CHAPTER 2

REVIEW OF LITERATURE

2.1 Historical background with diversity study

The word "lichen" (Gk.) was first used by Theophrastus around 300 BC, to describe the outgrowth that appeared on the bark of olive tree (Hawksworth and Hill, 1984). In the later part of the 19th century, Swiss botanist, Simon Schwendener realized that lichens are composed of dual organisms (algae and fungus) (Stahl, 1877; Muggia *et al*., 2009). Linnaeus (1753) provided the first comprehensive study on lichen taxa which included 80 species under the 24th class of Cryptogamie algae in "Species Plantarum". Acharius, a Swedish botanist, known as the father of lichenology, described numerous new genera and new species based only on their outward morphology in his massive publications, Methodica Lichenum, Lichenographia Universalis, and Synopsis Methodica Lichenium. There were floral accounts of lichen throughout the majority of regions of Europe, America, and Australia (Purvis *et al*., 1992). Most of the studies were conducted with the goal of identifying lichenologically rich locations for their conservation (Mc Cune, 2000; Peterson and Mc Cune, 2003); while few researchers evaluated the distribution and ecology of common epiphytic lichens and worked to conserve specific rare and endangered species (Bruteig, 1993).

The lichen contribution by Vondrak *et al*. (2015) represents 230 epiphytic and epixylic species from Slovakian Stuzica forest, which is dominated by old-growth beech trees; of the total species, 115 microlichen were identified new to Stuzic. Käffer *et al*. (2015) reported 43 corticolous microlichen from South America and Brazil; of these, 31 species being found as new reports to Southern Brazil, six to South America, two to Brazil and four were new occurrence for the state, Rio Grande do Sul. A total of 54 species were discovered under 30 genera and 16 families in Nakhon Ratchasima, Thailand (Dathong, 2016). Singh and Nayaka (2017) listed the occurrence of 69 lichen species from Schirmacher Oasis and 25 species from the area of Larsemann Hills, Antarctica, both locations presented mostly microlichens. In South Korea, 22 taxa of *Arthonia*, and a new lichenized fungus, *Arthonia ulleungdoensis* were reported from Ulleung Island (Lee and Hur, 2019). Of the 284 specimens collected, Umaña-Tenorio (2020) identified 25 taxa upto genus and 148 upto species, including 40 species that are new to Costa Rica. Diederich and Ertz (2020) enumerated a total of 56 lichenized taxa that have been reported as new for the Island of Mauritius, where they described two new genera (*Baidera* and *Serusiauxia*) and eight new species viz. *Baidera mauritiana*, *Biatoropsis millanesiana*, *Chapsa alletii*, *Collemopsidium mauritiae*, *Nyungwea pyneei*, *Porina florensii*, *Pyrenula muriciliata*, and *Serusiauxia inexpectata.* Park *et al*. (2022) conducted a survey on lichens of Gwangneung Forest and found 68 taxa in 11 orders, 22 families, and 39 genera. Orock and Fonge (2022) reported 89 lichen species from Mount Cameroon in South West Region of Cameroon. The lichen flora on *Quercus* supported 68 epiphytic species across 36 genera and 23 families (Belguidoum *et al*., 2022).

In India, lichenological research has been conducted since the time of Carl Linnaeus by European lichenologists. Although Indians began exploring lichen in later part of the 20th century but were identified by the Europeans. In the following seven decades, Awasthi played a significant role in initiating an effective school of lichenology in India and successfully built a solid foundation of the subject. Later other experts at CSIR-National Botanical Research Institute, Lucknow introduced various lichenology related concepts, including biomonitoring, biodeterioration, and bioprospecting, as well as both classical and modern taxonomy (Nayaka and Upreti, 2021). Lichens collected from Ceylon (Sri Lanka) by Thwaites and described by Leighton (1869), comprised 196 species of which 44 were new. Nylander (1900) described 159 species of lichen that were collected by Almquist in 1879, of which 48 species were new. Besides Stirton (1876), Patwardhan and Makhija (1981), Awasthi (1986), Nayaka *et al*. (2001), Bajpai *et al*. (2008), Logesh *et al*. (2014), numerous experts continued their research on lichenology. Similarly, Vinayaka *et al*. (2016) examined diversity and distribution of lichen in Mid-Elevation Wet Evergreen Forest of Southern Western Ghats, and discovered 40 species that belonged to 18 genera and 15 families; majority of the lichens were corticolous accounting to 80%, followed by saxicolous and terricolous 12.5% and 7.5% respectively. Mohabe *et al*. (2017) identified 15 new lichen taxa, including first records of the genera *Chapsa*, *Fissurina*, and *Thecaria* from Andhra Pradesh. Ingle *et al*. (2018) reported 10 species of *Pyrenula* viz. *Pyrenula andina*, *P. atropurpurea*, *P. bahiana, P. caracasana*, *P. macrospora, P. massariospora*, *P. pseudobufonia*, *P. subducta*, *P. subgregantula* and *P. tristissima* as new records to Indian lichen biota and provided an updated key to the species under *Pyrenula* from India.

A total of 411 species of Indian lichen biota were included in the checklist prepared by Sinha *et al*. (2018) during the period 2010–2017. Pallavi *et al*. (2018) enumerated 99 species from Cotigao Wildlife Sanctuary, Goa belonging to 43 genera and 25 families; of these, 21 species being new record for the sanctuary and 15 new to the state. Bajpai *et al*. (2018) investigated lichen diversity of Tawang district, Arunachal Pradesh and enumerated 122 species under 47 taxa and 24 families. Gupta *et al*. (2020) added eleven species of graphidoid and thelotremoid lichens as new to Indian lichen biota namely *Chapsa cinchonarum*, *C. farinosa*, *Diorygma sticticum*, *Fissurina albocinerea, Graphis bungartzii*, *G. discarpa*, *G. nigririmis*, *Ocellularia alba*, *Phaeographis pseudostromatica*, *Sarcographa verrucosa* and *Thelotrema crassisporum*. Thangjam *et al*. (2020) reported 22 lichen species as new additions to Mizoram and *Pyrenula dissimulans* as a new record for India. Haridas and Aliyarukunju (2021) reported 11 new records of lichen under 7 genera and 6 families in Kerala; the species included *Bacidia personata*, *Chrysothrix chlorina*, *Cryptothecia candida*, *C. emergens*, *C. nilghiriensis*, *Letrouitia aureola*, *Ochrolechia africana*, *Pertusaria coronata*, *P. quassiae*, *P. subdepressa*, and *Phyllopsora manipurensis*. Nayaka *et al*. (2021) identified 95 species of lichen from Sirumalai hills in the Eastern Ghats, six of the species were fresh records, and *Japewiella* was determined to be a new genus for India.

Assam, a prominent state in North-east India is sandwiched between two biodiversity hotspots. The state is geographically positioned between 24°2′–27°6′N latitudes and 89°8′–96°E longitudes covering an area of 78,523 km^2. Although the state has witnessed a fast pace of urbanization in the recent years, but still harbors about 34% (26,832km^2) of forest area with rich floral and faunal diversity (Gupta and Sinha, 2018). Stirton (1881) pioneered the study of lichen in Assam and presented 39 species from tea plants. Later contributors to the periodicals included Smith (1926), Santesson (1952), Upreti (1993, 1998), and Makhija and Patwardhan (1993, 1995). Rout *et al*. (2005) reported 24 species of lichens within 12 genera and 8 families from NIT Campus of Southern Assam, of which 18 species were crustose and 6 foliose. Singh and Sinha (2010) listed 141 species of lichens, native to Assam in their book 'Indian lichens –An annonated checklist'. The occurrence of 37 lichen species belonging to 16 genera and 10 families have been documented by Rout *et al*. (2012) growing on the bark of *Areca catechu* in Southern Assam. The family, *Graphidaceae* was dominant with 13 species followed by

Trypetheliaceae with 6 species. An account of 67 species of lichen under 24 genera and 12 families have been enumerated by Daimari *et al*. (2014) from Baksa, Kamrup and Sonitpur district of Assam; of the total species, 41 were new additions to the state. Hojai sub-division of Nagaon district, Assam have been represented by 31 species of lichen under 16 genera and 11 families. Dey *et al*. (2015) observed the dominance of crustose lichen (16 species) followed by foliose (14) and fruticose with a single member. Choudhury *et al*. (2016) studied the lichens growing on monuments and various historical sites in Sonitpur district and discovered 38 lichen taxa belonging to 21 genera and 15 families. The family, Physciaceae had the maximum species (11) followed by Teloschistaceae, Lecanoraceae, and Verrucariaceae, each with four, three, and three species respectively. Daimari *et al*. (2017) reported the occurrence of 45 species of lichen from Sonitpur district, 25 of which were new to the state and included 16 pyrenocarpous lichen. Gupta and Sinha (2018) in their book, "Lichen Flora of Assam", listed 300 lichen taxa from Assam that falls under 83 genera and 26 families. Subsequently, 25 lichen species were added as new to the state by Gogoi *et al*. (2019). Mishra *et al*. (2019) investigated the lichens of Dima Hasao district resulting in the discovery of 142 taxa, 98 species of which were new to Assam and *Multiclavula vernalis*, a new record for the entire nation. Behera *et al*. (2021) identified 138 species from Goalpara, Kamrup, Nagaon, Karbi Anglong, and Golaghat districts of Assam, whereby 37 taxa were described as new to the state. After compilations of all the published data, Gogoi *et al*. (2022) provided a checklist of lichens in Assam that included 657 species under 146 genera and 41 families.

2.2 Antioxidant activity of lichen

Lichens are one of the best sources of natural antioxidants, imposes protective function against oxidative stress. Lichen produces numerous polyphenolic substances, such as dibenzofurans, pulvinic acid derivatives, depsides, and depsidones. Phenolics, the primary secondary components of lichens, plays a critical role in controlling the growth and development of lichens under stressful and unfavourable environmental conditions. Phenols in lichen are usually produced by the mycobiont partner and left as crystals on the surface of fungal hyphae and derived from shikimic acid pathway. It consists of two monocyclic phenols connected by an ester bond, as in depsides, or by both an ester and an ether bond as in depsidones, or by furan heterocyclic bond, as in dibenzofurans, like usnic acid. The presence of phenolic group is associated with antioxidant activity of lichen (Paudel *et al*. 2012; Kumar *et al*. 2014; Watson 2014). Aoussar *et al*. (2017) quantified phenolic and flavonoid contents and determined the antioxidant activities of dichloromethane, acetone, and methanol extracts of *Evernia prunastri* and *Pseudevernia furfuracea*, where he showed a significant relationship between antioxidant capacities and total phenolic contents. Nguyen *et al*. (2019) assayed antioxidant activity of methanol extract of 14 lichen species, among which *Parmotrema tinctorum* demonstrated highest polyphenol and flavonoid content and free radical scavenging activity, with an IC50 of 59.9±4.65 mg/ml. Shcherbakova *et al*. (2021) compared the total phenol and antioxidant activity of hexane, dichloromethane, and acetonitrile extracts of *Evernia prunastri*; hexane extract displayed highest phenolic content and antioxidant activity compared to other two extracts. Volatile compounds in *Lichina pygmaea* were examined by Sanad *et al*. (2022) and exhibited intriguing scavenging properties on 2,2-diphenyl-1-picrylhydrazyl radical with an IC50 of 0.21 mg/ml. Tartouga *et al*. (2022) evaluated antioxidant activity using DPPH, ABTS, CUPRAC, β-carotene-linoleic acid, reducing power, total phenolic and flavonoid contents of the ethanol extract of *Parmotrema hypotropa*; the findings revealed higher concentration of phenolic content compared to flavonoids, and their antioxidant properties prevented protein denaturation in a dose-dependent manner and decreased intracellular reactive oxygen species.

2.3 Cytotoxic activity of lichen

Lichen compounds such as atranorin, gyrophoric acid, parietin, usnic acid etc. are known to possess cytotoxic effect and hence are of utmost importance in pharmaceutic industry (Bačkorová *et al*., 2012). The use of lichen secondary products as anticancer drugs dates back to the late 1960s, when activity of lichen polysaccharides against tumor cells was initially explored (Fukuoka *et al*., 1968; Shibata *et al*., 1968). The molecular mechanisms behind causing cell death by lichen compounds included cell cycle arrest, apoptosis, necrosis, and inhibition of angiogenesis (Brisdelli *et al*., 2013). Kupchan and Kopperman (1975), were the first to report tumor inhibitory activity of usnic acid extracted from *Cladonia* sp. against Lewis lung carcinoma. Felczykowska *et al*. (2017) reported the acetone extracts of *Caloplaca pusilla*, *Protoparmeliopsis muralis* and *Xanthoria* *parietina* to have potential anticancer activity against MCF-7 (human breast adenocarcinoma), PC-3 (human prostate cancer) and HeLa (human cervix adenocarcinoma) cancer cells; *X. parietina* cultured on PDA and G-LBM media decreased HeLa and MCF-7 cancer cells viability with an IC50 value of about 8 μg/ml, while *C. pusilla* grown on G-LBM media showed the highest potency in decreasing MCF-7 (7.29 μg/ml), PC-3 (7.96 μg/ml) and HeLa (6.57 μg/ml) cancer cells viability; increasing concentrations of *C. pusilla* extract induces apoptosis in HeLa, PC-3 and MCF-7 cell lines. Paluszczak *et al*. (2018) identified importance of lichen derived caperatic and physodic acid which can inhibit wnt signalling β-catenin in HCT116 and DLD-1 colorectal cancer cell. Nguyen *et al*. (2019) examined the anticancer activity of 14 lichen extracts, of which methanol extract of *Usnea flammea* had the most cytotoxic effect on MCF-7 cancer cell lines. Yurdacan *et al*. (2019) investigated usnic acid for its effectiveness on viability of HEPG2 hepatocellular carcinoma cell lines (HCC) in a dose-dependent way; usnic acid caused apoptosis in HCC cells with cell cycle arrest at G0/G1 and G2/M phase depending on the genetic profile of each cell type. Popovici *et al*. (2021), evaluated methanol, ethanol, acetone, and ethyl acetate extracts of *Usnea barbata* using MTT assay against CAL-27 cancer cell; the most potent anticancer property was displayed by acetone and ethyl acetate extracts, clonogenic assay showed that the extracts of *U. barbata* decreased the ability of cancer cells to form colonies compared to untreated cells, suggesting a potential anti-tumorigenic property of tested extracts. Mohammadi *et al*. (2022) studied the cytotoxic evaluation of gyrophoric acid from *Umbilicaria muhlenbergii*; GA reduced cell viability of breast cancer MCF-7 cell line by 98%, which specifies GA to have a strong cytotoxic property and significant potential to serve as a potent anticancer drug.

2.4 Antimicrobial activity of lichen

Antimicrobial activities of lichen are due to secondary metabolites such as phenols, flavonoids, saponins, terpenoids, alkaloids, tannins, coumarins and quinones produced by the mycobiont partner (Gyawali and Ibrahim, 2014). Secondary metabolites of lichens have been investigated as potential scaffolds for drug development (Müller, 2001; Zambare and Christopher 2012; Ranković and Kosanić, 2015). Specific natural compounds display significant role in modern drug development, especially as antimicrobial agents, due to the significant emergence of multidrug-resistant pathogenic microorganisms. Isolation of bioactive compounds from natural resources is thus a popular approach to develop new drugs as they often play an important role in treatment of many diseases with minimal side effects. Cankılıç *et al*. (2017) screened the antimicrobial activity of methanol, acetone and chloroform extracts of lichen *Usnea florida* using disc-diffusion method; strong activity against the tested bacteria and yeasts were observed in all extracts. However, no significant activity was observed against filamentous fungi. Aydin and Kinalioglu (2018) evaluated antimicrobial activity of crude extracts of *Protoparmeliopsis muralis* and *Parmotrema perlatum* by disc-diffusion and microbroth dilution methods; *P. muralis* possessed higher antimicrobial activity compared to *P. perlatum*; minimum inhibitory concentration values for bacteria was comparatively higher than that of fungi. Abdallah (2019) estimated antimicrobial activity of *Platismatia glauca* using disc-diffusion, MIC, MBC and MFC method against bacteria *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *S. saprophyticus*, *Streptococcus pneumoniae*, and one yeast *Candida albicans*. Amongst the investigated bacteria, the maximum inhibiting zone was shown in *S. saprophyticus* (18.5±1.0 mm) while lowest zone was observed in *E. coli* (11.0±0.0 mm); its antifungal efficacy against *Candida albicans* is also high (22.5±0.5 mm). Yadav *et al*., (2021) examined the antimicrobial activity of *Usnea longissimi* extracted in methanol, ethanol, ethyl acetate and acetone against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Fusarium oxysporum*; the highest zone of inhibition was obtained in *Escherichia coli* (15 mg/ml methanolic extract, 34 mm) followed by *Staphylococcus aureus* (10 mg/ml methanolic extract, 30 mm), *Pseudomonas aeruginosa* (10 mg/ml ethyl acetate, 16 mm), and *Fusarium oxysporium* (0.5 mg/ml ethanolic extract, 14 mm). Sanad *et al*. (2022) reported the volatile compounds of *Lichina pygmaea* to have considerable antimicrobial efficacy against the organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* with minimum inhibitory concentration values between 1.69–13.5 mg/ml.