Antioxidant activity of the mangrove endophytic fungus (*Trichoderma* sp.)

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1. Introduction

Mangrove environments are a rich source for discovery of the new microorganisms with applications in pharmaceutical science[1–6]. Marine derived fungi are known to produce secondary metabolites higher than fungi of terrestrial origin[7]. Endophytic fungi derived secondary metabolites have a high antioxidant activity against foreign particles[8]. The marine fungi produce bioactive compounds with antibacterial, anticancer, antifungal, and antiviral and anti-inflammation properties to cure the human diseases[9–11]. Mangrove derived endophytic fungus, *Ipex hydnoides* is shown to have a significant cytotoxicity against Hep2 cell line[12]. Marine fungus of *Trichoderma* species is reportedly significant in *silico* anti cancer activity against human skin and breast cancer protein[13]. However, endophytic fungi are poorly investigated microorganisms for medicinal property[14]. Hence the present work was undertaken to test antioxidant property of mangrove derived endophytic *Trichoderma* species.

2. Materials and methods

2.1. Collection of the mangrove leaves

Healthy young mangrove leaves were collected from the 12 species of mangroves (*Lumnitzera littorea* (Jack) Voigt, *Scyphiphora hydropthyllacea* (*S. hydrophyllacea*), *Xylocarpus mekongensis* Pierre, *Excoecaria agallocha* Linn, *Nisperos chrysotrichus* (Koenigi) Koenigi, *Kurmangium horsfieldii* (Dyer) Foss, *Avicennia marina* Forst, *Sonneratia alba* (Thunb) Miers, *Sonneratia caseolaris* Lam, *Bruguiera gymnorrhiza* (L.) K. Schum, *Bruguiera glauca* (L.) R. Br., *Bruguiera parviflora* (Poirier) Poirier, *Avicennia officinalis* L.) from the forests of Andaman and Nicobar Islands.
2.2. Isolation and maintenance of endophytic Trichoderma

The mangroves leaves were surface serialized by the method of Suryanarayanan et al[13]. Fresh leaf samples were washed five times with sterile distilled water. In each species, 1 g of leaf samples was taken and then crushed with 10 mL of sterile distilled water. A total of 1 mL of extract was serially diluted into 10⁻² to 10⁻⁵ and inoculated in Petri dishes containing Trichoderma selective medium by spread plate method[16], and then incubated at 28 °C for 12 d for enumeration and the counts were calculated using the following formula and are expressed as colony forming units (CFU) in 1 g of fresh leaf tissue.

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\text{Trichoderma counts (CFU/g of leaves)} = \frac{\text{Sample of volume plated (0.1mL)} \times \text{Total volume (mL)}}{\text{Total number of colonies}}
\]

2.3. Extraction of crude secondary metabolite compounds and antioxidant assays

Trichoderma biomass was harvested and extracted in 80% methanol according to the method described by Saravankumar et al[13]. The crude extracts were assessed for antioxidant activity following seven assays: total phenol content assay[17], radical scavenging activity assay using 1, 2-diphenyl-1-H-picrylhydrazyl (DPPH)[18], total antioxidant capacity assay[19], measurement of reducing power[20], nitric oxide radical scavenging assay[21], and hydrogen peroxide radical inhibition assay[21,22].

2.4. Gas chromatography–mass spectrometer (GC–MS) analysis of crude secondary metabolic compound of Trichoderma EMFCAS8

GCmate II GC–MS (Agilent) was used for the analysis of the crude secondary metabolites present in the Trichoderma EMFCAS8 extract by using HP–5 capillary column. About 1 µL of the extract was injected in the injection port with the temperature of 220 °C and helium as the carrier gas. Compounds were identified by matching with known compounds in library of the instrument.

3. Results

Counts of endophytic Trichoderma species from 12 mangrove species are shown in Figure 1. The counts significantly varied among the species (P<0.01). The high count was recorded as 256.5 CFU×10⁶/g in A. corniculatum, whereas low count was recorded in S. hydrophyllacea, R. stylosa and A. marina.

3.1. Antioxidants assays

The mangroves derived crude extracts were tested for total phenol, total antioxidant activity, DPPH radical scavenging activity, NO₂ radical scavenging activity, H₂O₂ radical scavenging activity and total reducing power. Total phenol content significantly varied among the fungal strains or concentrations of the extracts (P<0.01). Trichoderma strain of EMFCAS8 was rich in total phenol content and other strains were considerable in the content which also varied with concentrations of the extracts (Figure 2).
fungal strains or concentrations of extracts. The higher NO2 radical scavenging activity, H2O2 radical scavenging activity and total reducing power were observed to be high in the EMFCAS8 strain at high concentration and in the EMFCAS6 strain at lower concentration. Other strains also showed the significant activities (Figures 5 and 6). The total reducing power was higher in the EMFCAS8 and lower in the EMFCAS3 (Figure 7).

3.2. GC-MS analysis

The potent antioxidants isolate Trichoderma EMFCAS8 derived secondary metabolites was analyzed by using GC. GC chromatogram of EMFCAS 8 showed the four peeks (Figure 8) indicating the presence of Pregnane–3,20β-diol, 14α,18α–[4-methyl–3–oxo–(1-oxa–4-azabutane–1,4-diyl)], diacetate; 4–piperidineacetic acid,1–acetyl–5–ethyl–2–[3–(2-hydroxyethyl)–1–H–indol–2–yl]–a–methyl, methyl ester; Corynan–17–ol, 18,19–didehydro–10–methoxy and oleic acids.

Figure 3. Total antioxidant activity of mangrove derived endophytic Trichoderma.

Figure 4. DPPH radical scavenging activity of mangrove derived endophytic Trichoderma.

Figure 5. NO2 radical scavenging activity of mangrove derived endophytic Trichoderma.

Figure 6. H2O2 radical scavenging activity of mangrove derived endophytic Trichoderma.

Figure 7. Total reducing power of mangrove derived endophytic Trichoderma.

Figure 8. GC–MS chromatogram of EMFCAS8.
4. Discussion

In the present study the endophytic colonization of *Trichoderma* was found higher in the mangrove leaves of *A. corniculatum* than the other mangroves. This result can be attributed to the biochemical variations in the mangrove species, as the endophytic fungal colonizations are highly dependent on the biochemical characteristics of host leaves[23]. The ecological roles of endophytes are diverse, and they play a protective role against insect herbivory and serve as potential producers of antimicrobial metabolites[24].

*Trichoderma* strain EMFCAS8 exhibited the maximum antioxidant activities. In general, antioxidants activities in removal of toxic free radicals are positively related to the content of phenolic derivatives[25-28]. There is a positive relationship between total phenol content and antioxidants activity[25]. This is in support of the present study that *Trichoderma* strain EMFCAS8 which was rich in total phenol content showed the maximum antioxidant activity.

DPPH is an excellent simple method for identification of the potential antioxidants compounds and also it is not affected by metals and enzyme inhibition[29]. *Trichoderma* extract displayed high free radicals scavenging activity. Hydroxyl radicals are sensitive and bring the damage to the adjacent molecules[29,30]. *Trichoderma* showed increasing hydroxyl scavenging activity with increasing concentration of the extract. Thus hydroxyl scavenging activity is highly dependent on concentrations of the crude extract. Mangrove–derived endophytic *Trichoderma* species also showed the significant reducing sugar activity. The presence of pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], diacetate; 4-piperidineacetic acid,1- acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1-H-indol-2-yl]-a- methyl, methyl ester; corynan-17-ol, 18,19-didehydro-10-methoxy and oleic acids in the trchodemra EMFCAS8 was confirmed by GC–MS analysis, These metabolites may be a reason for the higher antioxidant activity of strain EMFCAS8. One of the metabolites, pregnane-3,20β-diol, 14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)], diacetate is reported as potential hepatoprotective compound[31]. This study revealed that mangroves derived endophytic *Trichoderma* was a potent source for the discovery of the antioxidant compounds.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Comments**

**Background**

It has been proved that some microorganisms, including fungi and bacteria, have antioxidant activities. Certain plant endophytic fungi derived secondary metabolites have antioxidant activities, which can be used as a source of novel antioxidants for pharmaceutical applications.

**Research frontiers**

The present research work describes the isolation of endophytic *Trichoderma* strains from leaves of different species of mangroves and the assessments of the crude extracts from the endophytic *Trichoderma* strains for antioxidant activities.

**Related reports**

It is reported that plant endophytic fungi can produce pharmaceutically important and valuable compounds like glycoside digoxin. Some of the endophytic fungi like *Phyllosticta* sp. and *Trichoderma* sp. have been found to show antioxidant activities.

**Innovations and breakthroughs**

This study depicts that the endophytic fungi isolated from mangroves plant exhibit antioxidant activities, which indicates that mangroves plant derived endophytic fungi may be a sources of compounds with antioxidant activities.

**Applications**

The research work suggests a potential approach to seek novel antioxidants from endophytic fungi inhabiting in mangroves plants.

**Peer review**

In this work, the authors have described the isolation of endophytic fungi from mangroves plants and the antioxidant activities from the isolates. It suggests a potential approach to seek novel antioxidants for pharmaceutical applications from endophytic fungi inhabiting in mangroves plants.

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