

Initial Screening of Mangrove Endophytic Fungi for Antimicrobial Compounds and Heavy Metal Biosorption Potential

(Saringan Awal Kulat Bakau Endofit untuk Potensi Sebatian Antimikrob dan Bioserapan Logam Berat)

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ABSTRACT

Endophytic fungi provide protection to their host plant and the fungi often produce antimicrobial compounds to aid the host fighting off pathogens. These bioactive compounds were secondary metabolites which were often produced as waste- or by-products. In the present study, endophytic fungi isolated from mangrove plants and soils were characterized and their antimicrobial production and bioremediation potential of heavy metals copper (Cu) and zinc (Zn) were assessed. Twelve (12) isolated and identified endophytic fungi belonged to seven species; Penicillium, Curvularia, Diaporthe, Aspergillus, Guignardia, Neusartorya and Eupenicillium. Antimicrobial activities of these 12 fungal endophytes were tested against Gram negative bacteria; Bacillus subtilis, Staphylococcus aureus, Gram positive bacteria; Escherichia coli and fungi; Candida albicans and Aspergillus niger among others. Two isolates (related to Guignardia sp. and Neusartorya sp.) showed strong antimicrobial (and antifungal) activity whereas the rest showed no activity. Compounds were isolated from both isolates and screened using HPLC. Both isolates displayed chemically very interesting chromatograms as they possessed a high diversity of basic chemical structures and peaks over a wide range of polarities, with structures similar to Trimeric catechin and Helenalin among others. For bioremediation assessment, the results showed maximum biosorption capacity for two isolates related to Curvularia sp. and Neusartorya sp., with the former removing 25 mg Cu/g biomass and the latter removing 24 mg Zn/g biomass. Our results indicated the potential of mangrove endophytic fungi in producing bioactive compounds and also highlighted their potential for the treatment of heavy metal-contaminated wastewater.

Keywords: Antimicrobial; bioactive compounds; biosorption; endophytic fungi; heavy metals; mangroves

ABSTRAK

Kulat endofit memberi perlindungan kepada perumah mereka dan seringkali menghasilkan sebatian antimikrob untuk membantu perumah melawan patogen. Sebatian bioaktif ini adalah metabolit sekunder yang sering dihasilkan sebagai bahan buangan atau keluaran sampingan. Dalam kajian ini, kulat endofit yang diasingkan daripada tumbuhan bakau dan tanah telah dikelaskan dan pengeluaran serta potensi bioremediasi logam berat tembaga (Cu) dan zink (Zn) telah dinilai. Duabelas (12) kulat endofit telah dipencilkan dan dikenal pasti terdiri daripada tujuh spesies; Penicillium, Curvularia, Diaporthe, Aspergillus, Guignardia, Neusartorya dan Eupenicillium. Aktiviti antimikrob daripada 12 kulat endofit ini telah diuji terhadap Gram bakteria negatif; Bacillus subtilis, Staphylococcus aureus, Gram bakteria positif; Escherichia coli dan kulat; Candida albicans dan Aspergillus niger. Dua pencilan (daripada Guignardia sp. dan Neusartorya sp.) telah menunjukkan aktiviti antimikrob yang kuat (dan anti-kulat) sedangkan yang lain tidak menunjukkan sebarang aktiviti. Sebatian telah diasingkan daripada kedua-dua pencilan diasing dan disaring menggunakan HPLC. Kedua-dua pencilan menunjukkan kromatogram yang sangat menarik kerana mereka mempunyai kepelbagaian yang tinggi dalam struktur kimia asas dan punca kepelbagai polariti, dengan struktur yang sama dengan Trimeric catechin dan Helenalin antara lainnya. Bagi penilaian potensi bioremediasi, keputusan menunjukkan keupayaan bioserapan maksimum pada dua pencilan yang berkaitan dengan Curvularia sp. dan Neusartorya sp., dengan Curvularia sp. mengeluarkan 25 mg Cu/g biojisim dan Neusartorya sp. mengeluarkan 24 mg Zn/g biojisim. Keputusan kami menunjukkan potensi kulat endofit bakau dalam menghasilkan sebatian bioaktif dan juga menyerlahkan potensi mereka untuk rawatan air yang tercemar dengan sisa logam berat.

Kata kunci: Antimikrob; bakau; bioserapan; kompaun bioaktif; kulat endofit; logam berat

INTRODUCTION

Mangrove forests in Malaysia cover an estimated total acreage of 5650 km², constituting of about 4% of the world's mangroves (FAO 2007). They are unique for their well-known adaptation towards their extreme environmental conditions of high salinity, changes in sea

level, high temperatures and anaerobic soils (Shearer et al. 2007). As mangroves are situated at the interface between land and sea, they are directly affected by disturbances to both land and sea. Mangrove forests in Malaysia are for example threatened by heavy metal pollution, resulting from industrial waste water pollution and urbanization

(Ayub et al. 1998). All these harsh conditions make mangrove forests ideal environments in the hunt for novel and unique endophytic fungi (Xing et al. 2011). Endophytic fungi are commonly referred as fungi residing in living tissues of plants without causing any adverse effects towards the host plant itself (Kaul et al. 2008; Palombo et al. 2010). The relationship between endophytes and their hosts is, however, still poorly understood and an endophytic fungi strain can for example present itself as a pathogen; however still contribute to plant defense against damage by herbivores by rendering the plant less desirable as a food source (Rodriguez & Redman 2008). Several studies have been conducted on endophytic communities of mangrove plants found along the coastlines of the Indian, Pacific and Atlantic Ocean (Xing et al. 2011), however not along Sarawak coast. Mangrove endophytic fungi are increasingly recognized for their bioactive compounds production. Bioactive compounds isolated have been found to possess anti-cancer, anti-diabetic and many other properties that were useful in biomedical research and drug development (Lu et al. 2010; Strobel & Daisy 2003; Wong et al. 2015a).

Besides being producers of bioactive compounds, endophytic fungi have also been found capable of removing heavy metal ions from water. The very common *Aspergillus niger* for example has been shown capable of removing lead, cadmium, copper and nickel ions from waste water (Kapoor et al. 1999). Members of the *Pestalotiopsis* have been found to biosorb copper, lead, chromium and zinc (Choo et al. 2015) and *Xylaria* sp. and *Phomopsis* sp. have been shown to be able to biosorb copper using dead as well as living biomass (Wong et al. 2015b). Heavy metals are bioaccumulative and toxic as they do not readily degrade in the environment. Accumulation in the human or ecological food chain can be hazardous to humans and the environment (Tumin et al. 2008).

Biosorption systems involve the use of microbial cells such as algae, fungi and bacteria (live or dead) to absorb and accumulate heavy metals and can thus reduce environmental contamination (Say et al. 2003). The advantages of biosorption include a high efficiency, cost effectiveness, the possibility of recovering the metal of interest and regeneration of the biosorbent (Kratochvil & Volesky 1998).

This study aimed to identify endophytic fungi associated with the mangrove plant *Avicennia* sp.; to evaluate their antimicrobial compounds; and to assess their potential to biosorb the heavy metals copper (Cu) and zinc (Zn).

MATERIALS & METHODS

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI

The collected plant materials (*Avicennia* sp.) were cut into 1 cm long fragments before being surface sterilized by immersing them sequentially in 70% ethanol for 3 min and 0.5% sodium hypochlorite for 1 min. Thereafter, the

fragments were rinsed thoroughly with sterile distilled water and surface-dried before being placed onto Potato Dextrose Agar (PDA) (Difco). The plates were incubated at 28°C for 1 week. After incubation period, hyphal tips of fungi growing out from the plant fragments were transferred to new PDA plates.

The endophytic fungi were identified based on molecular characterization. Genomic DNA was extracted from 5-day old fungi cultures grown on plates using a modified thermolysis method (Zhang et al. 2010). Fungal DNA was amplified using universal primers of fungal DNA ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). Each sample ready for amplification contained 2 µL of 10× PCR buffer (Fermentas), 1.2 µL of dNTP mixture (2.5 mmol l⁻¹ each), 0.8 µL of deionized formamide, 0.4 µL of MgCl₂ (25 mmol l⁻¹), 0.8 µL of each primer (10 µmol l⁻¹), 0.2 µL of Taq DNA polymerase (5 U µL⁻¹) and 1 µL of genomic DNA (20 ng/mL) in a total volume of 20 µL. DNA fragments were purified using Pure Link PaCR purification kit (Invitrogen, U.S.) and then sequenced, followed by analysis using the Basic Local Alignment Search Tool (Altschul et al. 1990), Chromas 2.22 and MEGA 5 (Tamura et al. 2011). Phylogenetic reconstruction was carried out based on maximum likelihood method according to the Tamura-Nei model and dendrograms for fungi were generated (Figure 1).

SCREENING OF ANTIMICROBIAL ACTIVITY

For the antimicrobial assay, test organisms used were: *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* (Gram positive bacteria); *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio anguillarum* (Gram negative bacteria) and *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* (Fungi). The test organisms were prepared in selected medium broth and incubated at 30°C for 24 h. Cylindrical pieces of 1 × 1 cm size (agar plugs), were cut from well grown and sporulated culture of one week old fungi strains. These pieces were placed on the agar previously streaked with test organisms. For the antibacterial activity, plates were incubated for 24 h. For antifungal activity, agar plugs of the investigated fungi strains were placed opposite of the fungi test pathogen and incubated for one week at 25°C. Inhibition zones were measured after the incubation period. All tests were done in triplicates.

EXTRACTION OF BIOACTIVE COMPOUNDS

A single cylindrical block (agar plug) from well grown and sporulated fungal cultures was inoculated into 20 mL of potato dextrose broth (PDB) and incubated for one week at 25°C. After the incubation period, 20 mL of ethyl acetate were added into the broth and left standing for 2 h. Then the mixture was filtered. The filtrate was then centrifuged at 8000 rpm for 10 min and the top layer (Ethyl acetate phase) was removed and transferred to new tubes. The extraction

was repeated three times. The ethyl acetate extract was then dried to give a solid and oily residue and the dried extract was stored at -20°C until further use.

HEAVY METAL BIOSORPTION BY DEAD FUNGAL CELLS

To obtain dried biomass, dead fungal cells were dried and then grind using mortar and pestle to obtain 0.1 g and then passed through a 0.45 µm sieve to standardize the particle size. For the biosorption assay, 0.1 g of the prepared dried biomass was then inoculated into the working standards (heavy metal ion solution) and incubated at 150 rpm and 30°C for 72 h in the dark. Samples were filtered using sterile filter paper (Whatman filters No.1) and cell-free filtrates obtained were analyzed for the remaining Cu (µg/mL) using atomic absorption spectrometry (AAS) (Kannan et al. 2011). Bioadsorption capacity was measured based on the amount of metal ions (mg) bioadsorbed per gm (dry mass) of biomass calculated using the following equation:

$$Q = [(C_i - C_f)/m] V,$$

where Q is the mg of metal ion bioadsorbed per gm of biomass; C_i is the initial metal ion concentration, mg/L; m is the mass of biomass in the reaction mixture gm; and V is the volume of the reaction mixture (L).

RESULTS AND DISCUSSION

IDENTIFICATION OF ENDOPHYTIC FUNGI

A total of 12 endophytic fungi were isolated from the plant samples (*Avicennia* sp.). The 12 isolates were identified and found belonging to seven families; *Penicillium*, *Curvularia*, *Diaporthe*, *Aspergillus*, *Guignardia*, *Neosartorya*, *Cladosporium* and *Eupenicillium* (Figure 1). Indeed, the fungi population isolated from the species *Avicennia* sp. commonly consists of *Penicillium*, *Curvularia* and *Aspergillus* as reported by Madavasamy and Panneerselvam (2012). Naikwade et al. (2012) reported on a total of 17 species of fungi being isolated from leaves of the mangrove plant *Cerriopstagal*, out of which nine fungal species belonged to *Aspergillus*, making it the dominant genus. Some species of *Penicillium* were well known for their activities to produce antibiotics (Phuwiwat & Soyong 2001). *Aspergillus flavus*, isolated from mangrove plant *Avicennia officinalis*, was associated with antioxidant potency, which might be responsible for the mutualistic association of plant and endophyte against various biotic and abiotic stresses (Ravindran et al. 2012). Isolate 6 was grouped with *Aspergillus* sp. Da91 (Figure 1) however it was the only isolate among twelve belonging to the genus *Aspergillus*. *Avicennia* species therefore seem to harbour distinctively different endophytic fungal communities.

Curvularia sp. is one of the marine-derived fungi, which have been known as rich source of biologically active secondary metabolites for instance lunatin and curvularin (Geetha et al. 2011). It has also been reported

by Madavasamy and Panneerselvam (2012) as one of the endophytic fungi out of twenty two species isolated from the leaves of *Avicennia marina*. Isolate 2 was identified as *Curvularia* sp. based on the similarity comparison of ITS sequences (Figure 1). *Curvularia* sp. has been reported to possess the potential of degrading polycyclic aromatic hydrocarbons (PAH) a group of environmental pollutants that can be found as contaminants at industrial sites, especially those associated with petroleum or gas production and wood preserving processes (Juckpech et al. 2012). Endophytic fungi seem to hold promise for bioremediation of plastic compounds as also recently shown by Bong et al. (2015) who obtained endophytic *Pestalotiopsis* from pitcher plants capable of degrading polyurethane, PUR.

Isolates 3 and 4 were linked to *Diaporthe* sp. (Figure 1), which is an important fungal group able to degrade fibre and commonly derived from marine algae, mangrove plants, seawoods and rotten wood (Lin et al. 2005). This genus has commonly found in mangrove fungal communities and has been described as an antibiotic producer (Sebastianes et al. 2012).

The strain *Guignardia* sp. was isolated for the first time from *Undaria pinnatifida*, a type of seaweed in Changdao Sea (Wang 2012). The genus *Guignardia* is also one of the endophytic fungi commonly isolated from mangrove forests and known for their cytotoxic activities (Bhimba et al. 2011). Isolate 7 was grouped with *Guignardia camelliae* strain B-15 [JQ086349] (Figure 1).

The genus *Neosartorya* (family *Trichocomaceae*) was first established by Malloch and Cain in 1972 to allow teleomorphs of species belonging to the *Aspergillus fischeri* series of the *Aspergillus fumigatus* species group (Varga et al. 2000). This genus was reported with a higher frequency of occurrence (%) in rhizome (11.1%) compared to in stems (3.7%) of mature *Cyperus malaccensis* which dominate about one-third of estuaries and mangroves (Karamchand et al. 2009). In this study, both Isolate 8 and Isolate 13 (*Neosartorya hiratsukae*, Figure 1) were found in roots of *Avicennia*.

The genus *Eupenicillium* was introduced by Ludwig in 1892 for an ascomycete species (Houbraken & Samson 2011). It also belongs to the family, *Trichocomaceae* (Aly et al. 2010), similar to the genus *Neosartorya*. *Trichocomaceae* comprise of a relatively large family of fungi, with the most well-known species belonging to the genera *Aspergillus*, *Penicillium* and *Paecilomyces*. They were well-known for their secretion of secondary metabolites that are known as mycotoxins while others were used as pharmaceuticals, including antibiotics such as penicillin (Houbraken & Samson 2011). Isolate 9 was related to *Eupenicillium* sp. 5 JH-2010 (Figure 1); however, it did not show antimicrobial activity in our tests.

The genus *Cladosporium* is one of the largest genera of dematiaceous hyphomycetes where most of the species belonging to this genus are characterized by a coronate scar structure (Bensch et al. 2010). *Cladosporium cladosporioides* was reported as an endophyte isolated from leaves of the mangrove plant *Rhizophora apiculata*

(Kumaresan & Suryanarayanan 2002). Besides, as reported earlier on *Curvularia*, *Cladosporium* has also been reported by Madavasamy and Panneerselvam (2012) as one of the endophytic fungi isolated from the leaves of *Avicennia marina*. Isolate 12 was related to *Cladosporium sphaerospermum* strain SCSGAF0054 confirming previous findings and indicating a common distribution of *Cladosporium* in *Avicennia*.

SCREENING FOR ANTIMICROBIAL COMPOUNDS

Two isolates out of the twelve showed antibacterial and antifungal activity; Isolate 7 (*Guignardia camelliae*) showed antibacterial activity against Gram positive bacteria

(*Bacillus cereus* and *Bacillus subtilis*) and Gram negative bacteria (*Vibrio anguillarum*) and *Neosartorya hiratsukae* showed antibacterial activity against Gram positive bacteria (*Micrococcus luteus*) and antifungal activity against fungi (*Candida albicans*; Table 1). This shows that the antibacterial activity of the isolates were more common towards the Gram positive bacteria compared to Gram negative bacteria indicating a higher resistance level towards Gram negative compared to Gram positive as also reported by Alias et al. (2010). Several studies support the results obtained. *Guignardia* sp., an endophyte isolated for the first time from the plant *Undaria pinnatifida*, was reported to possess antibacterial and antifungal activity

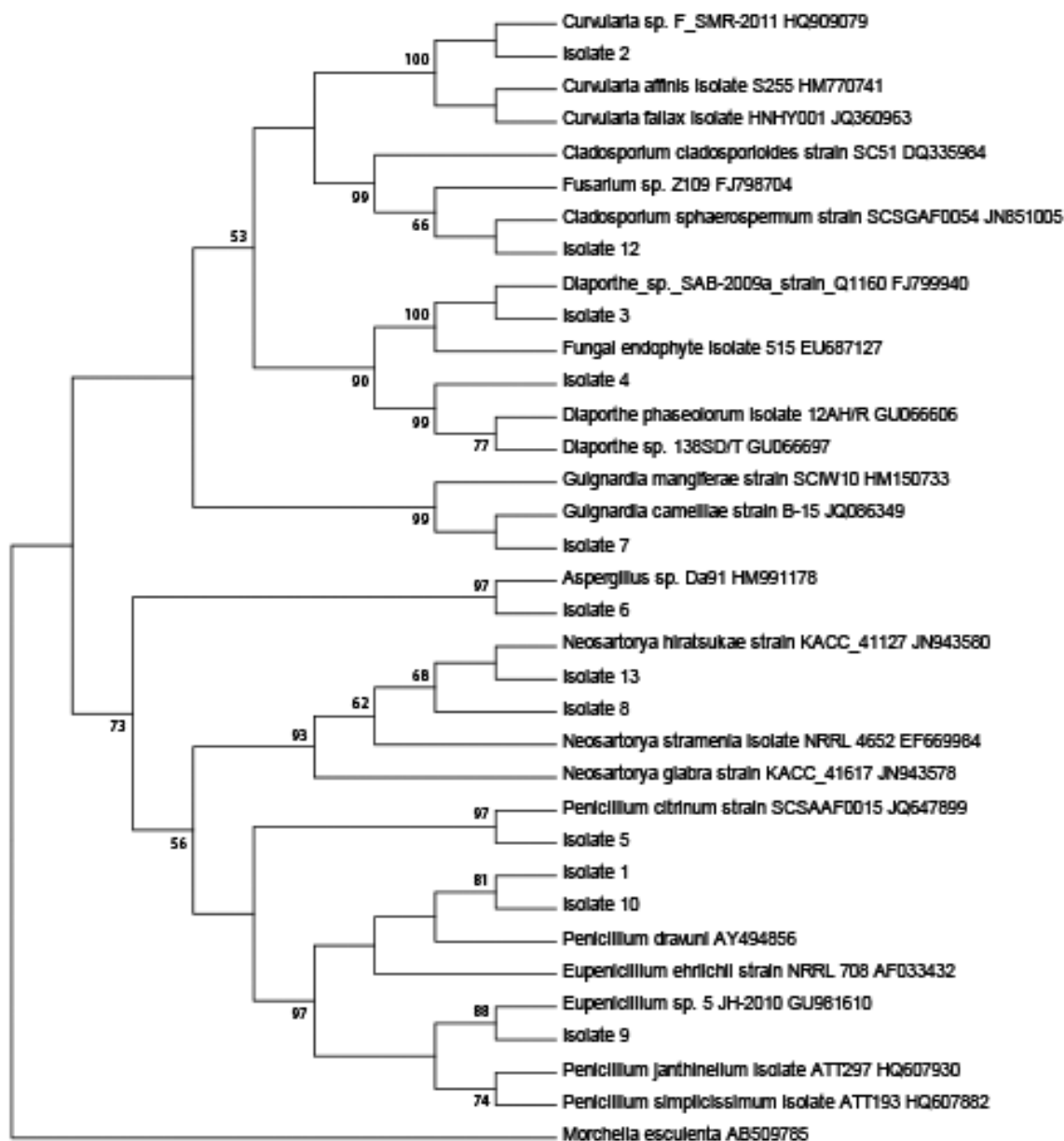


FIGURE 1. 18S gene-based phylogenetic tree representing the twelve endophytic fungal isolates. The phylogenetic tree was generated with distance methods, and sequence distances were estimated with the neighbour-joining method. Bootstrap values ≥ 50 are shown and accession numbers for the reference sequences are indicated

TABLE 1. Antimicrobial activity of endophytic fungi strains

| | Zone of inhibition (mm) (Mean \pm SD) | | | | | | | | | |
|-------------------|---|--------------------|-----------|--------------------|-----------|-----------|--------------------|---------------------|-----------|-----------|
| | BC | BS | SA | ML | EC | PA | VA | CA | SC | AN |
| <i>Isolate 7</i> | 7.33 \pm 0.58 | 7.00 \pm 1.00 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 7.67 \pm 0.58 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| <i>Isolate 13</i> | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 9.67 \pm 1.53 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 10.67 \pm 0.58 | 0 \pm 0 | 0 \pm 0 |

Zone of inhibition is measured in mm and the Mean \pm Standard Deviation are displayed. Only the strains that showed activity are displayed, the others have been omitted for reasons of clarity. BC: *Bacillus cereus*; BS: *Bacillus subtilis*; SA: *Staphylococcus aureus*; ML: *Micrococcus luteus*; EC: *Escherichia coli*; PA: *Pseudomonas aeruginosa*; VA: *Vibrio anguillarum*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; AN: *Aspergillus niger*

(Wang 2012). Galgoczy et al. (2011) reported a novel antifungal peptide isolated from *Neosartorya fischeri* and this antifungal peptide exhibited high antifungal activity against filamentous fungi within broad pH and temperature ranges. Both, *Guignardia camelliae* and *Neosartorya*

hiratsukae, were then fermented and their bioactive compounds extracted.

Both isolates displayed chemically very interesting HPLC chromatograms as they possess a high diversity of basic chemical structures and peaks over a wide

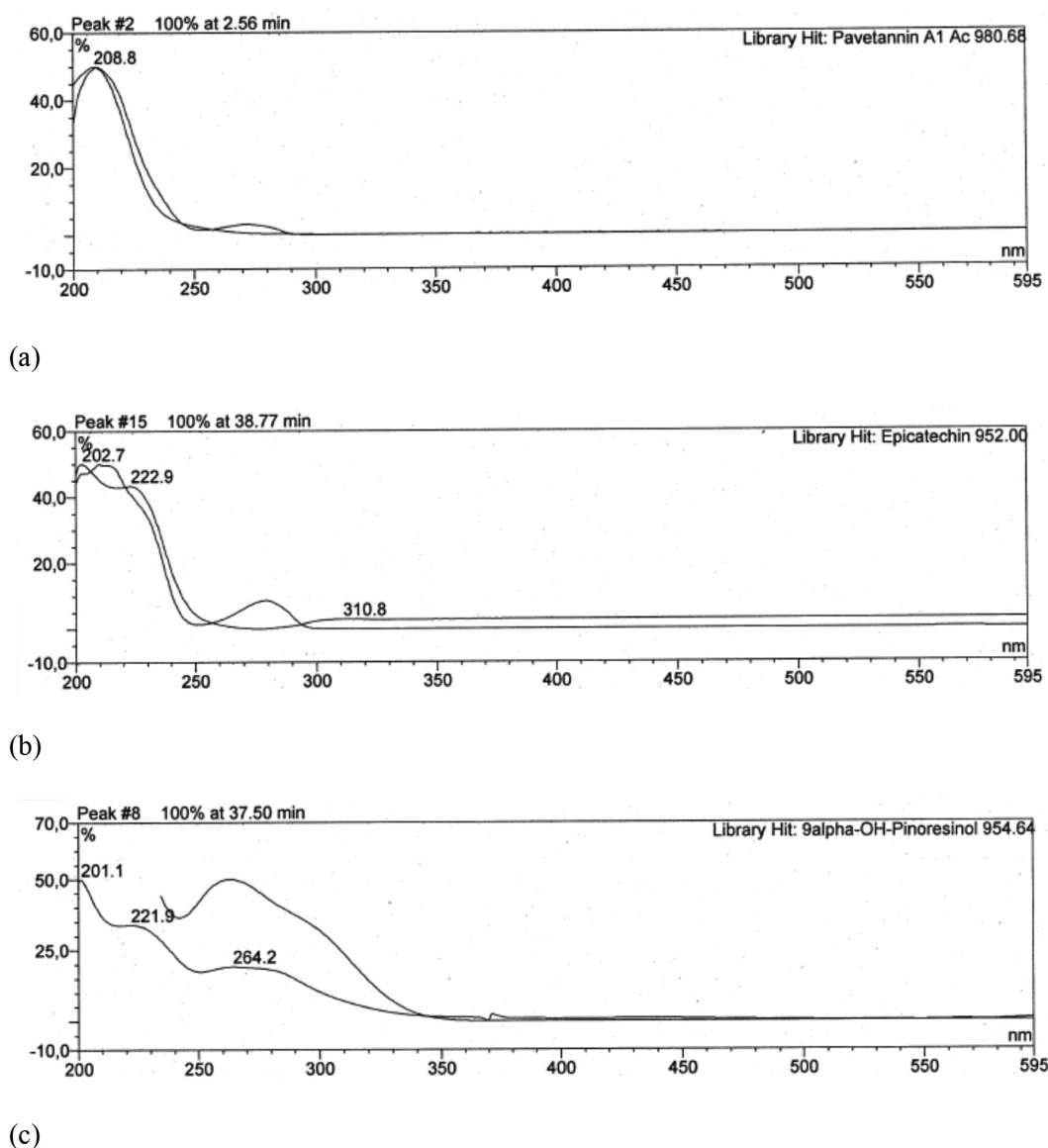


FIGURE 2. HPLC chromatograms of compounds from Isolate 7 that had similar structures to (a) Pavetannin A1 Ac, (b) Epicatechin and (c) 9alpha-OH-Pinoresinol. Chromatograms were recorded at 235 nm and library hits are indicated at the top right of the picture

range of polarities, with structures similar to a variety of compounds. In the ethyl acetate extract of *Guignardia camelliae*, three compounds with structures similar to Pavetannin A1 Ac (with a retention time of 2.56 min, Figure 2(a)), Epicatechin (with a retention time of 38.77 min, Figure 2(b)), and 9 α -OH-Pinoresinol (with a retention time of 37.50 min, Figure 2(c)) were identified. Pavetannin A1, has previously been reported from studies on antiviral properties of *Pavetta wariensis* and showed activity against *Herpes simplex* (Arnasan et al. 1995). Epicatechin is a flavanoid that has been reported to be responsible for antibacterial activity against Gram-positive and Gram-negative bacteria. This compound was isolated by Masika et al. (2004) from *Schotialatifolia*, a plant commonly used in folkloric medicine. 9 α -OH-Pinoresinol was reported as a lignin with anticancer activity

(Chunsriimyatav et al. 2009). These findings might help explain why *Guignardia camelliae* shows activity towards a wide range of organisms (Gram positive and Gram negative bacteria).

The ethyl acetate extract of *Neosartorya hiratsukae* also contained three compounds that displayed structures similar to known ones; Trimeric Catechin with a retention time of 37.53 min (Figure 3(a)), Epicatechin with a retention time of 38.76 min (Figure 3(b)) and Helenalin with a retention time of 40.88 min (Figure 3(c)).

Neosartorya hiratsukae was found to display antibacterial activity against Gram-positive bacteria (*Micrococcus luteus*) which might again be attributed to the compound with a similar structure as epicatechin. Trimeric catechin is catechin in its trimeric form (also known as oligomeric form). Catechins are polyphenols

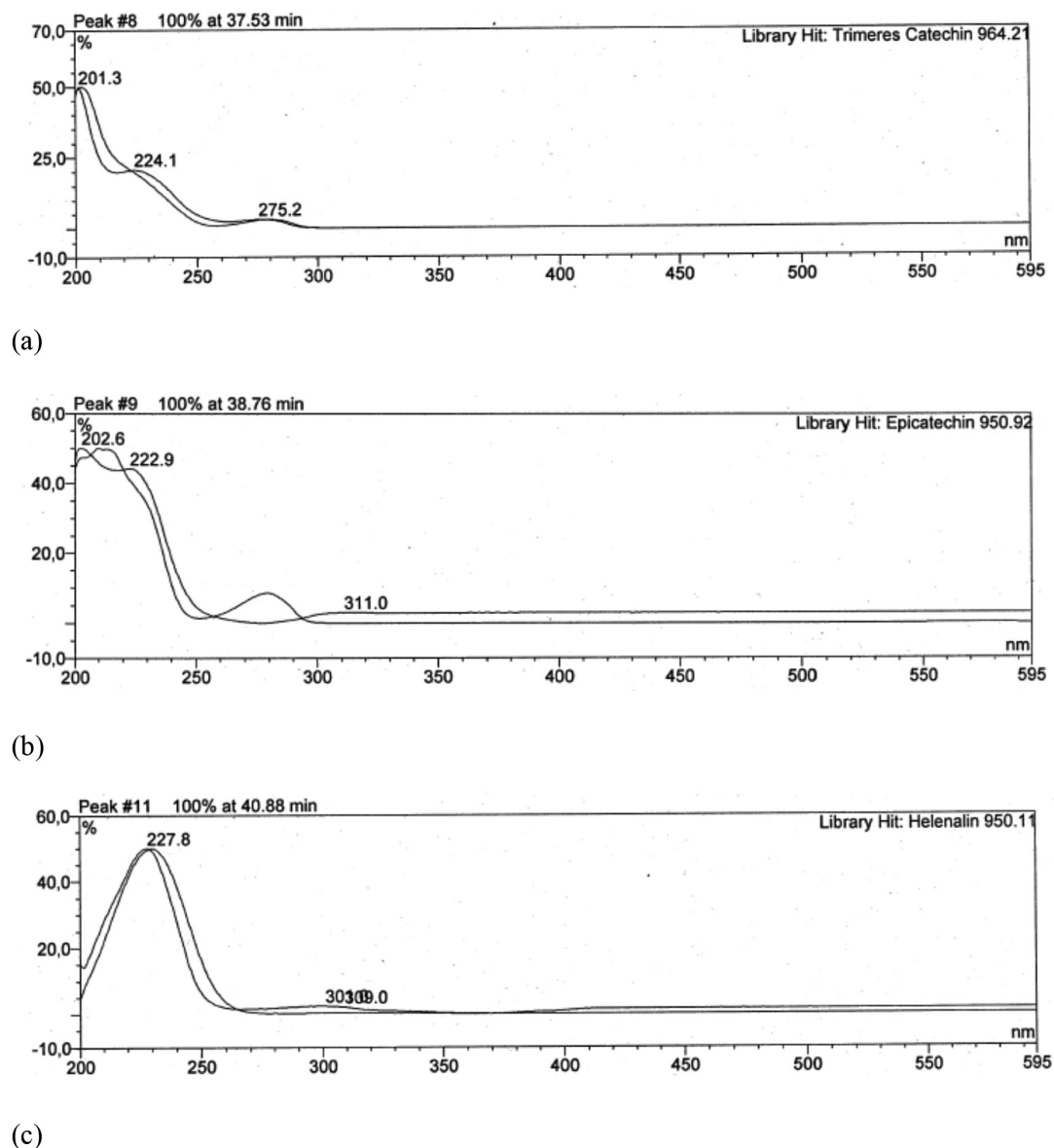


FIGURE 3. HPLC chromatograms of compounds from Isolate13 that had similar structures to (a) Trimeric Catechin, (b) Epicatechin and (c) Helenalin. Chromatograms were recorded at 235 nm and library hits are indicated at the top right of the picture

TABLE 2. Cu Biosorption capacity by dead fungal cells

| | Fungal strains | | | | | | | | | | | |
|---------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|------------|
| | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 | Isolate 5 | Isolate 6 | Isolate 7 | Isolate 8 | Isolate 9 | Isolate 10 | Isolate 12 | Isolate 13 |
| Q value | 18 | 25 | 11 | 5 | 8 | 1 | 8 | 15 | 4 | 0 | 15 | 7 |

TABLE 3. Zn Biosorption capacity by dead fungal cells

| | Fungal strains | | | | | | | | | | | |
|---------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|------------|
| | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 | Isolate 5 | Isolate 6 | Isolate 7 | Isolate 8 | Isolate 9 | Isolate 10 | Isolate 12 | Isolate 13 |
| Q value | 3 | 6 | 8 | 15 | 16 | 21 | 16 | 24 | 14 | 16 | 14 | 24 |

and components of condensed tannins which display antibacterial activity by precipitating proteins of pathogenic bacteria through direct binding (Shimamura et al. 2007). Besides, catechin was also reported to possess antifungal activity against *Candida albicans* (Hirasawa & Takada 2004). These findings were in agreement with our results as *Neosartorya hiratsukae* displayed activity against Gram positive bacteria as well as fungi. Helenalin, a sesquiterpene lactone was reported commonly isolated from plant families such as *Acanthaceae*, *Anacardiaceae*, *Apiaceae*, *Euphorbiaceae*, *Lauraceae* and *Magnoliaceae* (Chaturvedi 2011) with anti-inflammatory and antineoplastic activity.

HEAVY METAL BIOSORPTION

Based on Tables 2 and 3, three isolates were observed with maximum biosorption capacity, with *Curvularia affinis* in removing 25 mg Cu/g biomass (Table 2) and two other strains, *Neosartorya stramenia* and *Neosartorya hiratsukae* strains in removing 24 mg Zn/g biomass (Table 3). *Curvularia affinis* was found to be the most efficient in removing Cu/g biomass, however, to the best of our knowledge, this is the first report on the ability of *Curvularia* species in removing heavy metal using dead biomass.

Neosartorya stramenia and *Neosartorya hiratsukae* showed highest efficiency in Zn/g biomass removal (Table 3). Heavy metal removal using non-living biomass is less complicated, due to the absence of metabolic activity, hence this might explain for the close proximity of heavy metal removal capabilities for the same genus but different isolates. However, findings by Simonovicova (2008) reported results on non-living biomass of *Neosartorya fisheri* having the highest efficiency of removing Cu and the lowest efficiency in removing Zn.

CONCLUSION

Our results indicated the potential of mangrove endophytic fungi to produce bioactive compounds and also highlight their potential for the treatment of heavy metal-contaminated wastewater. Future work should

include more in-depth studies regarding the compounds involved such as solvent-solvent extraction and structure elucidation of the bioactive compounds. The endophytic fungi with heavy metal biosorption potential can be studied further using other heavy metals that are known for their high levels of toxicity, for instance mercury and lead. Besides, they can also be tested for their ability in removing radioactive substances.

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