SUMMARY AND CONCLUSION

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The present study revealed diversity of endophytic fungi colonizing three ethno-medicinal plants of Assam namely *H. cordata*, *E. foetidum* and *Z. oxyphyllum* and their antimicrobial potential against some clinically significant human test pathogens. A total of 214 endophytic fungi belonging to the classes Ascomycetes, Zygomycetes, Hyphomycetes and Deuteromycetes were isolated from surface sterilized leaf tissues of the three selected plant species. The dominant endophytic fungi belonged to class Deuteromycetes and the genus *Colletotrichum* was found to have maximum colonization frequency (50%). Other endophytic fungal genera include non- sporulating isolates categorized as Mycelia Sterilia (25.7%), *Purpureocillium* (6.54%) and *Curvularia* (5.61%). Besides, isolates belonging to the genera *Corynespora*, *Fusarium*, *Cladosporium*, *Stemphylium*, *Penicillium*, *Scopulariopsis*, *Alternaria*, *Aspergillus*, *Bipolaris* and a dimorphic yeast, *Pseudozyma* were also obtained as endophytes from the studied plant species. The highest recovery of endophytic fungi from *H. cordata* and *E. foetidum* was found in PDA medium while the maximum number of endophytic fungi from *Z. oxyphyllum* was isolated in medium amended with Plant Leaf Extract and Agar (PEA). The crude secondary metabolites obtained from the fungal isolates were screened for antimicrobial activity against four human test pathogens. It was found that 78.57% of isolates showed antimicrobial activity against by inhibiting at least one of the four test pathogens whereas 58.57% of the isolates showed antimicrobial activity against all the test pathogens. Preliminary antimicrobial assay revealed six endophytic fungal isolates namely, *Colletotrichum* sp. (HCS3), Morphotype (HCS6), *Scopulariopsis brevicaulis* (EF1), *Purpureocillium lilacinum* (EF6), *Penicillium chrysogenum* (EFB9) and *Colletotrichum gloeosporioides* (ZOB3) showed promising antimicrobial activity against

all the clinically significant test pathogens. The endophytic fungi that showed significant antimicrobial activity were grown in different nutritional and environmental conditions for enhanced antimicrobial metabolites production. The effects of different conditions like culture media (PDB, MEB, CDB and OMB), incubation periods (7, 14 and 21 days), pH (5, 7 and 11) and temperatures (25ºC, 30ºC, 35ºC and 40ºC) were observed for maximum metabolites production. The result indicated that most of the isolates showed enhanced antimicrobial activity when grown on PDB medium and incubation period of 14 days. However, *Penicillium chrysogenum* showed enhanced activity in 21 days of incubation period in PDA medium. The findings correspond with earlier works that reported isolates grown on PDB medium with varied incubation periods showed maximum metabolite production. The isolate *Purpureocillium lilacinum* EF6, showed maximum antimicrobial activity when grown in CDB when incubated for 14 days. Amongst the isolates, four endophytic fungi, namely, *Colletotrichum* sp., *Scopulariopsis brevicaulis*, *Penicillium chrysogenum* and *Colletotrichum gloeosporioides* showed optimum metabolites production in neutral pH while the other two endophytic isolates, Morphotype HCS6 and *P. lilacinum* showed enhanced activity in alkaline pH. It was observed that all the isolates showed maximum antimicrobial activity when cultivated at a temperature of 30ºC. None of the isolates showed better activity at lower temperatures and also could not withstand higher temperatures. Two of the endophytic fungi (*Colletotrichum* sp. HCS3 and Morphotype HCS6) that showed promising antimicrobial activity were further identified up to species level through molecular technique using ITS rDNA sequence analysis. Since the ITS2 region has been reported to vary in both primary and secondary structures, it is often used as molecular marker in systematic and phylogenetic reconstruction. Therefore, in order to validate the phylogenetic relationship

and for species level confirmation, phylogenetic trees and RNA secondary structures using ITS2 sequences were generated. Homology search and phylogenetic analysis using ITS and ITS2 sequence of the isolate HCS3 revealed it to be *Colletotrichum coccodes*. Furthermore, comparison of the ITS2 RNA secondary structures revealed its close affinity with endophytic *C. coccodes* and thus confirmed its endophytic lifestyle. Similarly, the homology search and phylogenetic analysis using both ITS and ITS2 sequence of the isolate Morphotype HCS6 revealed it to be *Phyllosticta capitalensis*. The ITS2 RNA secondary structure of the isolate showed its close affinity with an endophytic *Phyllosticta capitalensis*. The ITS2 phylogenetic trees and RNA secondary structures thus provided a better phylogenetic resolution and the study showed distinct structural similarities and variations when compared with similar isolates of different lifestyles. Further, the crude EtOAc derived organic extracts of the six isolates were characterized using different analytical spectroscopic techniques. FTIR analysis of the isolates revealed the presence of common compound classes like alkanes, alkenes, aromatic esters, tertiary alcohols, fluoro compounds, phenols, alcohols and primary amines and alkyl aryl ethers. Some of the isolates like *C. coccodes*, *P. capitalensis*, *S. brevicaulis* and *P. lilacinum* indicated presence of ester compounds and δ- lactones, which have been reported to have antimicrobial and antitumor properties. Other compound classes obtained from EtOAc extract of *C. coccodes* were sulphonamides and nitro groups. These compounds are reported to be used as effective antimicrobial drugs. Similar study of the EtOAc extract obtained from endophytic fungus *P. capitalensis* indicated presence of amines and α, β-unsaturated esters which are reported to be antimicrobial agents. GCMS analysis of the crude extracts of all the six isolates revealed presence of wide range of bioactive compounds. Some of the compounds have been reported to exhibit antimicrobial,

antioxidant, anti-inflammatory and cytotoxic activities like Geranyl geraniol, Squalene, Phenol,2,4-Bis (1,1-Dimethyl ethyl), Dibutyl Phthalate, Oleic Acid, 1-Octacosanol, Undecanoic acid, Hexacosanol Acetate. These metabolites were commonly obtained from endophytic fungi *C. coccodes*, *P. capitalensis* and *P. chrysogenum*. Interestingly, compounds like Undecanoic acid and N-Hexadecanoic acid which were reported in the extract of *H. cordata* were also revealed in the crude extract of *C. coccodes*. Similarly, bioactive compounds Hexadecanoic acid and Tetradecanal were also revealed to be present in the fungal extract of *P. chrysogenum* which has also been reported to be present in the leaves of *E. foetidum*. This supports the view that endophytes from medicinal plants produce bioactive metabolites similar to the host plant or vice-versa. Amongst the isolates, crude metabolites obtained from *C. coccodes* showed significant antimicrobial activity against the test pathogens. Therefore, it was further purified and active fraction was characterized which resulted in the yield of polyphenolic compound identified to be 'Tyrosol' based on its NMR analysis. Tyrosol is a well-known secondary metabolite produced by both plants and fungi. Although this compound is reported from *Colletotrichum* (*C. gleosporoides* and *C. crassipes*). However, isolation of this compound from *C. coccodes* is limited. Literature survey revealed that tyrosol possess antimicrobial and antioxidative properties, inhibit biofilm formation and is an anti Quorum-Sensing(QS) molecule. To our knowledge, this is the first report of this compound from endophytic fungus, *C. coccodes*. The present findings suggest that *H. cordata* harbour endophytic fungi, ability to produce bioactive metabolites. The study also supports the possibility that the antimicrobial activity of the medicinal plant may be attributed to the presence of endophytic fungi which synthesize wide range of bioactive metabolites. The findings also suggest that endophytic fungi inhabiting ethno-medicinal plants prove to be

a good source of antimicrobial metabolites. The results therefore justify the traditional use of the selected three medicinal plants against human pathogenic microorganisms although the crude use of them in traditional way needs refinement. It also justifies that the studies on isolation and identification of these bioactive compounds can be a crucial approach to the search of novel natural products.