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Anti-oxidant activity of selected endo lichenic fungi (ELF) in mangrove ecosystem of Puttalam lagoon

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Natural products based drug development has become an attractive area of research since there are limited options available to treat certain non-infectious diseases such as diabetes. Among these natural products, it has been reported that secondary metabolites of endolichenic fungi (ELF), have the ability to produce promising bioactive compounds. The objectives of this research were to isolate and identify ELF inhabiting mangroves in Puttalam lagoon, Sri Lanka using classical and DNA barcoding approach and to determine anti-oxidant activity of their secondary metabolites. Lichen hosts were collected from Puttalam lagoon in two different sites near, Athathale and around the NARA institute. The ELF were isolated following a standard procedure**:** a small piece of the thallus was surface sterilized, cut into pieces and dried on sterilized filter papers and then placed on malt extract agar in Petri dishes and incubated at room temperature (28 ^{0}C – 30 ^{0}C) . Once pure cultures were obtained, seven isolates were randomly selected for DNA extraction following standard procedures. Quality of DNA was checked by agarose gel electrophoresis. Fungal internal transcribed spacer (ITS) region was amplified using polymerase chain reaction (PCR) with universal ITS 1 and ITS 4 primers and PCR products were sequenced using Sanger *dideoxy* chain-*termination technology*. DNA sequences were edited using BioEdit software and compared with the available sequences in the GenBank using Basic Local Sequence Alignment Search Tool (BLAST). In addition, morphological characterization of each fungal isolate was also carried out. Secondary metabolites from each isolate were extracted with ethylacetate separately and the solvent was evaporated under reduced pressure to obtain the crude extract. Free radical scavenging activity of the extracts were evaluated using 2, 2-diphenyl-1-picrylhydrdrazyl (DPPH) assay. Based on the highest sequence similarity to the GenBank sequences, isolates were identified as *Diaporthe arengae* (98 %), *Neurospora crassa* (100%), *Lasiodiplodia theobromae* (100 %), *Schizophyllum commune* (98 %), *Diaporthe musigena* (98 %), *Hypoxylon anthochroum* (98 %) and *Nigrospora sphaerica* (98%). IC_{50} values of extracts of *Diaporthe arengae*, *Neurospora crassa* and *Lasiodiplodia theobromae* were 375.9± 0.062µg/mL, 304.9±0.057 µg/mL and 211.2± 0.086 µg/mL respectively. Since percent inhibitions of the rest of the isolates were less than 50 % in the test doses, IC_{50} values were not calculated. All of the values were compared with standard Butylated Hydroxy Toluene (BHT) (IC_{50}=108.0±0.072). Out of the seven ELF tested, *L. theobromae* showed the highest DPPH radical scavenging activity. Further testing of the rest of the isolates are being carried out and ELF may provide a good source of antioxidants for biotechnological applications.

Keywords: Anti-oxidant activity, Bioactive, Endolichenic fungi, Mangroves