

## SUMMARY AND CONCLUSION

The present thesis entitled “**Screening, Isolation and Identification of Antimicrobial and Antioxidant Substances from Some Common Lichens of Darjeeling Hills**” was conducted.

The study revealed the following results-

During antimicrobial screening broad spectrum antibacterial activity shown by ethanolic and methanolic extract of *U. baileyi*, *P. reticulatum*, *R. hossei* and *Everniastrum* sp. Out of the four medicinal plants which underwent antimicrobial screening ethanolic extract of *P. pseudoginseng* exhibited a good degree of antimicrobial activity, *U. dioica* was least active, *S. mukrossi* and *B. ciliata* was moderately active. All the lichens and medicinal plants used in this study exhibited some degree of antibacterial as well as antifungal activity against clinical and phytopathogens.

- Although some lichens and medicinal plants did not show any antimicrobial properties individually, but they exhibited antimicrobial property when their extracts were combined. This combined effect was manifested by ethanolic extracts of *P. pseudoginseng* + *Everniastrum* sp and methanolic extracts of *P. pseudoginseng* + *Everniastrum* sp with relatively strong antimicrobial activity evidenced by inhibition zones greater than the control antibiotic. Besides this ethanolic extract of (*U. baileyi* + *U. dioica*) and methanolic extract of (*U. baileyi* + *U. dioica*) also possessed synergistic effect. The activity of ethanolic and methanolic extract of *S. mukrossi* was enhanced by its combination with ethanolic extract of *Everniastrum* sp, methanolic extract of *Everniastrum* sp, and ethanolic and methanolic extract of *P. reticulatum*.
- A weak combination of ethanolic extract of *S. mukrossi* + *U. baileyi* was observed. This study gives possibility of use of these lichens which are available throughout the growing season.
- The MIC of the different extracts was within the range of 500-10000µg/ml. Among the tested lichen extracts methanolic extract of *U. baileyi*, ethanolic extracts of *P. reticulatum*, *S. pomiferum*, ethanolic and

methanolic extract of *Everniastrum* sp, ethanolic and methanolic extract of *R. hossei* exhibited MIC of 500µg/ml against test microorganisms.

- It was noted that the MIC value of medicinal plants ranged from 100 µg/ml (*S.mukrossi*) to 500µg/ml. In combination low MIC (500µg/ml) was exhibited by methanolic extract of *R. hossei* + *B. ciliata*, ethanolic extract of *R. hossei* + *B. ciliata*, methanolic extract of *Everniastrum* sp + *B. ciliata*, methanolic extract of (*Everniastrum* sp + *U. dioica*) and ethanolic extract of (*Everniastrum* sp + *U. dioica*).
- In our study higher total antioxidant activity (Phosphomolybdenum method) was shown by ethanolic extracts of *R. hossei* 3.19µg/ml (p<0.05) and *U. baileyi* 2.12 µg/ml (p<0.05) α-tocopherol equivalent/mg. Among the medicinal plants under study ethanolic and methanolic extract of *B. ciliata* exhibited antioxidant activity 5.43 µg equivalent/mg of plant extract and 7.03(p<0.05) µg/α-tocopherol equivalent/mg. The best synergistic total antioxidant activity was shown by ethanolic extracts of (*U. baileyi* + *P. pseudoginseng*) and (*U. baileyi* + *B. ciliata*).
- Ethanolic extract of *Everniastrum* sp proved to be a best free radical scavenger (p<0.05) followed by ethanolic extract of *U. baileyi*, methanolic extract of *P. reticulatum*, ethanolic extracts of *Everniastrum* sp, *R. hossei* and *P. reticulatum* which showed quite appreciable amount of radical scavenging activity. A low radical scavenging activity was shown by ethanolic and methanolic extract of *S. pomiferum* compared to the other lichen extracts.
- Methanolic extract of *R. hossei* demonstrated strongest reducing power, 0.72±0.007 (p<0.05) followed by ethanolic extract of *Everniastrum* sp 0.61 ±0.0040 which was nearer to the reducing power of standard BHT (0.89±0.0010).
- Among the medicinal plant extracts methanolic extract of *U. dioica* and *B. ciliata* possessed a good radical scavenging activity (p<0.05), which was quite near to the standard compound. Ethanolic extract of *B. ciliata* and *U. dioica* were also potent radical scavengers. Methanolic extract of *P. reticulatum* + *B. ciliata* in combination possessed a DPPH radical scavenging value of 72.12±1.83 % which was greater than the standard

compound Ascorbic acid. Ethanolic extract of *P. reticulatum* + *B. ciliata* and methanolic extract of *U. baileyi* + *B. ciliata*, ethanolic extract of *S. pomiferum* + *U. dioica* and methanolic extract of *S. pomiferum* + *U. dioica* were also able to quench the free radicals.

- An appreciable amount of phenol content was found in all test lichens. Methanolic and ethanolic extracts *U. baileyi* (141.2±0.34 and 106±0.51) and *P. reticulatum* (143.23±1.30 and 108.4±1.50) ( $p < 0.05$ ), were recorded as highest. Phenolic content of methanolic extract of *Everniastrum* sp was 96.86±0.37µg tannic acid equivalents which are greater than or nearly equal to the standard compound tannic acid (97.40±1.53).
- The value of flavonoid content ranged from methanolic extract of *P. reticulatum* 1.41±0.01 to ethanolic extract of *R. hossei* 1.66±0.01 which was less than the standard (quercetin).
- The catalase activity of lichen was highest in case of *Everniastrum* sp as 1.68 enz/min/gm tissue and for *Parmotrema reticulatum* it was 1.57enz/min/gm tissue. High peroxidase activity was noted in case of *Usnea baileyi*, *Ramalina hossei* and *Stereocaulon pomiferum* as 2.058, 2.4 and 2.26 enz/min/gm of tissue respectively.
- The total protein content decreased with the increasing concentration of all test lichen extracts (from 0.1 to 0.5ml) extract during determination of total protein content of culture filtrate. The effect of lichen extracts on growth of microorganisms showed that the duration of log phase decreased with increasing concentration of different lichen extracts.
- It appeared that all the five lichen extracts with antimicrobial activity also possessed appreciable antioxidant activity with some altered results.
- The LCMS chromatograms of different lichens (methanolic extracts) have been found to contain a variety of secondary lichen substances which are responsible for antimicrobial and antioxidant activity of lichens. Twenty five such active compounds were isolated from methanolic extract of *Everniastrum* sp alone which includes mainly orcinol, depsidones and depside compounds with some aliphatic acids, xanthenes and Usnic acid derivatives. Methanolic extract of *S. pomiferum* mainly yielded orcinol depsidones and depside compounds with some aliphatic acids, xanthenes and usnic acid derivatives. Secondary metabolites such as orcinol

depside,  $\beta$ -orcinol depsidones, anthraquinones, usnic acid derivatives, and ergochromes were isolated from methanolic extract of *U. baileyi*.

- The chromatogram of methanolic extract of *R. hossei* revealed the existence of xanthenes, pulvinic acid derivatives, orcinol tridepsides, xanthenes, naphthaquinone, monocyclic aromatic derivatives, aliphatic acids, terpenoids, and pulvinic acid derivatives in the crude extract.
- In those cases where combined extracts of lichen and medicinal plant were used there is also a possibility of two or more compounds working in consortium to give antimicrobial activity. Combinations of antimicrobials that demonstrate an *in vitro* synergistic effect against microorganisms are more likely to result in successful therapeutic application. The results obtained of these studies may be helpful in developing the plant based natural antimicrobial agents, fungicides and insecticides for preventing and curing the common diseases of humans and to reduce the pathogen population.
- The presence of compounds indicated after LCMS data seem to be responsible constituent or one of active principles of the lichens for its antimicrobial as well as antioxidant properties.

## **Recommendations**

The present investigation dealt with only five lichen specimens of Darjeeling Hills, but other lichen samples are also abundantly present. Hence it is recommended to conduct such studies on other lichen species also.

- Altogether eight microorganisms including Gram positive bacteria, Gram negative bacteria and fungus was taken for determination of antimicrobial activity but other microorganisms can be also taken for antimicrobial assay against lichen extract.
- Four medicinal plants were taken for the determination of synergistic activity with lichens. However other locally available medicinal plants can be explored for the antimicrobial potentiality.

- Only few parameters are taken for determination of antioxidant activity other parameters like lipid peroxidation assay, nitric oxide scavenging assay, hydroxyl radical scavenging assay, hydroxyl radical scavenging assay can be also further performed.
- Lastly, the results obtained in the present study could be used as a database for further use of lichens for medicinal purpose.

Consequently, the antimicrobial effect of lichens and plants tested can be further authenticated with new studies by taking other clinical pathogens and conducting the pharmacological tests. The findings of this study is the database for further research for search and isolation of the lichen metabolites, greater detail investigation in the action of lichen substances for their application is essential. Further clinical trials are warranted beyond this thesis.