Lichen diversity of Ultapani Forest Range, Manas Biosphere Reserve, Assam and biological activity of selected species

Abstract

The present work entitled “Lichen diversity of Ultapani Forest Range, Manas Biosphere Reserve, Assam and biological activity of selected species”, was carried out during the period 2019–2023. Study on the lichens of Ultapani Forest Range revealed 217 species under 31 families, 69 genera and a genus under the order Arthoniales. The study area is dominated by crustose lichen with 172 species (79%), followed by foliose with 34 species (16%), squamulose with six species (3%), fruticose with three species (1%), and leprose with two species (1%). The lichen family Graphidaceae is the most dominant and diverse group in UFR with 45 species under 10 genera followed by Diploschistaceae and Malmideaceae with 22 and 21 species, respectively. Among the various genera *Malmidea* is the most dominant genus with 21 species followed by *Pyrenula* and *Graphis* with 16 and 15 species, respectively. The lichen community is also represented by good diversity of the genera viz. *Hemithecium*, *Ocellularia* and *Porina*. The species, *Agonimia bryophilopsis*, a squamulose lichen was previously reported as terricolous lichen (growing on soil) from Finland, Europe but it is found to be corticolous lichen (growing on the bark of tree) as reported in the present study. A total of 27 species of lichen are identified as new records to Country; 78 species as new additions to the state, including first record of 12 genera out of 69 genera, *Agonimia*, *Anzia*, *Aptrootia*, *Bactrospora*, *Crocynia*, *Eugeniella*, *Lithothelium*, *Myriostigma*, *Nadvornikia*, *Parmeliella*, *Rhabdodiscus* and *Sclerophyton* from the present study. The number of 172 species are found as new distributional records from Bodoland Territorial Region. Of the species identified, 27 are endemic due to their limited distribution. The study also encompasses employment of lichen species, *Anzia ornatoides* for the evaluation of antioxidant, cytotoxic and antimicrobial activities. Crude extraction of the lichen *A. ornatoides* was done using five different solvents viz. hexane, diethyl ether, ethyl acetate, methanol and water in increasing polarity using Soxhlet apparatus. Preliminary screening was done to investigate presence of alkaloid, carbohydrate, flavonoid, glycoside, phenol, and saponin. The presence of 21 volatile compounds were identified through GC-MS among which the top three major compounds were methyloxyolivetol (78.91%), imidazole 2-t-butyl-1,4-dimethyl-5-phenyl (6.32%) and benzoic acid 2,4-dihydroxy-3,6-dimethyl-methyl ester (4.77%). Total phenolic content was determined by Folin-Ciocalteau assay while flavonoid

content was estimated using aluminium chloride assay. The antioxidant activity of the extracts was analysed by phosphomolybdenum assay, FRAP, DPPH, ABTS, and lipid peroxidation inhibition. All the extracts showed good antioxidant activity and there was a statistically significant difference at $P < 0.05$ and $P < 0.01$. The effectiveness of lichen extracts against six cancer cell lines, PC-3, OVCAR-3, MCF-7, HeLa, Hep-G2, and h-1299 was investigated. From the study it was evident that the methanol extract suppresses maximum cancer cell growth compared to other extracts. Effectiveness of cell death was directly proportional to the concentration of the extracts. The cell line, OVCAR-3 exhibits high induction of cancer cell death. IC50 values for PC-3, OVCAR-3, MCF-7, HeLa, Hep-G2, and h-1299 were 52, 38, 48, 47, 52, and 78 µg/ml, respectively. Further the methanolic extract was used to determine apoptotic potential and FACS study to see the significant death of OVCAR-3 cancer cells. The percentage of apoptotic cells were 55%, which was greater than untreated cells (5%). In-vitro cytotoxicity assay was conducted with the non-cancer cell lines HEK-293, L-929, hMSCs, and MCF-10A. No significant toxicity was detected in in-vitro cultured non-cancerous cells. The antimicrobial potential of *A. ornatoides* was determined by disc-diffusion, agar-well diffusion assay, MIC, MBC and MFC using human pathogenic (MDR) four bacteria viz. *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and four yeasts namely *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*. In disc-diffusion assay, *S. aureus* was the most susceptible bacterium that recorded 24.00±0.00 mm zone of inhibition in methanol extract; in agar-well assay, the highest zone of inhibition was observed in water extract against *S. aureus* (25.33±0.33 mm) at the maximum concentration of 25 mg/ml. Among the yeasts, in disc-diffusion assay, *C. albicans* showed highest zone of inhibition (23.00±0.00 mm) against the methanol extract whereas in agar-well assay, highest zone of inhibition was obtained for water extract (23.00±0.00 mm) at the concentration of 25 mg/ml. Extracts of *Anzia ornatoides* were inactive against the other three yeasts, C. *glabrata*, *C. krusei*, and *C. tropicalis*. The MIC of all the extracts against the bacterial and yeast strains were within the range of 1.56–6.25 mg/ml. The MBC and MFC values of the extracts against the strains were between 3.125–25 mg/ml. Notably, among the bacterial strains, water extract of *A. ornatoides* demonstrated considerable efficacy against *S. aureus* and *K. pneumonae* whereas methanol extract showed significant effectiveness against *E. coli*. However, the extracts of diethyl ether and methanol showed significant effect against *C. albicans* and recorded the lowest MIC and MFC value.