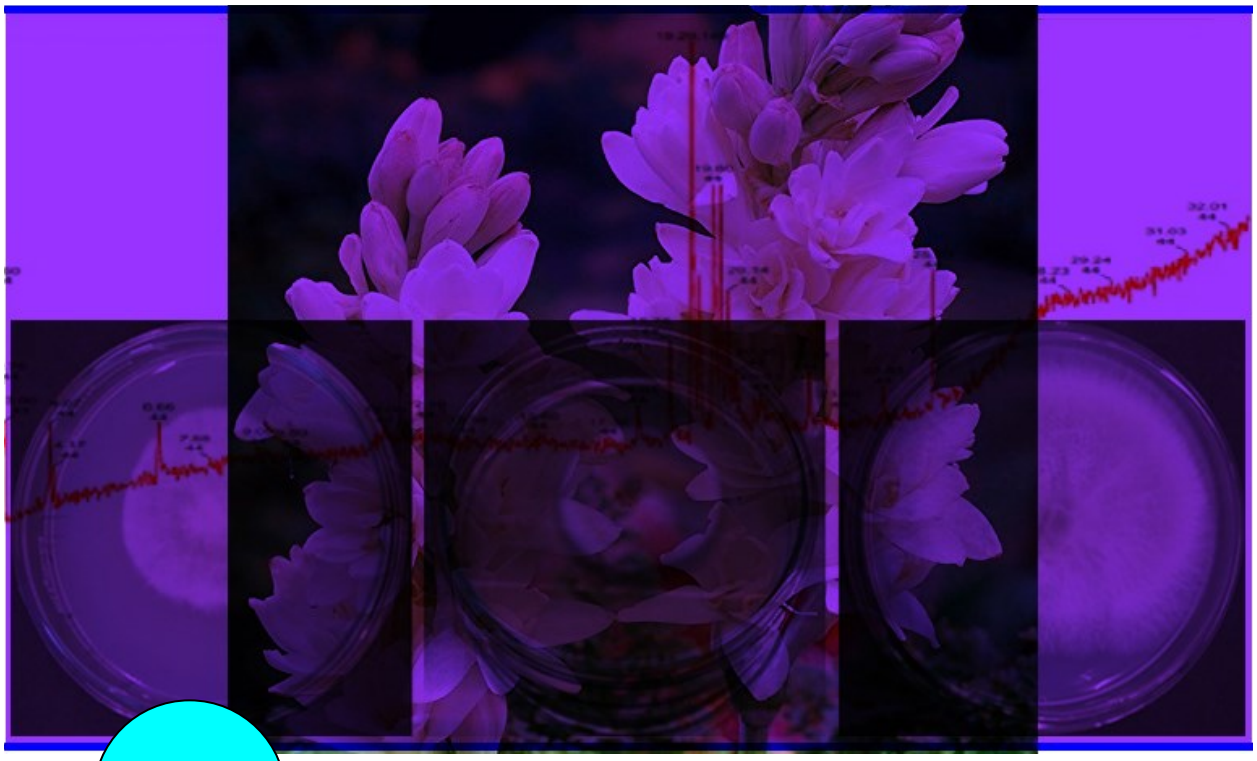

BJP

Bangladesh Journal of Pharmacology

Research Article

Effect of endophytic fungi *Daldinia eschscholtzii* against multidrug resistant pathogens

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info; www.bdpsjournal.org
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2023; 18: 17-23
ISSN: 1991-0088

Effect of endophytic fungi *Daldinia eschscholtzii* against multidrug resistant pathogens

Ranjitha Dhevi V. Sundar[1] and Sathiavelu Arunachalam[2]

[1]Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore 14, India; [2]Department of Agriculture microbiology, Vellore Institute of Technology School of Agricultural innovations and Advanced Learning, Vellore Institute of Technology, Vellore, India.

Article Info

Received: 21 November 2022
Accepted: 11 January 2023
Available Online: 13 January 2023
DOI: 10.3329/bjp.v18i1.62914

Cite this article:
Sundar RDV, Arunachalam S. Effect of endophytic fungi *Daldinia eschscholtzii* against multidrug resistant pathogens. Bangladesh J Pharmacol. 2023; 18: 17-23.

Abstract

This study aimed to explore the *in vitro* antibacterial potential of endophytic fungus isolated from the roots of *Polianthes tuberosa* against multidrug-resistant pathogens. Fungal isolates were screened for their antibacterial activities by disc diffusion, agar plug diffusion, MIC, and MBC method. GC-MS was carried out to determine the crude extract's chemical profile. The highest antagonistic effect in the disc diffusion method was seen in the ethyl acetate extract of PTR3, with an inhibitory zone ranging from 14-18 mm. Therefore, the potent isolate was identified as *Daldinia eschscholtzii*. The maximum inhibition of 18 mm and 20 mm was observed against MRSA (ATCC 43300) (ATCC 700699), followed by VRE (14 mm). The MIC and MBC were between 3.12-25 µg/mL and 6.25-50 µg/mL, respectively. 1,2-Benzene dicarboxylic acid and diheptyl ester were the primary chemical constituents present in the extract. These findings confirm that *D. eschscholtzii* PTR3 crude extract shows antibacterial activity.

Introduction

Natural products are most likely to afford a wide range of difficult-to-synthesize structures, making them the prospective sources of novel antibiotic classes (Bate et al., 2020).

Fungi are the most often isolated endophytes. Opportunities exist for retrieving unknown fungi, as most of the plants endophytes have not yet been studied (Strobel and Daisy, 2003). Endophytic fungi are well-known biological agents that live inside the plant's internal tissues and cause no harm to their hosts (Khan et al., 2021). Endophytes have a mutualistic relationship with their host plants by receiving protection and nutrition from a host and providing increased resistance to pathogens, herbivores, and abiotic stresses. Depending on the growth phase of the fungus and host, host defense response and environmental factors, some endophytic fungi can become plant pathogens (Hema et al., 2015). They are the reservoir of various phytochemicals like alkaloids, quinones, terpenoids, tannins, steroids, phenolic acids, and saponins (Verma et al., 2022). Endophytes-derived secondary metabolites can inhibit the mechanism of resistance by incapacitating pathogenic invasion (Manganyi and Ateba, 2020). Thus, the compounds derived from endophytes exhibit a broad range of activities in human diseases, including immunomodulatory, anti-diabetic, antifungal, antioxidant and anti-cancer (Verma et al., 2022). The bioactive metabolites produced by the fungal endophytes were divided into four groups, including terpenes, alkaloids, polyketides and non-ribosomal peptides based on their biosynthetic pathway and chemical structures (Khan et al., 2021). In ethnobotanical aspects of plant protection, conservation and sustainable use, researchers have been interested in isolating microbes associated with plants.

Endophytic fungi meet this demand and serve as the best substitute source of compounds with a range of bioactivity (Verma et al., 2022).

The ethyl acetate extracts obtained from *Pestalotiopsis sp*. Eef-9, *Chaetomium sp*. Eef-10 and *Lophiostoma sp*. Eef-7 of *Eucalyptus exserta* exhibited potent antibacterial activitiy against some pathogenic bacteria (Ababutain et al., 2021). Cyperaceae plants harbor numerous endophytes that produce antibacterial metabolites active against both Gram positive and, to a lesser extent, Gram negative bacteria (Ratnaweera et al., 2018). There is report of antimicrobial effects of endophytic fungi from *E. chrysantha. (*Zhang et al., 2015*).*

Due to the increased demand for alternative therapy for infectious diseases, the present research focused on isolating and identifying endophytic fungi from the *Polianthes tuberosa* plant. It belongs to the family of Amaryllidaceae and is an ornamental plant (Sundar and Arunachalam, 2022). The antibacterial activities, including Kirby Bauer disc diffusion, minimum inhibitory and bactericidal concentration and time-kill study of the endophytic fungal extracts against Gram-positive resistant pathogens, were investigated.

Materials and Methods

Isolation and identification of endophytes

P. tuberosa plant materials were collected and bought in a polythene bag to the laboratory from the Vellore District. The collected samples were washed thoroughly in running tap water to remove the surface adhere dust or soil particles. Surface sterilization was processed under the laminar hood using ethanol (70%) for 30 sec, followed by 3-5 min washing in 1% sodium hypochlorite solution. Later, washed two to three times under sterilized distilled water. The segment was placed aseptically on a potato dextrose agar medium and incubated for a week at 27 ± 2°C. Mycelial tips were subcultured on potato dextrose agar to produce pure colonies and stored for further analysis. Fungal isolates were morphologically identified by colony characteristics such as hyphal features, reproductive structures and spores arrangement. Lacto phenol cotton blue staining was carried out for microscopic identification. 18S rRNA ITS molecular sequencing identification was carried out for the molecular identification of the fungus (Roopa et al., 2015).

Mycelial growth rate

The isolated endophytic fungus was cultured and incubated at 27 ± 2°C. After 7 days, the mycelial colony diameter of all three fungi was measured every 3 days to determine the growth characteristics of the fungal isolates (Techaoei et al., 2020).

Preparation of biomass filtrate of active fungus

Fungal endophytes were grown in potato dextrose agar (500 mL) in a 1L Erlenmeyer flask at 27 ± 2°C for 21 days. The fermented broth of each isolate was thoroughly blended with solvents of different polarities, such as hexane, dichloromethane, ethyl acetate and *n*-butanol, sequentially in equal volumes. The obtained crudes were dried and the resultant dissolved in dimethyl sulfoxide to prepare a stock solution of 1 mg/mL and used for antibacterial studies (Palanichamy et al., 2018; Elghaffar et al., 2022).

Kirby Bauer disc diffusion assay

The endophytic fungal isolates that exhibited a broader antibacterial spectrum in the initial screening were taken further for secondary investigation by following the disc diffusion protocol. The test bacteria were uniformly seeded on the surface of Mueller-Hinton agar. About 6 mm sterile discs loaded with different extract concentrations (25, 50, 75, 100 µg/mL) prepared from the stock solution were placed on the medium. Inoculated plates were incubated overnight at 37°C and measured for inhibition zone to evaluate their anti-

Box 1: Agar Plug Diffusion Assay

Principle

The agar plug diffusion method was primarily used to determine the antibacterial activity.

Requirements

Muller Hinton agar, Potato dextrose agar, Tryptic soy broth, Brain heart infusion broth, Test pathogens (methicillin-resistant *Staphylococcus aureus*-MRSA (ATCC 43300), (ATCC 700699), Vancomycin-resistant *Enterococci*-VRE (ATCC 51299), *S. aureus* (ATCC 25923), *S. aureus* (MTCC 3160) and *Enterococcus faecalis*

Procedure

Step 1: The isolated fungal colonies were grown on the PDA medium at 27 ± 2°C for 7 days

Step 2: From the actively growing culture, 1 cm (diameter) and 4 mm (thickness) agar plugs were bored and transferred onto the MHA medium spread with the test microbes

Step 3: The plates were sealed and initially kept overnight at 4° C to allow the diffusion of bioactive compounds

Step 4: Post incubation, the plates were incubated at 37°C for 12 hours. The clear zone around the agar plugs was measured

Reference

Taufiq and Darah, 2018

References (Video)

Rattanasuk et al., 2021; Semerci et al., 2020; Krishnan et al., 2019

bacterial action. Dimethyl sulfoxide (5%) and oxacillin (1 µg) were included in the plates as a negative and positive control (Katoch et al., 2017; Marcellano et al., 2017). In addition, the activity index for each extract was calculated (Singh and Kumar, 2012).

Activity index = Inhibition zone of the sample / Inhibition zone of the standard

Minimum inhibitory concentration

The two-fold micro broth dilution method recommended by CLSI was used to determine the minimum inhibitory concentration (MIC) of ethyl acetate crude extracts that displayed the highest antagonistic activity. First, 50 to 0.39 µg/mL extract concentration was prepared from the stock solution. Then, Muller Hinton broth (100 µL) and extract (different dilution) were added to the 96-well microtiter plate. About 10 µL of bacterial inoculum was added and the setup was incubated at 37°C overnight. The lowest concentration was recorded with no visible bacterial growth (Ohikhena et al., 2017).

Minimum bactericidal concentration

The different turbidity sample (6.25 and 3.12 µg/mL concentration) from the MIC microtiter plate was used to determine the minimum bactericidal concentration (MBC) value. The samples were streaked on the Mueller-Hinton agar medium and incubated at 37°C for 18 hours. Post incubation, the lowest concentration showing no visible growth of bacteria was noted as the MBC of the extract (Ohikhena et al., 2017).

GC-MS analysis

Ethyl acetate derived from PTR3 containing different metabolites was subjected to GC-MS analysis. The study was carried out in Perkin Elmer Clarus 680 equipped with Clarus 600 mass spectrometer that was fitted with 30 m, 0.25 mm ID, 250 µm df Elite-5Ms capillary column. Initially, the GC oven was maintained for 3 min at 55°, ramp 10°C/min - 300°C for 6 min. Helium is used as a carrier with a flow rate of 1 mL/min. The mass transfer line and source temperature are set at 240°C. Turbo mass version 5.4.2 is used for spectral studies. The obtained unknown spectrum was compared with known spectrum components stored in the NIST library 2008 for structure identification (Liu et al., 2021).

Results

Isolation and antibacterial screening of endophytic fungi

Three endophytic fungi were isolated from the root part and coded PTR1, PTR2 and PTR3 (Figure 1). Each of these isolates showed different morphological and microscopic features. Then the isolates were subjected to antibacterial potential against the selected resistant pathogens. The isolated PTR3 had a remarkable inhibitory ability against the pathogens developing a clear zone in primary screening. Further, the crude extracts obtained from PTR3 were tested by the agar disc diffusion method.

Table I shows the antibacterial activities exhibited by the active fungal crude extracts against the tested bacteria at 100 µg/disc concentration. All four solvent crude extracts prepared from the potent isolate demonstrated promising antagonistic effects. Of them, ethyl acetate extract exhibited potent inhibition against the test pathogens.

Furthermore, it revealed the highest inhibition zone (IZ) of 20 mm with activity index (AI) 3.3 and (IZ 18 mm; AI 3) against MRSA (ATCC 700699) and (ATCC 43300), which was 2 to 3 times higher than the positive control Linezolid. Moreover, for VRE (IZ 14 mm; AI 1.4) zone was recorded (Table I). Thus, it indicates the isolate was a promising source for a novel antibiotic agent.

Morphological and molecular identification of the PTR3 isolate

The 18S rRNA sequencing identified the potent fungus as *Daldinia eschscholtzii* and submitted it to GenBank (accession No. OP800285). Figure 2 shows the phylo-

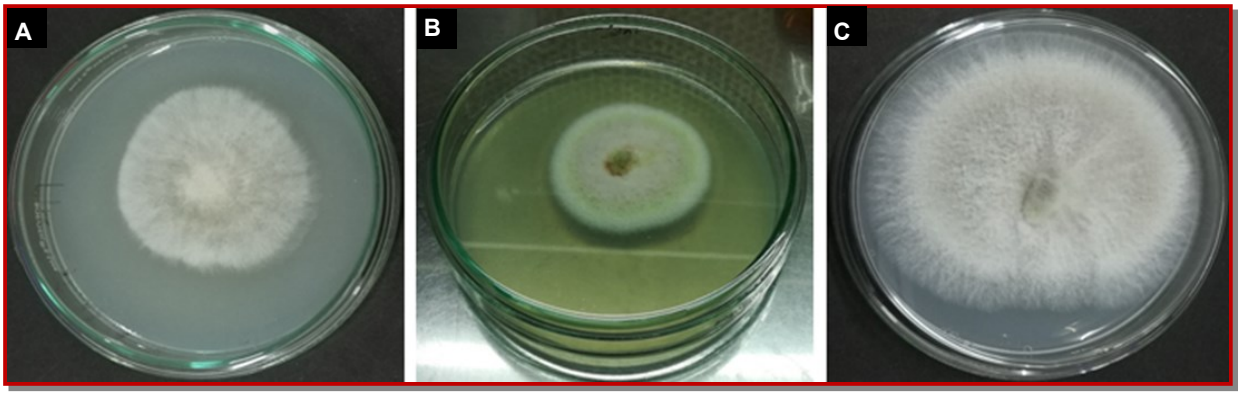

Figure 1: Endophytic fungal colony characteristics in PDA plates PTR1 (A), PTR2 (B), PTR3 (C)

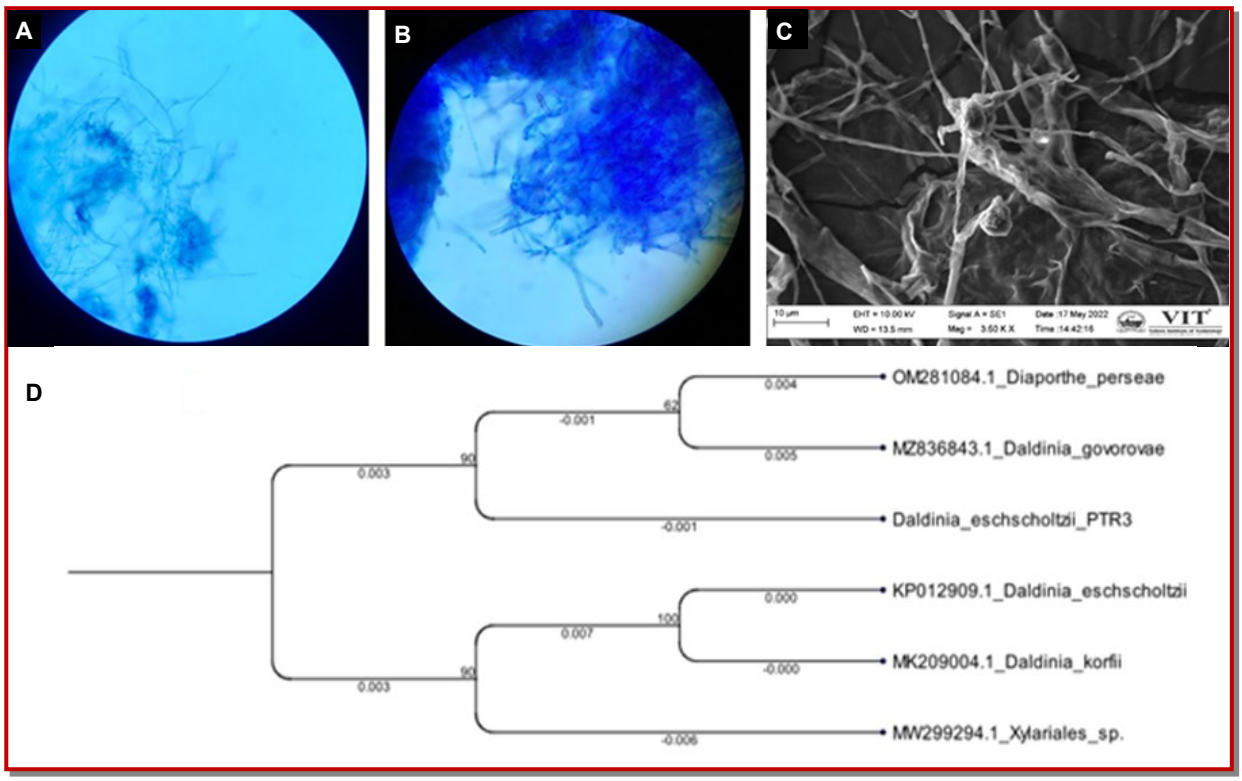

Figure 2: LCB stained at 40x (A) and 100x (B), SEM (C), Phylogenetic tree (D) generated by maximum likelihood of *Daldinia eschscholtzii* PTR3

genetic tree and morphological SEM study of PTR3.

Evaluation of MIC and MBC of active extract

The *D. eschscholtzii* PTR3 ethyl acetate extract that showed the maximum antagonistic effect was subjected further to determine their MIC and MBC. The extract showed a MIC of 6.25 and 3.12 µg/mL, an MBC of 50 and 25 µg/mL against two MRSA (i.e., ATCC 43300, ATCC 700699), respectively (Table II). Whereas, against VRE, the MIC and MBC were 12.5 and 50 µg/mL.

Growth rate of mycelia

The radial growth of the PTR3 fungus was more rapid when compared with PTR1 and PTR2 isolates, representing that nutrient-rich media could enrich mycelial density and the growth rate (data not shown).

Table II

Minimum inhibitory and minimum bactericidal concentration of PTR3 ethyl acetate extract

Test pathogens	Concentration (µg/mL)	
	MIC	MBC
MRSA (ATCC 43300)	6.25	50
MRSA (ATCC 700699)	3.12	25
S. aureus (ATCC 25923)	3.12	6.25
S. aureus (MTCC 3160)	3.12	12.5
VRE (ATCC 51299)	12.5	50
Oxacillin	-	-
Vancomycin	25	-

MIC means minimum inhibitory concentration; MBC means minimum bactericidal concentration

Table I

Average inhibition zone and activity index of PTR3 ethyl acetate extract (100 µg/mL)

Pathogens	Average inhibition zone (mm)							
	Hexane		Dichloromethane		Ethyl acetate		*n*-Butanol	
	ZOI	AI	ZOI	AI	ZOI	AI	ZOI	AI
MRSA (ATCC 43300)	6.5	1.1	15	2.5	18	3	10	1.7
MRSA (ATCC 700699)	-	-	12.5	2.1	20	3.3	6.8	1.1
S. aureus (ATCC 25923)	13	0.8	17	1.1	19	1.2	11	0.7
S. aureus (MTCC 3160)	14	0.9	16	1.0	20	1.3	17	1.1
VRE (ATCC 51299)	-	-	10.2	1.0	14	1.4	-	-

ZOI means zone of inhibition; AI means activity index

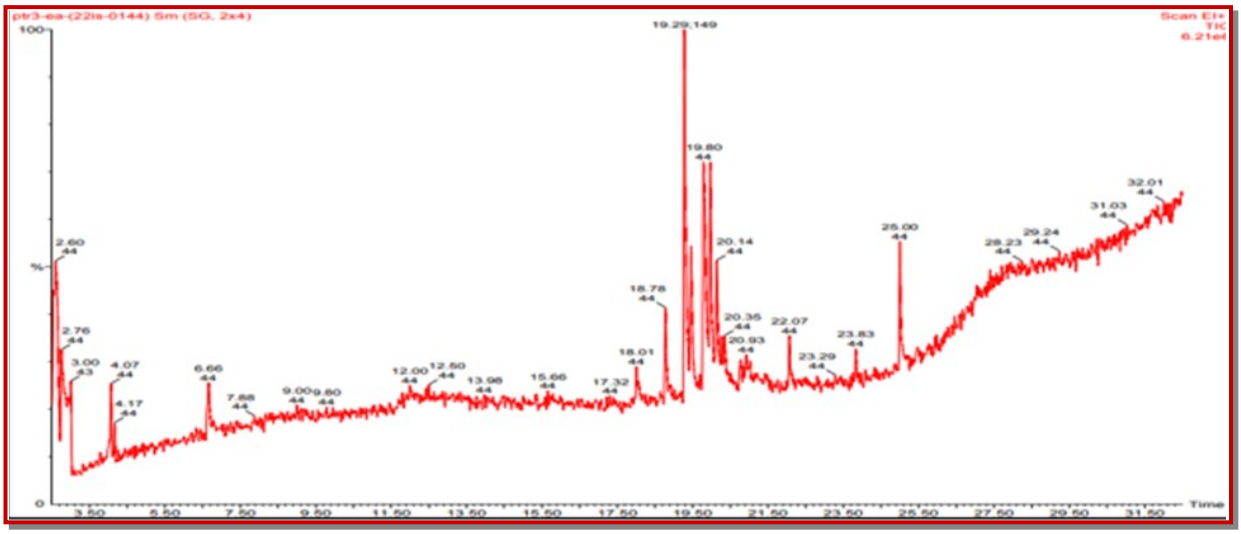

Figure 3: Chromatogram analysis of ethyl acetate extract

GC-MS of potent extract

As seen in Figure 3, 1,2-benzene dicarboxylic acid and diheptyl ester were the primary compound present in two different retention times of 19.3 and 19.8 min with an area percentage of 24.1% and 19.2% respectively.

Discussion

The present study demonstrated significant antibacterial activity of *D. eschscholtzii* isolated from *P. tuberosa* against drug-resistant pathogens.

The previous study reported that at 1 mg/mL concentration, ethyl acetate crude extracts of *D. eschscholtzii* isolated from the plant *Musa paradisiaca* displayed substantial antibacterial activity against *P. aeruginosa* (5 mm), *E. coli* (7 mm) and *B. subtilis* (3 mm). It also inhibited extended spectrum beta lactamase, a resistant bacterial strain of *E. coli*, with a 4 mm zone of inhibition (Victor et al., 2020). The ethyl acetate extract obtained from *D. eschscholtzii* of *Psidium guajava* has antibacterial activity against B. *subtilis, S. aureus, E. faecalis, P. aeruginosa, E. coli, P. aeruginosa* and *P. vulgaris*. *E. faecalis* was the most sensitive to the extract, with an inhibition zone of 15.7 ± 0.6 mm and a MIC of 1.25 mg/mL (Chutulo and Chalannavar, 2020). The broth ethyl acetate extract of *D. eschscholtzii* FK511P from Gorgonian *Annella* sp. inhibited Gram-positive bacteria *S. aureus* and MRSA with 8.3 ± 0.5 and 11.8 ± 1.0 mm inhibition, respectively, and Gram-negative *E. coli* with 9.0 ± 0.5 mm inhibition. Furthermore, mycelia methanol extract inhibited *S. aureus* and MRSA with inhibition zones of 8.1 ± 1.0 and 17.1 ± 1.2 mm, respectively, and *E.coli* with an inhibition zone of 12.1 ± 1.6 mm (Kandou et al., 2021). Nodulisporin H and 8-O-methyl-nodulisporin F, two new polyketides isolated from the ethanolic extract of the mangrove-derived fungus *D. eschscholtzii* HJ004, demonstrated moderate antibacterial activity against *S. aureus*, MRSA and *Bacillus cereus*, with MIC ranging from 6.25 to 12.5 µg/mL. The compound 5-hydroxy-2-methoxy-6,7-dimethyl-1,4-naphthoquinone had antibacterial activity against *B. cereus* with a MIC value of 12.5 µg/mL (Liao et al., 2019). Endophytic *D. cf concentrica* and its volatile metabolites from *Olea europaea* have shown antibacterial efficacy against plant pathogenic fungi. Similarly, *D. sacchari* metabolites from sugarcane in South India displayed antibacterial action against fungus and Gram-positive bacteria (Chutulo and Chalannavar, 2020).

Previous studies reported that 1,2-benzene dicarboxylic acid, mono(2-ethylhexyl) ester isolated from leaves of *Stevia rebaudiana* showed antifungal activity against *S. sclerotiorum* with an inhibition zone of 29 mm after 48 hours. Similarly, the compound 1,2-benzene dicarboxylic acid, (2-ethylhexyl) ester produced by the marine *Burkholderia cepacia* showed potential antibacterial activity against *Aeromonas hydrophila, Edwardsiella tarda* and *Vibrio ordalii* (Verma et al., 2014). 1,2-Benzene dicarboxylic acid, bis (2-ethyhexyl) ester and 1,2-benzenedicarboxylic acid, dibutyl ester derived from ethyl acetate fraction of *Alternaria sp.* isolated from *Salvadora persica* showed antimicrobial and antioxidant activity (Elgorban et al., 2019). And also, 1,2-benzene dicarboxylic acid and dioctyl ester isolated from the soluble subportion of ethyl acetate extract of the *Nauclea latifolia* unripe fruits displayed antibacterial activity against Gram-positive bacteria.

This is the first report on endophytic fungi *D. eschscholtzii* isolated from a root of *P. tuberosa* and their antibacterial activity.

Conclusion

This study shows the significant antibacterial activity of the endophytic fungi *D. eschscholtzii* isolated from the

root of *P. tuberosa* against antibiotic-resistant pathogens.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

The authors thank Vellore Institute of Technology's management for providing the facilities to carry out this study.

References

Ababutain IM , Aldosary SK, Aljuraifani AA, Alghamdi AI, Alabdalall AH, Al-Khaldi EM, Aldakeel SA, Almandil NB, Sayed AbdulAzeez S, Borgio JF. Identification and antibacterial characterization of endophytic fungi from *Artemisia sieberi*. Int J Microbiol. 2021; 2021.

Bate PNN, Orock AE, Nyongbela KD, Babiaka SB, Kukwah A, Ngemenya MN. *In vitro* activity against multi-drug resistant bacteria and cytotoxicity of lichens collected from Mount Cameroon. J King Saud Univ Sci. 2020; 32: 614-19.

Chutulo EC, Chalannavar RK. *Daldinia eschscholtzii*: An endophytic fungus isolated from *Psidium guajava* as an alternative source of bioactive secondary metabolites. Asian J Mycol. 2020; 3: 376-98.

Elghaffar RYA, Amin BH, Hashem AH, Sehim AE. Promising endophytic *Alternaria alternata* from leaves of *Ziziphus spina-christi*: Phytochemical analyses, antimicrobial and antioxidant activities. Appl Biochem Biotechnol. 2022; 194: 3984-4001.

Elgorban AM, Bahkali AH, Farraj DAA, Abdel-Wahab MA. Natural products of *Alternaria sp*., an endophytic fungus isolated from *Salvadora persic*a from Saudi Arabia. Saudi J Biol Sci. 2019; 26: 1068-77.

Hema P, Murali M, Thriveni MC, Prathibha M, Jayaramu SC, Amruthesh KN. Phytochemical analysis and antibacterial activity of endophytic fungi isolated from *Basella rubra* L. - A medicinal plant. J Pure Appl Microbiol. 2015; 9: 2971-78.

Kandou FEF, Mangindaan REP, Rompas RM, Simbala HI. Molecular identification and antibacterial activity of marine-endophytic fungi isolated from sea fan *Annella* sp. from Bunaken waters, Manado, North Sulawesi, Indonesia. AACL Bioflux. 2021; 14: 317-27.

Katoch M, Phull S, Vaid S, Singh S. Diversity, phylogeny, anticancer and antimicrobial potential of fungal endophytes associated with *Monarda citriodora* L. BMC Microbiol. 2017; 17: 1-13.

Khan MS, Gao J, Munir I, Zhang M, Liu Y, Moe TS, Xue J, Zhang X. Characterization of endophytic fungi, *Acremonium sp.*, from *Lilium davidii* and analysis of its antifungal and plant growth-promoting effects. Biomed Res Int. 2021; 1-14.

Krishnan M, Dey DK, Sharma C, Kang SC. Antibacterial activity of *Weissella confusa* by disc diffusion method. Bangladesh J Pharmacol. 2019; 14: 117-22.

Liao HX, Shao TM, Mei RQ, Huang GL, Zhou XM, Zheng CJ,Wang CY. Bioactive secondary metabolites from the culture of the mangrove-derived fungus *Daldinia eschscholtzii* HJ004. Mar Drugs. 2019; 17: 1-9.

Manganyi MS, Ateba CN. Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms. 2020; 8: 1934.

Mao Z, Zhang W, Wu C, Feng H, Peng Y, Shahid H, Cui Z , Ding P, Shan T. Diversity and antibacterial activity of fungal endophytes from *Eucalyptus exserta*. BMC Microbiol. 2021; 2021.

Marcellano JP, Collanto AS, Fuentes RG. Antibacterial activity of endophytic fungi isolated from the bark of *Cinnamomum mercadoi*. Pharmacogn J. 2017; 9: 405-09.

Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitate* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tea, using the dilution techniques. Sci World J. 2017; 1-8.

Palanichamy P, Krishnamoorthy G, Kannan S, Marudhamuthu M. Bioactive potential of secondary metabolites derived from medicinal plant endophytes. Egypt J Basic Appl Sci. 2018; 5: 303-12.

Ratnaweera PB, Walgama RC, Jayasundera KU, Herath SD, Abira S, Williams DE, Andersen RJ, de Silva ED. Antibacterial activities of endophytic fungi isolated from six Sri Lankan plants of the family Cyperaceae. Bangladesh J Pharmacol. 2018; 13: 264-72.

Rattanasuk S, Boongapim R, Phiwthong T. Antibacterial activity of *Cathormion umbellatum*. Bangladesh J Pharmacol. 2021; 16: 91-95.

Roopa G, Madhusudhan MC, Sunil KCR, Lisa N, Calvin R, Poornima R, Zeinab N, Kini KR, Prakash HS, Geetha N. Identification of taxol-producing endophytic fungi isolated from *Salacia oblonga* through genomic mining approach. J Genet Eng Biotechnol. 2015; 13: 119-12.

Semerci AB, İnceçayır D, Mammadova V, Hoş A, Tunç K. Antimicrobial activities of *Allium staticiforme* and *Allium subhirsutum*. Bangladesh J Pharmacol. 2020; 15: 19-23.

Singh G and Kumar P. Antibacterial potential of alkaloids of *Withania somnifera* L. & *Euphorbia hirta* L. Int J Pharm Pharm Sci. 2012; 4: 78-81.

Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003; 67: 491-502.

Sundar RDV, Arunachalam S. Anti-MRSA activity of *Polianthes tuberosa* leaf extracts. Bangladesh J Pharmacol. 2022; 17: 11-13.

Taufiq MMJ, Darah I. Fungal endophytes isolated from the leaves of a medicinal plant, *Ocimum sanctum* Linn and evaluation of their antimicrobial activities. Afr J Microbiol Res. 2018; 12: 616-22.

Techaoei S, Jirayuthcharoenkul C, Jarmkom K, Dumrongphuttidecha T, Khobjai W. Chemical evaluation and antibacterial activity of novel bioactive compounds from endophytic fungi in *Nelumbo nucifera*. Saudi J Biol Sci. 2020; 27: 2883-89.

Victor C, Moses O, Eze A, Festus O, Charles E. Isolation, identification, and evaluation of biological activities of *Daldinia eschscholtzii*, an endophytic fungus isolated from the leaves of *Musa paradisiaca*. GSC Biol Pharm Sci. 2020; 12: 216-28.

Verma A, Johri BN, Prakash A. Antagonistic evaluation of bioactive metabolite from endophytic fungus, *Aspergillus flavipes* KF671231. J Mycol. 2014; 2014.

Verma A, Gupta P, Rai N, Tiwari RK, Kumar A, Salvi P, Kamble SC, Singh SK, VG. Assessment of biological activities of fungal endophytes derived bioactive compounds isolated from *Amoora rohituka*. J Fungi. 2022; 8: 285.

Zhang H, Ruan C, Bai X. Isolation and antimicrobial effects of endophytic fungi from *Edgeworthia chrysantha*. Bangladesh J Pharmacol. 2015; 10: 529-32.

Author Info
Sathiavelu Arunachalam (Principal contact)
e-mail: asathiavelu@vit.ac.in