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Molecular identification and screening of anti-obesity activity and anti-diabetes property of selected endolichenic fungi 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 and obesity. Among these natural products, it has been reported that secondary metabolites of fungi, especially 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-diabetes and anti-obesity activities of their secondary metabolites.

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 then it was 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 procedure. 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). Further morphological characterization of each fungal isolate was also carried out. Secondary metabolites from each isolate were extracted with ethyl acetate separately and the solvent was evaporated under reduced pressure to get the crude extract. Anti-obesity and anti-diabetes activity of the extracts were evaluated using Lipase inhibitory assay and α-amylase inhibitory assay, respectively.

Based on the highest sequence similarity to the GenBank sequences, isolates were identified as *Hypoxylon anthochroum* (100%), *Xylaria feejeensis* (100%), *Daldinia eschscholtzii* (100%), *Endomelanconiopsis endophytica* (100%), *Neosartorya hiratsukae* (99%), *Neurospora crassa* (100%) and *Xylariaceae* sp (100%). According to the results of the amylase inhibition assay, maximum percentage inhibition for the highest dose of *H. anthochroum*, *X. feejeensis*, *D. eschscholtzii*, *E. endophytica*, *N. hiratsukae*, *N. crassa* and *Xylariaceae* sp were 10.50±2.53, 7.61±0.41, 1.29±0.27, 8.21± 0.67, 6.76± 1.39, 1.57± 0.08 and 0.69± 0.02, respectively. Acarbose was used as positive control and its maximum percentage inhibition was 55.27±4.13. According to the results of the lipase inhibition assay maximum percentage inhibition for the highest dose of *H. anthochroum*, *X. feejeensis*, *D. eschscholtzii*, *E. endophytica*, *N. hiratsukae*, *N. crassa* and *Xylariaceae* sp were 10.00 ±0.83, 19.71±0.97, 39.72±1.86, 30.76±4.04, 5.62±0.65, 37.71±2.31 and 24.67±2.16, respectively. Orlistat was used as positive control and its maximum percentage inhibition was 45.60±4.18 at 100µg/mL. In lipase assay, percentage inhibition of all tested ELF were less than that of the positive control. Considering the α-amylase assay results all tested ELF didn't show significant activity comparing with standard acarbose.

References

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