ABSTRACT

Due to wide aspects of biological activities of lichens and the concern in human health for discovering new sources of biomedical agents with fewer side effects, in this research work, biological activities of some lichen extracts along with their phytochemical analysis were studied.

Lichens were collected from Ooty, Tamil Nadu, India and were identified on the basis of their morphology and characterizing their chemical constituents. Eight lichen species were identified as Cladonia subradiata, Heterodermia leucomelos, Parmotrema crinitum, Parmotrema reticulatum, Herpothallon sp. Parmotrema tinctorum, Leptogium sp. and Ramalina celastri. Identified samples were extracted by hexane, ethyl acetate, methanol and ethanol according to increase polarity to obtain active metabolites during fractionation process. Methanol could extract more yields among other solvent used in this study.

All lichen extracts were subjected to phytochemical analysis by quantitative and qualitative methods. Qualitative investigations showed that all lichen extracts are rich in flavonoids, phenols and carotenoids. Quantitative study was done by estimating total phenol, flavonoids, carotenoids and antioxidant contents in each lichen extract. Methanol extract of P. crinitum had highest amount of phytochemical. Highest carotenoid content was observed in R. celastri. A TLC bio-autography method showed the antioxidant compounds of lichen extracts. P. crinitum and R. celastri had highest amount of antioxidant content. A positive correlation between phenol and flavonoids as well as antioxidant and phenols and flavonoids was observed while there were no relation between carotenoid with phenols and antioxidant components.

Anti-bacterial activity of lichen extracts was determined by a preliminary agar-disc diffusion assay. Minimum Inhibitory Concentration (MIC) and Non-Inhibitory Concentration (NIC) of all tested extracts were determined by Gompertz model. Their Minimal Bactericidal Concentration (MBC) was determined by culture dilution method. Growth curves of treated bacteria were constructed by an O.D./time model and two predictive biological models. Constructed graphs were compared with untreated bacterial growth curves. From these models, growth rate ($\lambda$), Lag Phase Duration (LPD) and generation time (t), Fractional Area (FA), Area Under Curve (AUC) and percent inhibition of each bacterial suspension before and after treatment was measured and it was confirmed that lichen extracts are bactericidal, concentration dependent agents. There
was a reverse correlation between LPD with growth rate and a positive relation between growth rate and FA. The bactericidal components in lichen extracts were detected by TLC-bioautography assay.

Anti-diabetic activity of lichen extracts were carried out by measuring percentage of inhibition in two key enzymes, which cause type-2 Diabetes by glucose formation; α-Amylase and β-glucosidase. Anti-amylase activity was estimated in three concentrations (5, 10, 15 mg/mL) for all lichen extracts. A dose dependent, moderate to great inhibitory activity was seen ranging from 47.9 % by *Leptogium* sp. to 99.2 % inhibition in case of *H. leucomelos*. β-Glucosidase inhibitory activity of 20μg/mL lichen extracts showed than lichen’s ethanolic extracts have higher percent inhibition. Highest inhibition was seen in *R. celastri* by 91%. There was a positive correlation between phenolic content of lichen extracts and their glucosidase activity. glucosidase inhibitory compounds were detected using a TLC-bioautography method.

Anticancer activity of lichen extracts was investigated on three cancerous cell lines and one normal cell line to compare the efficacy on normal cells. Viable cells were seeded by desired concentration in ELISA plates and cytotoxicity assay was done by MTT colorimetric assay. It was observed that these extracts are highly toxic to cancerous cell lines but less active on normal cells. In order to know the mechanism of cell death, DNA of NIC-H713 cell (colorectal cancerous cell) as the most affected cell by *P. crinitum* after 24hrs of treatment was isolated and it was seen that all lysed cells were eradicated after 24hrs and only DNA from trace amount of non-inhibited cells were remaining. Cell cycle analysis showed that 100% of cells were arrested in G1 phase while in untreated cell, 95% of cells were in S phase. Morphological studies clearly proved that lichen extracts had induced an apoptosis event which caused cell death. Overall results suggested an early apoptosis event, so NIC-H713 cells treated with *P. crinitum* were analyzed by Annexin V-FITC method and it was showed that about 50% of cells were undergone early apoptosis by only 4hrs of treatment.

The methanolic extract of *P. crinitum* as a potent biologically active extract was subjected to characterization of its active components. Three fractions were isolated by column chromatography and were analyzed by LCMS, GCMS, 1H NMR, 13C NMR and COESY, HMBC and HSQC as 2D NMR. Chemical structures of obtained fractions were elucidated as 1-Hydroxy 3-methoxy 6-methyl anthraquinone, constictic acid and stictic acid.