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SHORT COMMUNICATION

Antibacterial activity of endophytic fungi isolated from *Sceletium tortuosum* L. (Kougoed)

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Abstract

Endophytic fungi have the ability to co-exist with their host plants without causing any harm and are beneficial to both the plant and the fungi. The current study determined the antimicrobial properties and identify the chemical compounds of secondary metabolites produced by endophytic fungi isolated from *Sceletium tortuosum* L. A total of 60 endophytic fungi produced secondary metabolites that were detected after fermentation and extraction. Antibacterial properties of the secondary metabolites were determined using the disc diffusion assay against pathogenic environmental Gram-positive and Gram-negative bacteria as well as control stains. The chemical compounds were characterized by GC-MS. Overall, 15% of fungal extracts displayed narrow spectrum of activity against the bacteria strains. Despite this, none of the fungal extracts inhibited growth of *Enterococcus faecalis* (ATCC S1299) and *Enterococcus gallinarum* (ATCC 700425) while *Bacillus cereus* (ATCC 10876) was the most susceptible against the fungal extracts. *Fusarium oxysporum* (GG 008) with accession no. KJ774041.1 displayed significant antibacterial activity that was linked to high levels of 5-hydroxymethylfurfural (HMF) and octadecanoic acid as revealed by GC-MS. This study revealed the presence of bioactive secondary metabolites with antibacterial activities from fungi isolated from *Sceletium tortuosum* L.

Keywords Endophytic fungi · *Sceletium tortuosum* · Secondary metabolites · Bioactive compounds · GC-MS analysis · *Fusarium oxysporum*

Findings

In developing countries including Southern Africa, approximately 80% of the population depend on medical plants to cure diseases caused by pathogens (WHO 2004; Ekor, 2013). In addition, the efficacy of modern medicines is dramatically reducing especially due to the ever-increasing bacteria resistance that is currently an issue of great public health concern (Alpert 2017). Moreover, the misuse of antibiotics and poor hygienic conditions as well as the continuous movement of travelers significantly contribute towards the development and dissemination of resistant determinants (Von Nussbaum et al. 2006). The aforementioned indicate the urgent need to intensify studies that are designed to discover new, efficacious, effective, and affordable antimicrobial agents by exploring new niches and habitats (Lahlou 2013).

Sceletium tortuosum L. is a flowing succulent plant that belongs to the family Mesembryanthemaceae, and it is indigenous to South Africa (Gericke and Viljoen 2008). The plant is widely used by San and Khoikhoi natives for treating complications in humans (Smith et al. 1996). These communities use *Sceletium tortuosum* (L.) in creating a sense of wellbeing, reducing stress and for treating anxiety, depression in patients (Gericke and Viljoen 2008). Plants including *Sceletium*

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Table 1 Antimicrobial activity of extracts produced by endophytic fungal isolated from *Sceletium tortuosum*

Sample no.	Sample ID	Probable name	Zone of Inhibition (mm)					
			E. coli ATCC 25922	*E. coli* ATCC 0177	*B. cereus* ATCC 10876	*E. faecalis* ATCC S1299	*E. faecium* ATCC 700221	*E. gallinarum* ATCC 700425
1	GG 008	*Fusarium*	–	++ (7)	–	–	++ (9)	–
2	GG 009	*Neurospora*	–	–	++ (9)	–	–	–
3	GG 012	*Fusarium*	–	++ (7)	–	–	–	–
4	GG 013	*Aspergillus*	–	–	++ (7)	–	–	–
5	ND 19	*Fusarium*	–	++ (8)	–	–	–	–
6	DR 006	*Fusarium*	++ (7)	–	–	–	–	–
7	DR 017	*Fusarium*	–	–	+ (2)	–	–	–
8	DR 020	*Fusarium*	++ (8)	–	–	–	–	–
9	DR 023	*Fusarium*	–	–	++ (9)	–	–	–

Zone of inhibition: –: no activity; +: slight activity (< 5 mm); ++: good activity (6–10 mm); +++: very good activity (> 10 mm)

tortuosum L. may be inhabited by fungi in which case they exist in beneficial relationships.

Endophytic fungi colonize both inter- and intracellular tissues of plants without causing any apparent damage, nor symptoms of diseases (Rodriguez et al. 2009). The symbiotic association may be attributed to the production of bioactive compounds that function in growth promotion, competitiveness, and protection of the host against herbivores and pathogens (Porras-Alfaro and Bayman 2011). Endophytic fungi inhibit plants having a broad spectrum of environmental habitats and thus require diverse adaptative potentials due to their exposure to these unusual environments. These characteristics permit them to be ideal candidates for bio-control processes and thus are applicable in pharmaceuticals as well as medical and agricultural industries (Rai et al. 2014; Gouda et al. 2016; Fareed et al. 2017). In addition, endophytic fungi are generally known to produce secondary metabolites (4-hydroxybenzoic acid, indole-3-acetic acid (IAA) and gibepyrone D) that exhibit biological activities against a wide variety of microbes (Deshmukh et al. 2014; Bogner et al. 2017).

In the present study, endophytic fungi were isolated from *Sceletium tortuosum* (L.) and the secondary metabolites were screened against resistant Gram-positive and Gram-negative bacteria. The identities of secondary metabolites were determined using analytic gas chromatography-mass spectrophotometry (GC-MS).

A total of sixty (60) endophytic fungi were successfully isolated from healthy *Sceletium tortuosum* plants in South Africa. The identities of the fungal isolates were determined through morphological and molecular identification using internal transcribed spacer (ITS) and elongation factor 1-alpha (EF-1α) primers as reported in a previous finding (Manganyi et al. 2018a). All nucleotide sequences were deposited in the National Centre for Biotechnology Information (NCBI, GenBank) and unique accession numbers were provided. Bacteria control strains obtained from American Type Culture

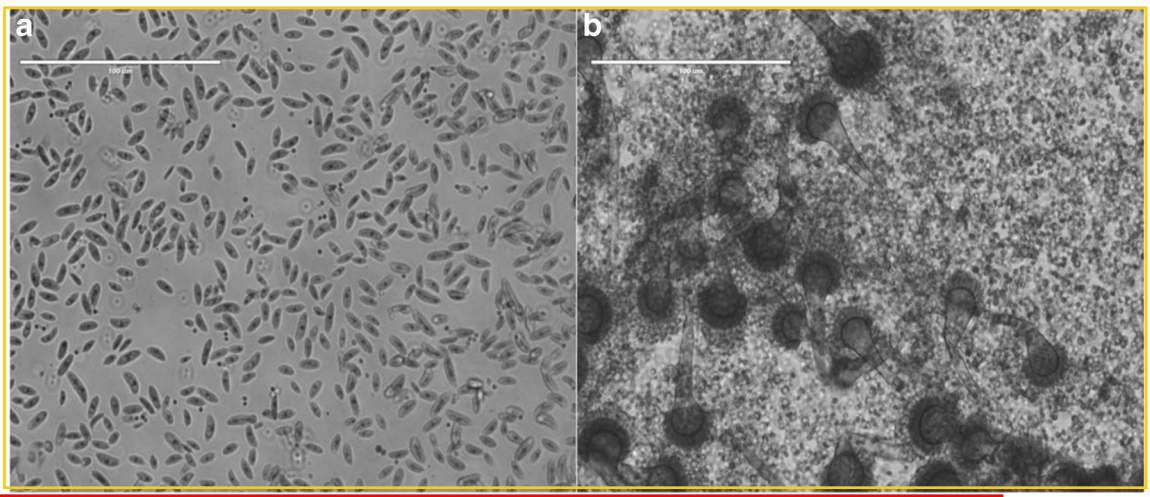

Fig. 1 Morphological structures of the most active genus **a** *Fusarium* and **b** *Aspergillus* observed under a microscope. Bar, 100 μm

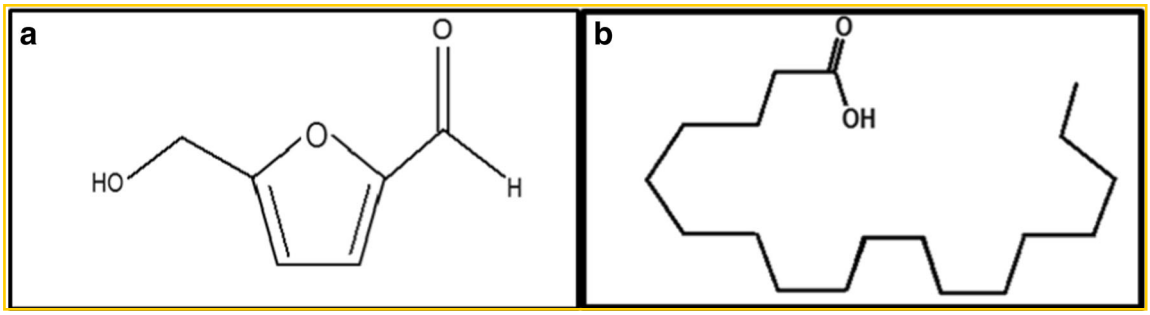

Fig. 2 Chemical structure of the most abundant compound extracted from endophytic *F. oxysporum* **a** 5-hydroxymethylfurfural and **b** octadecanoic acid (Images from National Institute of Standards and Technology 2018 and de Melo et al. 2014)

Collection ATCC (*Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, *Enterococcus gallinarum* ATCC 700425) as well as environmental strains (*E. coli* O177) from cattle feces, *Enterococcus faecalis* (accession no. S1299), and *Enterococcus faecium* (accession no. 700221) from ground water were used in the study to assess the bioactive potential of the secondary metabolites. The potential of all the 60 fungal isolates to produce secondary metabolites was assessed using a fermentation process. To achieve this, each of the isolates was inoculated in 50 mL of malt extract broth (Merck, Darmstadt, Germany) contained in 250-mL Erlenmeyer flasks. The isolates were incubated at 25 °C for 5 days in a rotary shaker at 150 rpm. After incubation, the inoculated broth was filtrated through a 0.45-μm PALL Sterile Acrodisc Syringe Filter obtained from Separations, South Africa (Manganyi et al. 2018b). Secondary metabolites were screened for antibacterial activity against the test bacteria isolates using the agar disc diffusion assay (Bauer et al., 1966). The discs were prepared by punching Whatman No. 3 filter paper (Separations, South Africa) and sterilizing them twice. About 1×10^7 cells/mL of bacteria suspensions for each isolate was used spread-plated on Nutrient agar. Triplicate plates per isolate were prepared in Ahmad et al. (2013). The discs were soaked in fungal extracts for several minutes before placing them on the inoculated plates in order to prepare a bacteria lawn. The inoculated plates were incubated aerobically at 37 °C for 24 h. The inhibition bacteria growth inhibition zone diameter data was measured in millimeters. Gas chromatography-mass spectrometry analysis of secondary metabolites was performed using the Shimadzu GC-MS-TQ8050 with Multifunctional Autosampler AOC-6000. The GC-MS analysis was set using capillary column (RTX-5 60 m × 0.25 mm × 0.25 μm) with an initial temperature of 70 °C that was maintained for 2 min. The oven program was then elevated to 180 °C for 2 min and later raised to 310 °C for 10 min at 1 °C/min intervals. The injection port temperature was 250 °C and Helium flow rate was 1 mL/min as described by Sharma et al. (2017). The results were compared with those previously submitted in the database from National Institute of Standards and Technology, (NIST05), US. The findings of the study revealed that a total of 9 out of 60 fungal extracts showed effective antibacterial activities against the test bacteria isolates. The antibacterial activities of fungal extracts that produced activity against the tests isolates are shown in Table 1. Despite the fact that the overall proportion of the extracts that exhibited inhibitory activities against the test isolates was significantly low (15%), fungi belonging to the genus *Fusarium* produced the highest proportion (seven out of the nine fungal extracts) of biologically active secondary metabolites. This was followed by isolates belonging to the genus *Neurospora* and *Aspergillus* that exhibited one each of the nine biological activities. Figure 1 represents the morphological features of isolates belonging to the genus *Fusarium* and *Aspergillus* that exhibited the highest biological activities as identified under a 100-μm bar magnification. The findings of this study are in agreement with a previous report (Zhang et al. 2016), in which endophytic belonging to the genus *Fusarium* sp. also demonstrated the strongest antimicrobial potential against *Pseudomonas aeruginosa*. In the later study, it was reported that the

Table 2 List of secondary metabolites produced and identified from of *F. oxysporum* by GC-MS

Number	Metabolite name	Retention time (min)	Area	Height
1	Ethanol	1.948	11,472,806	1,284,622
2	Octadecanoic acid, phenylmethyl ester	2.197	1,222,848	223,452
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	2.355	1,146,050	162,938
4	5-Hydroxymethylfurfural	2.431	2,249,944	305,544
5	Acetamide-N-D, N,N′-1,3- cyclohexane	2.617	475,548	88,881

antibacterial effect was mediated by increasing the permeability of the cell membrane (Zhang et al. 2016). None of the secondary metabolite was capable of inhibiting the growth of *E. gallinarum* (ATCC 700425) control strain and *E. faecalis* (accession no. S1299) environmental strain respectively. Despite this, the highly pathogenic environmental atypical *E. coli* (strain O177) and *B. cereus* (ATCC 10876) were most often susceptible to the secondary metabolites. With the exception of an extract from a *Fusarium oxysporum* (GG 008; accession no. KJ774041.1), that exhibited activity against two isolates, all the secondary metabolites exhibited biological activity against only one bacteria test isolate. These findings concur with those previously reported by Musavi and Balakrishnan (2014) which indicated that an extract from *Fusarium oxysporum* (NFX06) isolated from the leaves of *Nothapodytes foetida* exhibited strong antibacterial properties against pathogenic *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 69548).

GC-MS chromatograms of fungal extract from *F. oxysporum* revealed the presence of five secondary metabolites whose identities based on the NIST mass spectral database comprised ethanol, saturated hydrocarbons, and acetamide just to mention a few. However, 5-hydroxymethylfurfural was the most abundant secondary metabolite produced by *F. oxysporum* (GG 008, accession no. KJ774041.1) and displayed the highest peak area followed by octadecanoic acid (Fig. 2). The list of secondary metabolites identified from the extract of *F. oxysporum* is shown in Table 2 and Fig. 1. Sharma et al. (2016) reported that 5-hydroxymethylfurfural was one of the major bioactive compounds produced by the endophytic fungus *Pestalotiopsis neglecta* (BAB-5510) isolated from leaves of *Cupressus torulosa* D.Don.

Recently, bacterial infections associated with antibiotic resistant strains remain an issue of severe public health concern (Morris and Materton 2002). In order to address this problem, there is a need to search and develop new and highly effective antimicrobial agents (Wang et al. 2004). To date, extensive data has been generated on the occurrence of bioactive compounds from medicinal plant extracts while very little exists on the isolation and characterization of secondary metabolites from endophytic fungi. This therefore amplifies the need for the study whose findings have revealed potentially important and biological active secondary metabolites with antimicrobial activity against both Gram-negative and Gram-positive bacteria isolates.

In conclusion, these findings suggest that endophytic fungi from *S. tortuosum* L. that is indigenous to South Africa produced secondary metabolites that exhibited highly effective antibacterial activity against multi-drug resistant bacterial strains, and these isolates could serve as potential sources for the isolation of novel antimicrobial agents that may contribute in the fight against antibiotic strains. To the best of our knowledge, this is the first report on secondary metabolites from endophytic fungi and *F. oxysporum* (GG 008, accession no. KJ774041.1) in particular that was isolated from *S. tortuosum* L. plants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Not applicable (N/A).

Informed consent Not applicable (N/A). This research does not involve human participants and/or animals; therefore, no informed consent is needed.

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