Anti-MRSA Activity of Oxysporone and Xylitol from the Endophytic Fungus *Pestalotia* sp. Growing on the Sundarbans Mangrove Plant *Heritiera fomes*

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Heritiera fomes **Buch.-Ham., a mangrove plant from the Sundarbans, has adapted to a unique habitat, muddy saline water, anaerobic soil, brackish tidal activities and high microbial competition. Endophytic fungal association protects this plant from adverse environmental conditions. This plant is used in Bangladeshi folk medicine, but it has not been extensively studied phytochemically, and there is hardly any report on investigation on endophytic fungi growing on this plant. In this study, endophytic fungi were isolated from the surface sterilized cladodes and leaves of *H. fomes*. The antimicrobial activities were evaluated against two Gram-positive and two Gram-negative bacteria and the fungal strain, *Candida albicans*. Extracts of *Pestalotia sp.* showed activities against all test bacterial strains, except that the EtOAc extract was inactive against *E. coli*. The structures of the purified compounds, oxysporone and xylitol, were elucidated by spectroscopic means. The anti-MRSA potential of the isolated compounds were determined against various MRSA strains, i.e., ATCC 25923, SA-1199B, RN4220, XU212, EMRSA-15 and EMRSA-16, with MIC values ranging from 32-128 μg/mL. This paper, for the first time, reports on the anti-MRSA property of oxysporone and xylitol, isolation of the endophyte *Pestalotia sp*. from *H. fomes,* and isolation of xylitol from a *Pestalotia* sp.**

Keywords: Heritiera fomes; Pestalotia sp.; endophyte; oxysporone; xylitol; anti-MRSA

INTRODUCTION

Endophytic fungi are a diverse group of microorganisms that thrive asymptomatically in the healthy tissues of the host plant. Many of these endophytes biosynthesize a plethora of bioactive secondary metabolites that may assist the host in protection and survival against pathogenic microbial and insect attacks, stress tolerance and disease resistance (Zhang *et al.*, 2006; Rodriguez *et al.,* 2009). Moreover, some of these metabolites are useful as leads for novel drug discovery (Tan *et al.* 2001; Yu *et al.,* 2010; Yadav *et al.,* 2014).

The unique mangrove ecosystem adjacent to the coastal waters provides a wide variety of organic substrates and a significant salinity gradient caused by daily changes in the sea level (Shearer *et al.,* 2007). This constitutes an ideal environment for the bases of trunks and submerged aerating roots of mangrove plants, making mangrove forests an important source for unique endophytic fungi (Xing *et al.*, 2011). Mangrove fungi are the second largest group among the marine fungi (Hyde and Lee, 1995). Mangrove forest represents an ecosystem of high biodiversity (Bandarnayake, 1998). In addition, plants produce secondary metabolites under stressful conditions. Therefore, it is not surprising that mangrove plants, facing various ecological and environmental stresses, also biosynthesize a wide range of secondary metabolites of potential medicinal importance.

Mangrove plants are atypical from common terrestrial plants in that they can tolerate high salt concentration and remain submerged in saline water. Because of the scant distribution of the mangrove forests, mangrove plant species are still almost unacquainted to a vast population. Ancient people used mangrove plant species scarcely, because they could hardly enter these areas (Shilpi *et al.,* 2012). *Heritiera fomes* Buch. Ham. (Syn.: *Heritiera minor* or *Amygdalus minor*) is an evergreen moderate size tree growing abundantly in the Sundarbans (Pasha and Siddiqui, 2013). All plant parts of *H. fomes* are used in the treatment of different ailments; leaves and seeds are reported for their uses in the treatment of gastrointestinal disorders (diarrhoea, dysentery, constipation, acidity, indigestion and stomach ache) (Mollik *et al.,* 2010; Patra and Thatoi, 2011). The stem bark is a well-reputed remedy for diabetes and skin diseases (dermatitis, eczema, boils, abscess, acne, sores, and rash) (Mollik *et al.,* 2010). Local people use twigs to clean teeth and relive cough (Rahmatullah *et al.,* 2010).

Activity of many phytochemicals reported with various bioactivities have been found to be the products of endophytic fungus, not necessary products of biosynthesis in plants. The classic example is taxol, which is actually a compound from an endophytic fungus

Pestalotiopsis microspora growing on *Taxus wallacbiana*, not necessarily produced by the plant itself (Strobel *et al*., 1996). Since tolerance to biotic stress has been correlated with endophytic fungal natural products, it is likely that *H. fomes* would be a rich source of endophytic fungi, which produce chemically diverse and biologically active secondary metabolites enhancing the host allelopathic effects, and providing protection against phytopathogenic microbes (Wangensteen et al. 2009; Patra and Thatoi, 2013).

In this context, the objective of the present study was to investigate the antimicrobial activities, particularly potential anti-MRSA property, of the endophytic fungi from *H. fomes* and to purify compounds responsible for the activity. Therefore, we here report on the isolation of endophytic fungi from *H. fomes* growing in the Sundarbans mangrove forest in Bangladesh, the antimicrobial properties of the organic extracts, isolation and structure elucidation of the principal antimicrobial secondary metabolites (oxysporone and xylitol) and assessment of their anti-MRSA potential.

MATERIALS AND METHODS

General. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Dorset, UK). All solvents for extraction and chromatography were purchased from Fisher Scientific, (Loughborough, UK). NMR solvents were from GOSS Scientific (Crewe, UK). Mass spectroscopic analyses were performed on a Finnigan MAT 95 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded at 600 MHz and 150 MHz, respectively on an Ultrashield Bruker AMX 600 NMR spectrometer. Methyl, methylene and methane carbons were distinguished by DEPT experiments. Homonuclear ^{1}H connectivity was determined by using the COSY experiment. ^{1}H-^{13}C one-bond connectivity was established with HSQC gradient pulse factor selection. Two- and three-bond connectivity was confirmed by HMBC experiments. Chemical shifts are reported in δ (ppm) and coupling constants (*J*) were measured in Hz.

Collection of plant materials, and isolation and identification of endophytic fungi. Healthy leaves, bark and roots of *Heritiera fomes* Buch. Ham. were collected from the mangrove forest Sundarbans, Bangladesh. The samples were kept in tightly sealed polythene bags under humid conditions at room temperature. The plant was identified during collection following their morphological characters as outlined by Zabala (1990), and a voucher specimen (TRN-KU-2017011) of this collection has been retained at the herbarium of the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh. The isolation of the fungal endophytes commenced within 24 h of collection.

Prior to isolation of endophytes, the plant material was surface-sterilized. The bark, roots and leaves were washed thoroughly with water for 8, 10 and 3 min, respectively, immersed in 70% ethanol for 1-2 min, 5.25 % Sodium hypochlorite for 1-2 min and again in 70% ethanol for 30-60 sec (Pattanaik *et al*., 2008). Finally, the surface-sterilized plant parts were washed with sterilized distilled water and allowed to dry inside a laminar flow cabinet. Small pieces of tissue were cut from the surface-sterilized plant materials and placed on dishes with water agar (WA). The endophytic fungi that immerged from the tissues were transferred on to potato dextrose agar (PDA) dishes and sequential sub culturing was performed until pure cultures were obtained.

For calculation of colonization frequency, the number of endophytic fungi found in the water agar was calculated with the total number of tissue placed on the agar plate. The colonization frequency percentage and the dominant fungi percentage of the endophytic fungi

was calculated using the method described by Kumar, *et al*. (2009) utilizing the following equations.

Colonization frequency % = [(Number of segments colonized by an endophyte) / (Total number of segments analysed)] x 100, and
Dominant fungi % = [(Number of isolates collected from the samples) / Total number of bark)] x 100

Colony morphological features of isolated endophytic fungal strain were recorded, and examined under microscope leading to identification as a *Pestalotia* sp.

Extraction of secondary metabolites. Each pure fungal culture was grown in (5 × 250 mL) conical flasks containing potato dextrose broth (PDB) for around 28 days. Culture broths were separated from the mycelium by filtration, and the culture filtrates were extracted three times with an equal volume of ethyl acetate (EtOAc) in a separating funnel. The EtOAc layer was evaporated to dryness under reduced pressure at 40-45°C using a rotary evaporator to obtain a crude broth EtOAc extract. The mycelium was dried and extracted by methanol (MeOH) and filtered. The MeOH layers was evaporated to dryness under reduced pressure at 50-55°C using a rotary evaporator to obtain a crude broth methanolic extract.

Initial antimicrobial screening. The EtOAc and MeOH extracts of the endophytic fungi *Pestalotia* sp were screened for their potential antimicrobial activity against two Gram-positive, i.e., *Staphylococcus aureus* (NCTC 12981) and *Micrococcus luteus* (NCTC 7508) two Gram-negative, i.e., *Escherichia coli* (NCTC 12241) and *Pseudomonas aeruginosa* (NCTC 12903), bacterial strains, as well as against a fungal strain, *Candida albicans* (ATCC 90028) using the resazurin 96-well microtitre plate based *in vitro* antimicrobial assay (Sarker et al., 2007). These microbial strains were selected for initial screening because they are easily available, easy to grow, representative of two Gram stains and a fungus, provide a clear idea about potential antimicrobial activity of test samples, and proven and established organisms for initial antimicrobial screening as outlined in numerous publications.

All bacterial strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37^0C prior to MIC determination (resazurin assay). Ciprofloxacin was used as a positive control for bacterial strains, and nystatin for *C. albicans*. Resazurin solution, prepared by dissolving 4 mg

of resazurin in 20 mL of sterile distilled water, was used in this assay as an indicator of cell growth, and the method was as described by Sarker et al. (2007).

Briefly, plates were prepared under aseptic conditions. A sterile 96 well plate was labelled. A volume of 100 µL of test material in 10% (v/v) DMSO (10 mg/mL for crude extracts) was pipetted into the first row of the plate. To all other wells 50 µL normal saline was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. To each well of 30 µL nutrient broth and 10 µL of resazurin indicator solution was added. Finally, 10 µL of bacterial suspension (5×10^5 cfu/mL) was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control (usually ciprofloxacin in serial dilution), a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead. The plates were prepared in triplicates, and placed in an incubator set at 37°C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial strain.

Assessment of anti-MRSA activity. Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich Company Ltd., UK. Cation-adjusted Mueller-Hinton broth was obtained from Oxfoid and was adjusted to contain 20 and 10 mg/L of Ca^{2+} and Mg^{2+}, respectively. The *S. aureus* strains used in this study included ATCC 25923, SA-1199B, XU212, MRSA340702, and EMRSA-15. ATCC 25923 is a standard laboratory strain sensitive to antibiotics like tetracycline (Gibbons and Udo, 2000). SA-1199B overexpresses the NorA MDR efflux pump (Kaatz et al., 1993). XU212 is a Kuwaiti hospital isolate that is a MRSA strain possessing the TetK tetracycline efflux pump (Gibbons and Udo, 2000), whilst the EMRSA-15 strain (Richardson and Reith, 1993) was epidemic in the UK. All were obtained from the National Collection of Type Cultures (NCTC).

An inoculum density of 5X 10^5 colony-forming units of each bacterial strain was prepared in normal saline (9 g/L) by comparison with a 0.5 MacFarland turbidity standard. The inoculum (125 µL) was added to all wells, and the microtitre plate was incubated at 37^0C for the corresponding incubation time. For MIC determination, 20 µL of a 5 mg/mL methanolic

solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each of the wells and incubated for 20 min. Bacterial growth was indicated by a colour change. The minimum inhibitory concentrations (MICs) were determined using broth microdilution method according to National Committee for Clinical Laboratory Standards with modification using Nutrient broth as the medium. Norfloxacin, a well-known antibiotic, was used as the positive control.

Large-scale fermentation and extraction of secondary metabolites. The endophytic fungus *Pestalotia* sp, as its EtOAc and MeOH extracts showed significant antimicrobial activity, was subjected large-scale fermentation. The fungus was grown on PDA media for 3 days. The mycelium (5 mm) was transferred to 40 x 250 mL conical flasks containing the media. The flasks were placed in continuous shaking for around 28 days at 180 RPM at room temperature. The mycelium was separated from the media by filtration and extracted by equal volume of EtOAc. The mycelium then dried, extracted by MeOH and filtered. Both EtOAc and MeOH extracts were dried using a rotary evaporator.

Fractionation, isolation and structure elucidation of the bioactive compounds (oxysporone and xylitol). Reversed-phase analytical HPLC analysis of the EtOAc and the MeOH extracts, using a Phenomenex C_{18} reversed-phase column (250 mm x 4.6 mm; particle size 5 μm) on a Dionex Ultimate 3000 analytical HPLC-coupled with a photo-diode-array detector (mobile phase: standard gradient of 30-100% MeOH in water over 30 min, flow rate: 1 mL/min), revealed the presence of only two major metabolites (>90% of the extract), one in each extract. Therefore, the crude EtOAc and MeOH extracts were investigated directly by 1D and 2D NMR and MS spectral data analyses, revealing the identity of the major compounds as xylitol (**1**) from the EtOAc extract and oxysporone (**2**) from the MeOH extract.

RESULTS AND DISCUSSION

Pestalotia sp (family: Amphisphaeriaceae), which is a secondary pathogen, saprophytic on dead and dying tissues and weakly parasitic infecting wounds under moist conditions, was successfully isolated and identified as one of the major endophytic fungal species growing in various parts, *e.g.,* leaves, bark and roots of the mangrove plant *Heritiera fomes*. The identification of *Pestalotia* sp was based on its macroscopic and microscopic morphological features (**Figure 1**). The frequency of endophytic fungal growth was quite variable in various parts; from the primary culture (water agar medium) of fungi, the colonization frequency percentage and dominant fungi percentage were calculated using the method described by Kumar *et al.* (2009) and the frequencies of endophytic fungal growth in the water agar medium were 100, 60 and 67% in leaves, bark and roots, respectively.

(a) (b) (c)

Figure 1: (a) *Pestalotia* sp on PDA medium (b) Spore of the fungi at 100x (c) Mycelium of the fungi.

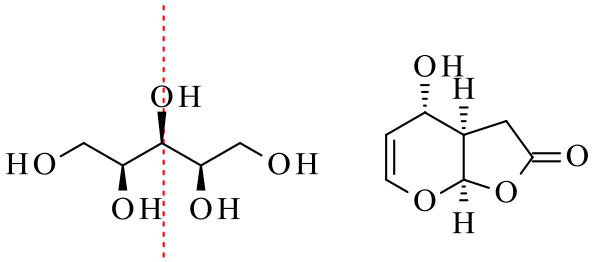
Large-scale culture and subsequent extraction of *Pestalotia* sp with EtOAc and MeOH yielded 0.6 and 1g of crude EtOAc and MeOH extracts, respectively. Initial assessment of the antimicrobial activity of the extracts using the resazurin microtitre assay (Sarker et al., 2007) demonstrated significant antimicrobial activity against all tested microorganisms (Table 1) with MIC values ranging from 0.00024 mg/mL to 0.25 mg/mL, except that the EtOAc extract was inactive against *E. coli*. The most remarkable activity was observed with the MeOH extract against *P. aeruginosa* (MIC = 0.00024 mg/mL), which was approximately half the potency of the positive control, ciprofloxacin (MIC = 0.00012207 mg/mL).

Table 1. Antimicrobial activity of the EtOAc and MeOH extracts of *Pistalotia* sp

Test organisms	Minimum inhibitory concentration (MIC) in mg/mL			
	Positive controls		Extracts	
	Ciprofloxacin	Nystatin	EtOAc	MeOH
Candida albicans (ATCC 90028)	NT	0.00097656	0.25	0.062
Escherichia coli (NCTC 12241)	0.00048828	NT	NA	0.25
Micrococcus luteus (NCTC 7508)	0.00097656	NT	0.25	0.062
Pseudomonas aeruginosa (NCTC 12903)	0.00012207	NT	0.125	0.00024
Staphylococcus aureus (NCTC 12981)	0.00097656	NT	0.25	0.125

NT = Not tested; NA = No activity at the highest test concentration

As both extracts showed significant antimicrobial activities, they were analysed by reversed-phase analytical HPLC coupled with a photo-diode-array detector. Each extract showed the presence of one major peak, accounting for >90% of the detected peaks in the extracts. However, the peak observed for the EtOAc extract almost co-eluted with the solvent front, right at the beginning of the chromatogram. Analytical HPLC analysis suggested that there was no need for further purification as it seemed the major compounds present in the extracts were of sufficient purity for further analysis. Therefore, the crude EtOAc and MeOH extracts were investigated directly by 1D and 2D NMR and MS spectral data analyses, revealing the identity of the major compounds as xylitol (**1**) from the EtOAc extract and oxysporone (**2**) from the MeOH extract (**Figure 2**). NMR and MS spectroscopic analyses established that xylitol (**1**) constituted more than 95% of the EtOAc, and as this compound does not have any chromophore, this compound was not really observed in the HPLC chromatogram of the EtOAc extract where PDA UV-Vis detector was used. The peak observed in the HPLC chromatogram of EtOAc extract was not due to xylitol (**1**), but for some highly UV sensitive minor compounds (<5%) of highly polar phenolic or polyphenolic compound(s) which almost co-eluted with the solvent front. Thus, the NMR and MS, together with the HPLC data, have established that the crude EtOAc and MeOH extract had >95% pure xylitol (**1**) and oxysporone (**2**), respectively, requiring no additional purification process, *e.g.*, chromatography, to obtain these compound in high yield and high purity directly from the crude (broth) extract.



1 **2**

Figure 2. Xylitol (**1**) and oxysporone (**2**) from *Pestalotia* sp

The ESIMS (positive and negative ion modes) analyses of the EtOAc extract comprising mainly one compound (**1**) revealed the $[M + H]^+$ ion at *m/z* 153 and the [M-H]- ion at *m/z* 151 confirming the molecular formula $C_5H_{12}O_5$ for the compound present in the EtOAc extract. The ^{1}H NMR spectrum (**Table 2**) showed several highly overlapped peaks in the region δ_H 3.60-3.80 ppm, assignable to oxymethine and oxymethylene protons, indicating that the compound was a polyhydroxylated compound. The ^{13}C NMR spectrum (**Table 2**) showed signals for only three carbons in the region δ_C 63.7-71.6 ppm, and a DEPTQ ^{13}C NMR revealed that two of the three signals (δ_C 70.0 and 71.6 ppm) were oxymethine carbons and one was an oxymethylene (δ_C 63.7 ppm), but there was no quaternary or methyl carbon. The ^{1}H-^{13}C HSQC NMR spectrum (**Table 2**) established the direct ^{1}H-^{13}C correlations and helped assignment of the overlapped protons. The number of ^{13}C NMR signals and their associated ^{1}H NMR signals could not support any three carbon based chemical compound, and also could not satisfy the MS data. The only way the situation could be explained was with the presence of a symmetry, where there were two sets of identical carbon atoms in the molecule. Therefore, instead of a compound containing three carbon atoms, it was actually a compound with five carbon atoms, and all carbon atoms were linked to oxygen atoms. Taking the NMR and MS data together, the compound could be identified as xylitol (**1**) (Biological Magnetic Resonance Data Bank, 2017), where a clear symmetry exists (**Figure 2**). The ^{1}H-^{13}C HMBC NMR spectrum established the ^2J and ^3J ^{1}H-^{13}C correlations (**Table 2**) and further confirmed the structure of xylitol (**1**). To the best of our knowledge, this is the first report on the isolation of xylitol from a *Pestalotia* sp.. However, this compound has previously been found in other fungal species, e.g., *Aspergillus* and *Candida* (Sampaio *et al*., 2003; Dalli *et al.*, 2017) and endophytic yeast (Buro *et al*., 2012).

Table 2. 1D and 2D NMR data of xylitol (1)

Position	Chemical shift δ in ppm		^{1}H-^{13}C HSQC direct correlation	^{1}H-^{13}C HMBC long-range correlations	
	^{1}H NMR[a] (Coupling constant *J* in Hz in parentheses)	^{13}C NMR[b]	^{1}J	^{2}J	^{3}J
1 and 5	3.62 and 3.78 m, 4H	63.7	C-1 and C-5	C-2 (C-4)	C-3
2 and 4	3.76 dt (6.9, 4.4), 2H	70.0	C-2 and C-4	C-1, C-3 (C-3, C-5)	C-4 (C-2)
3	3.66 m, 1H	71.6	C-3	C-2, C-4	C-1 (C-5)

[a]Obtained in CD_3OD, 600 MHz; [b]Obtained in CD_3OD, 150 MHz

The ESIMS (positive ion mode) analyses of the MeOH extract comprising mainly one compound (**2**) revealed the $[M + H]^+$ ion at *m/z* 157 confirming the molecular formula $C_7H_8O_4$ for the compound present in the MeOH extract. The ^{1}H NMR and ^{13}C NMR spectra of **2** (**Table 3**), revealed the presence of two olefinic methines (δ_H 6.46 and 5.05; δ_C 143.5 and 100.1), one highly deshielded oxymethine (δ_H 5.87; δ_C 96.0) and another oxymethine (δ_H 4.05; δ_C 60.0), a methine (δ_H 2.85; δ_C 41.8) and a methylene (δ_H 2.38 and 2.62; δ_C 29.5). The ^{13}C NMR spectrum (**Table 3**) also showed the presence of a lactone carbonyl signal at δ_C 175.3. All these data were comparable to the published NMR data for oxysporone (**2**) (**Figure 2**) (Adesogan and Alo, 1979; Venkatasubbaiah et al. 1991; Mazzeo et al., 2013). A combination of ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HSQC and HMBC NMR analyses further supported the identification of **2** as oxysporone and the unequivocal assignment of all data. Oxysporone (**2**) was first isolated from *Fusarium oxysporum* (Adesogan and Alo, 1979), but later, found in a number of other endophytic fungal strains including *Pestalotia* species (Sarker *et al.*, 2017). This is the first report on the isolation of *Pestalotia* sp growing on the mangrove plant, *Heritiera fomes*, as well as the isolation of oxysporone (**2**) from this endophytic fungus.

Table 3. 1D and 2D NMR data of oxysporone (2)

Position	Chemical shift δ in ppm		^{1}H-^{13}C HSQC direct correlation	^{1}H-^{13}C HMBC long-range correlations	
	^{1}H NMR[a] (Coupling constant *J* in Hz in parentheses)	^{13}C NMR[b]	^{1}J	^{2}J	^{3}J
2	6.46 d (6.0), 1H	143.5	C-2	C-3	C-4, C-6
3	5.05 ddd (6.0, 5.5, 1.0), 1H	100.1	C-3	C-2, C-4	C-5
4	4.05 dd (5.5, 2.0), 1H	60.0	C-4	C-3, C-5	C-6, C-7
5	2.85 m, 1H	41.8	C-5	C-4, C-6, C-7	C-3, C-8
6	5.87 d (4.5), 1H	96.0	C-6	C-5	C-7, C-8
7	2.38 and 2.62 dd (11.6, 9.2)	29.5	C-7	C-5, C-8	C-4, C-6
8	-	175.3	-	-	-

[a]Obtained in CD_3OD, 600 MHz; [b]Obtained in CD_3OD, 150 MHz

Once the structures of xylitol (**1**) and oxysporone (**2**) were confirmed, and their initial antimicrobial activity was assessed (Table 1), these compounds were subjected to evaluation of their potential anti-MRSA activity against five MRSA strains (**Table 4**), as the focus of this study was on anti-MRSA activity . Both compounds demonstrated considerable activity against all strains with MIC values ranging from 32-128 μM. Oxysporone (**2**) was most active against the MRSA strains, SA1199B and EMRSA-15 (MIC = 32 μM), and overall, was more active than xylitol (**1**). In fact, the anti-MRSA activity of oxysporone (**2**) against SA1199B and MRSA340702 was comparable with the positive control norfloxacin (**Table 4**).

Table 4. Anti-MRSA activity of xylitol (1) and oxysporone (2) from *Pistalotia* sp

Test organisms	Minimum inhibitory concentration (MIC) in μM		
	Positive control (Norfloxacin)	Test compounds	
		1	**2**
XU212	16	128	128
ATCC25923	2	128	64
SA1199B	32	64	32
EMRSA-15	1	64	32
MRSA340702	64	128	64

This is the first report on the anti-MRSA activity study on xylitol (**1**) and oxysporone (**2**). Bioactivity studies on oxysporone (**1**), surprisingly, have been limited to its phytotoxicity and antifungal property over the last few decades, despite the fact that it was first isolated as a potential cure for chronic dysentery (Adesogan and Alo, 1979; Andolfi et al., 2014; Sarker et al., 2017). Antimicrobial activity of *H. fomes* has previously been reported (Wangensteen et al., 2009), but only with the crude extracts. One might wonder, whether the antimicrobial activity, at least to some extent could be contributed by the antibacterial secondary metabolites, *e.g.*, xylitol (**1**) and oxysporone (**2**) produced by the endophytic fungus like *Pestalotia* sp growing in *H. fomes.* Xylitol (**1**) is a sugar alcohol, used as a sweetener in chewing gum, lozenges and nasal spray, and known to reduce the incidence of acute middle ear infection in healthy children, and to inhibit the growth of *Streptococcus pneumoniae*, as well as the attachment of *Haemophilus influenzae* on the nasopharyngeal cells (Reusens, 2004).

CONCLUSIONS

The present study has revealed that *H. fomes* harbours the endophytic fungus, *Pestalotia* sp. that produces potent antimicrobial substances, xylitol (**1**) and oxysporone (**2**). The findings of this study also suggests that endophytes from harsh and competitive environments, like mangrove eco system, might be an attractive source for new anti-infective compounds. Promising anti-MRSA activity shown by oxysporone (**2**) might be exploited further to generate analogues using oxysporone (**2**) structural template for anti-MRSA drug discovery and development. In fact, oxysporone (**2**) has a simple structure and the hydroxyl group at C-4 could provide an easy option for generating numerous synthetic analogues by simply incorporating various functionalities at C-4 whilst keeping the main skeleton intact.

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