0763, with molecular forms $C_{12}H_{14}O_5$ and $C_{15}H_{12}O_5$, respectively. According to the MS and NMR data and a substructure search in AntiBase (Laatsch 2012), compound **1** was identified as diaporthin, an isocoumarin with a 2-hydroxypropyl side chain, whereas compound **2** turned out to be its analogue de-*O*-methyldiaporthin, also known as orthosporin (Fig. 2) (Lee et al. 2006).

Antibacterial activity of isolated compounds

The antibacterial activity of crude extract and isolated compounds were evaluated against the bacteria *E. coli*, *M. luteus*, MSSA and MRSA, and the yeast *S. cerevisiae*. The crude extract presented high antimicrobial activity against MSSA and MRSA (Table 1), however, with lower values of inhibition zones comparing with the positive control, oxacillin (100 and 600 µg/mL). Diaporthin presented equivalent activity to the crude extract against *E. coli* and superior activity against *M. luteus* and the yeast *S. cerevisiae* (Table 1). However, the activity of secondary metabolites from LGMF907 against methicillin-sensitive and methicillin-resistant *S. aureus* was lower than the crude extract, which suggests a synergism of these compounds with other compounds present in lower amount (Table 1).

Discussion

In 2016, Santos et al. reported that the new species *D. terebinthifolli* LGMF907 had the ability to colonize citrus plants and the crude extracts produced by this endophytic fungus showed inhibition of mycelial growth and pycnidia formation of the phytopathogen *P. citricarpa*. Based on these data, we decided that it could be valuable to expand the studies about biological activity of strain LGMF907 against human pathogens and determine the major compounds produced by *D. terebinthifolli*.

In order to select the best culture conditions to produce active metabolites, the culture media were evaluated and the relevant result was observed using MEB medium, 10 days of culture, pH 5.8, using 120 rpm, at 28 °C. The lower activity when different sources of carbon and nitrogen than malt extract and peptone were used suggests a secondary metabolism depending on culture conditions (Tonial et al. 2016), and that growth conditions may limit

the production of antimicrobials by the organisms of this species. Fungi have a specific response to different growing environments that affects the biosynthesis of active secondary metabolites. Environmental changes can alter the chemical and biological profiles leading to metabolite diversification and, consequently, to novel pharmacological applications (Bracarense and Takahashi 2014; Ismaiel et al. 2010).

The isolated isocoumarin compound diaporthin (1) was firstly isolated from *Endothia parasitica* (Boller et al. 1957) and reported as phytotoxin from *Cryphonectria parasitica* (Sparapano et al. 1989). The first report of orthosporin (2) was from the plant pathogenic fungus *Drechslera siccanis* (Hallock et al. 1988). Isocoumarin derivatives are abundant in the fungal genera *Penicillium*, *Ceratocystis*, *Fusarium*, *Artemisia*, *Aspergillus*, *Cladosporium*, *Oospora*, and *Hydrangea* (Saeed 2016). In addition, both diaporthin and orthosporin were co-isolated from *Daldinia concentrica*, *Aspergillus ochraceus* (Miller et al. 2012; Huang et al. 2015), and in a hypovirulent strain of *Cryphonectria parasitica* (Arnone et al. 2002), and have their absolute optical rotary power and absolute configuration already described in the literature (Supplementary file, Fig. S6) (Ichihara et al. 1989; Tang et al. 2017).

In this study, we showed for the first time the bioactivity of both isocoumarins against gram-positive bacteria, *S. aureus* and *M. luteus*, the gram-negative *E. coli*, and the yeast *S. cerevisiae*. Diaporthin presented higher antimicrobial activity than orthosporin. Our data are supported by previous studies that demonstrated orthosporin correlated with lower activity against *Bacillus subtilis* 6633 B1, methicillin-resistant *Staphylococcus aureus* 134/93 R9 (MRSA), vancomycin-resistant *Enterococcus faecalis* 1528 R10 (VRE), and *Penicillium notatum* JP36 P1 (Barnes et al. 2016). Compounds diaporthin and orthosporin had activity against the clinical bacteria MSSA and MRSA. However, the crude extract showed higher activity than the isolated compounds, even superior to the antibiotic ampicillin. These data suggest that the activity observed in the extract is possibly due to the isolated compounds diaporthin and orthosporin, in synergism with other compounds that are present in small amounts in the extract (Table 1 and Supplementary file, Table S1). The synergism is also supported by the equivalent activity against MRSA and MSSA, produced by crude extract, which may suggest the presence of two different mechanisms of action (Redwan et al. 2016). Since diaporthin was described as a phytotoxic compound produced by *Sporothrix* sp. an endophytic fungus of *Kandelia candel* (South China Sea, China), showing toxicity to tumor cell line HepG2 (Barrow and McCulloch 2009), the antimicrobial activity observed can be related with the cytotoxicity of these compounds. Besides the low resistance, *E. coli*, *M. luteus*, and *S. cerevisiae* are associated with a wide range of infections with difficulty in treatment (Torres et al. 2005; Yang et al. 2001; Pérez-Torrado and Querol 2015). Specially, compounds with broad-spectrum activity have significant importance in the treatment of infection caused by those microorganisms and resistant strains, such as MRSA (Abdelmajid et al. 2016).

Interestingly, *D. terebinthifolli* was isolated the first time as an endophyte from *S. terebinthifolius*; however, in the culture conditions evaluated, this species produced two phytotoxic compounds as major metabolites. According to Strange (2007), plant pathogens produce phytotoxic compounds that are often crucial determinants of plant disease, and these compounds are usually produced in liquid media. An explanation for host survival can

be the ability to produce enzymes that inactivate or degrade the toxins (Takken and Joosten 2000).

In conclusion, this is the first study about the antibacterial activity and characterization of secondary metabolites produced by *D. terebinthifolii* LGMF907. Two isocoumarins were isolated and diaporthin had considerable higher activity than the related compound orthosporin. In addition, different from the isolated compounds, the crude extract showed a significant activity against the bacteria methicillin-resistant *S. aureus*, which suggested the production of other active compounds in small amounts that should be followed up in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Abdelmajid Z, Taieb M, Lamine MA, Cherif A, Jridil T, Mahjoubi B, Mbarek S, Fliss I, Nefzi A, Sebei K, Hamida JB. ANTISTAPHYBASE: database of antimicrobial peptides (AMPs) and essential oils (EOs) against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus*. Arch Microbiol. 2016; 1:1–18.
- Alvin A, Miler KI, Neilan BA. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol Res. 2014; 169(7–8):483–495. <https://doi.org/10.1016/j.micres.2013.12.009>. [PubMed: 24582778]
- Aly AH, Debbab A, Proksch P. Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol. 2011; 90(6):1829–1845. <https://doi.org/10.1007/s00253-011-3270-y>. [PubMed: 21523479]
- Arnone A, Assante G, Nasini G, Strada S, Vercesi A. Cryphonectric acid and other minor metabolites from a hypovirulent strain of *Cryphonectria parasitica*. J Nat Prod. 2002; 65(1):48–50. <https://doi.org/10.1021/np0103012>. [PubMed: 11809063]
- Atanasov AG, Waltenberger B, Pferschy-Wenzig EM. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv. 2015; 33(8):1582–1614. <https://doi.org/10.1016/j.biotechadv.2015.08.001>. [PubMed: 26281720]
- Barnes E, Jumpathong J, Lumyong S, Voigt K, Hertweck C. Daldionin, an unprecedented binaphthyl derivative, and diverse polyketide congeners from a fungal orchid endophyte. Chemistry. 2016; 22(13):4551–4555. <https://doi.org/10.1002/chem.201504005>. [PubMed: 26880363]
- Barrow RA, McCulloch MWB. Linear naphtha-gpyrones: a naturally occurring scaffold of biological importance. Med Chem. 2009; 9:273–292.
- Boller A, Gauman E, Hardegger E, Kugler K, Naef-Roth RM. Welkstotte und antibiotike: diaporthin ein welketoxin aus kultaren von *Endothia parasitica* (Murr.). Helv Chim Acta. 1957; 40(3):875–880. <https://doi.org/10.1002/hlca.19570400337>.

- Bracarense AAP, Takahashi JA. Modulation of antimicrobial metabolites production by the fungus *Aspergillus parasiticus*. 2014; 45:313–321.
- Carvalho MG, Melo AGN, Aragão CFS, Raffin FN, Moura TFA. *Schinus terebinthifolius* Raddi: chemical composition, biological properties and toxicity. Rev Bras Plant Med. 2013; 15(1):158–169. <https://doi.org/10.1590/S1516-05722013000100022>.
- Flores AC, Pamphile JA, Sarragiotto MH, Clemente E. Production of 3-nitropropionic acid by endophytic fungus *Phomopsis longicolla* isolated from *Trichilia elegans* A. JUSS ssp. *elegans* and evaluation of biological activity. World J Microbiol Biotechnol. 2013; 29(5):923–932. <https://doi.org/10.1007/s11274-013-1251-2>. [PubMed: 23296917]
- Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Fung Div. 2013; 60:161–170.
- Hallock YF, Clardy J, Kenfield DS, Strobel G. De-*O*-methyl-diaporthin, a phytotoxin from *Drechslera siccans*. Phytochemistry. 1988; 27(10):3123–3125. [https://doi.org/10.1016/0031-9422\(88\)80012-8](https://doi.org/10.1016/0031-9422(88)80012-8).
- Huang Q, An H, Song H, Mao H, Shen W, Dong J. Diversity and biotransformative potential of endophytic fungi associated with the medicinal plant *Kadsura angustifolia*. Res Microbiol. 2015; 166(1):45–55. <https://doi.org/10.1016/j.resmic.2014.12.004>. [PubMed: 25530313]
- Ichihara A, Hashimoto M, Hirai T, Takeda I, Sasamarua Y, Sakamura S, Sato R, Tajimi A. Structure, synthesis, and stereochemistry of (+)-orthosporin, a phytotoxic metabolite of Rhynchosporium orthosporum. Chem Lett. 1989; 3:1495–1498.
- Ismail AA, El-Sayed AES, Mahmoud AA. Some optimal culture conditions for production of cyclosporin A by *Fusarium roseum*. Braz J Microbiol. 2010; 41:1517–8382.
- Laatsch, H. AntiBase. Wiley-VCH; Weinheim: 2012.
- Lee IK, Seok SJ, Kim WG, Yun BS. Diaporthin and orthosporin from the fruiting body of *Daldinia concentrica*. Mycobiology. 2006; 34(1):38–40. <https://doi.org/10.4489/MYCO.2006.34.1.038>. [PubMed: 24039468]
- Miller KI, Qing C, Sze DM-Y, Roufogalis BD, Neilan BA. Culturable endophytes of medicinal plants and the genetic basis for their bioactivity. Microb Ecol. 2012; 64(2):431–449. <https://doi.org/10.1007/s00248-012-0044-8>. [PubMed: 22430508]
- NCCLS. Clinical and Laboratory Standards Institute document M2-A8. Clinical and Laboratory Standards Institute; Wayne: 2003. Performance standards for antimicrobial disk susceptibility tests; approved standard—8th edn.
- Pérez-Torrado R, Querol A. Opportunistic strains of *Saccharomyces cerevisiae*: a potential risk sold in food products. Front Microbiol. 2015; 6:1522. [PubMed: 26779173]
- Redwan EM, El-Baky NA, Al-Hejin AM, Baeshen MN, Al-mehdar HA, Elsayaw A, Gomaa ABM, Al-Masaudi SB, Universky VN. Significant antibacterial activity and synergistic effects of camel lactoferrin with antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA). Res Microbiol. 2016; 167(6):480–491. <https://doi.org/10.1016/j.resmic.2016.04.006>. [PubMed: 27130281]
- Saeed A. Isocoumarins, miraculous natural products blessed with diverse pharmacological activities. Eur J Med Chem. 2016; 116:290–317. <https://doi.org/10.1016/j.ejmech.2016.03.025>. [PubMed: 27155563]
- Santos PJC, Savi DC, Gomes RR, Goulin EH, Senkiv CC, Tanaka FAO, Almeida MR, Galli-Terasawa L, Kava V, Glienke C. *Diaporthe endophytica* and *D. terebinthifolii* from medicinal plants for biological control of *Phyllosticta citricarpa*. Microbiol Res. 2016; 186:153–160. [PubMed: 27242153]
- Silver LL. Natural products as a source of drug leads to overcome drug resistance. Future Microbiol. 2015; 10(11):1711–1718. <https://doi.org/10.2217/fmb.15.67>. [PubMed: 26517443]
- Sparapano L, Mairirotta P, Lerario P. Phytotoxic substances from virulent and hypovirulent strains of *Cryphonectria Parasitica*. Phyto Plant Pathol. 1989; 27:399–401.
- Strange RN. Phytotoxins produced by microbial plant pathogens. Nat Prod Rep. 2007; 24(1):127–144. <https://doi.org/10.1039/B513232K>. [PubMed: 17268610]
- Takken FLW, Joosten MHA. Plant resistance genes: their structure, function and evolution. Eur J Plant Pathol. 2000; 106(8):699–713. <https://doi.org/10.1023/A:1026571130477>.

- Tang JW, Wang WG, Li A, Yan BC, Chen R, Li XN, Du X, Sun HD, Pu JX. Polyketides from the endophytic fungus *Phomopsis* sp. sh917 by using the one strain/many compounds strategy. *Tetrahedron*. 2017; 73(26):3577–3584. <https://doi.org/10.1016/j.tet.2017.02.019>.
- Tonial F, Maia BHLNS, Gomes-Figueiredo JA, Sobottka AM, Bertol CD, Nepel A, Savi DC, Vicente VA, Gomes RR, Glienke C. Influence of culturing conditions on bioprospecting and the antimicrobial potential of endophytic fungi from *Schinus terebinthifolius*. *Curr Microbiol*. 2016; 72(2):173–183. <https://doi.org/10.1007/s00284-015-0929-0>. [PubMed: 26563302]
- Torres AG, Zhou X, Kaper JB. Adherence of diarrhea genic *Escherichia coli* strains to epithelial cells. *Infect Immun*. 2005; 73(1):18–29. <https://doi.org/10.1128/IAI.73.1.18-29.2005>. [PubMed: 15618137]
- WHO. World Health Organization. Antimicrobial resistance. 2017. Accessed: <http://www.who.int/mediacentre/factsheets/fs194/en/>
- Yang S, Sugawara S, Monodane T, Nishijima M, Adachi Y, Akashi S, Miyake K, Hase S, Takada H. *Micrococcus luteus* Teichuronic acids activate human and murine Monocytic cells in a CD14- and toll-like receptor 4-dependent manner. *Infect Immun*. 2001; 69(4):2025–2030. <https://doi.org/10.1128/IAI.69.4.2025-2030.2001>. [PubMed: 11254554]

Fig. 1. Inhibition zone of crude extracts from strain LGMF907 produced in different media, times, and pH against *Saccharomyces cerevisiae* (a), *Escherichia coli* (b), and *Micrococcus luteus* (c)

- 1:** R=CH₃; Diaporthin
2: R=H; Orthosporin

- 1:** (+)-HRESI-MS: m/z 273.0732 [M + Na]⁺
(Calcd. 273.0739 for C₁₃H₁₄O₅Na [M + Na]⁺)
- 2:** (+)-HRESI-MS: m/z 237.0763 [M + H]⁺
(Calcd. 237.0763 for C₁₂H₁₃O₅ [M + H]⁺)

Fig. 2.

Chemical structures and HR-MS data of diaporthin (**1**) and orthosporin (**2**) produced by the fungus *Diaporthe terebinthifolii* LGMF907

Table 1

Inhibition zone (mm) growth of clinical microorganisms after inoculation in medium culture with disks containing crude extracts and compounds (100 µg/disk) produced by *Diaporthe terebinthifolii* LGMF907

Microorganism	Methanol	Ampicillin 100 µg/mL	Oxacillin 100 µg/mL	Oxacillin 600 µg/mL	Amphotericin B	Diaporthin	Orthosporin	Crude extract
<i>Escherichia coli</i>	0.0D	3.83A	–	–	–	1.73B	1.03C	1.77B
<i>Micrococcus luteus</i>	0.0D	4.73A	–	–	–	2.47B	1.53C	1.47C
MRSA	0.0D	0.0D	15.67B	20.0A	–	9.50C	9.0C	16.33B
<i>Staphylococcus aureus</i>	0.0E	15.67C	26.67B	31.0A	–	9.0D	9.33D	16.0C
<i>Saccharomyces cerevisiae</i>	0.0D	–	–	–	2.07A	1.77B	1.87AB	1.37C

Letters represent Tukey test for average results among treatments, with significance of 0.05
(–) Compounds not evaluated