Chapter-1

INTRODUCTION
1.1 Lichens- A unique entity with variety of morphological, anatomical and physiological peculiarities

During the course of plant evolution, the lichen came as a result of mutual inter-relationship of algal and fungal cells. Thus, lichens can be defined as composite organism consisting of two distinct and dissimilar components- the mycobiont and the phycobionts. There are about 18,800 known lichen taxa. Since the fungal constituent usually dominates the association, lichens traditionally have been considered as a lifestyle of fungi (Feuerer and Hawksworth 2007).

Pioneering work on lichens of India was started by the “Father of Taxonomy”; Carolus Linnaeus (1707-1778), Swedish naturalist who mentioned *Lichen fusiformis* (L.) DC (*Roccellamontagneii* Bel.) in his momentous work “*Species Plantarum*” on plant taxonomy which is valuable even today. Linnaeus grouped all lichen species under the genus Lichen and gave it to one of his last students Erik Acharius for further study. Lichens described in *Species Plantarum* were the collection of Linnaeus himself. **Erik Acharius**, last student of Linnaeus, “The father of Lichenology”, was a Physician by profession, but studied the specimens collected by Linnaeus and created many new genera and documented his work in *Lichenographiae suecicae prodromus* (1798); *Methodus lichenum* (1803); *Lichenographia universalis* (1810) and *Synopsis methodicaLichenum* (1814). However, the first ever record of lichen collection from India is known to be Belanger (1834-38) from Pondicherry and Coromandal coast.
Introduction

Lichens have been problematic organisms since Simon Schwendener in the 1860s (Schwendener 1869) discovered their so-called “double nature”, and showed that they are a consortium of algae and fungi. Moreover, the present of more than two types of photobionts, precisely the chlorophycean alga and the cyanobacterial or blue green alga in some of the genera also for some years had put this group into a state of uncertainty (Jørgensen, 1998).

The emphasis on the conservation of biodiversity has been felt by the concerned working authorities, which have purposely opened many institutions in the country and the world. The Natural History Museum; London is one among them which has a section with relict specimens of lichens specially concentrating on the potential lichens which are used as bioindicator, used as fodder, used to study palaeogeographical evolution of any landmass etc. The museum has some very good collection of plant specimens including those of lichens, which were the pioneer collection of Linnaeus, Acharius and other
lichenologists. The Royal Society of England; reflected in its founding Charters of the 1660s, to recognize, promote, and support excellence in science and to encourage the development and use of science for the benefit of humanity. The Royal Botanical Garden, Kew, is the largest Herbarium and has a vast collection of algae, fungi, lichens, bryophytes, pteridophytes and angiosperm plants.

**Figure 1.3:** Illustrations from Shwendener’s drawing showing hyphal entanglement of algal cells

In India, the Central National Herbarium (CNH, Kolkata) attached to Head Quarters, Botanical Survey of India; Kolkata is the largest herbarium in the country with more than 5, 00, 000 total specimens present with many holotype specimens present there. All other zonal headquarters have also collection of plant specimens and lichen specimens, particularly, the BSI, Allahabad, Guwahati and Shillong have done excellent work in the field of lichen taxonomy and floral documentation, but biotechnological and modern scientific approach towards lichen study is still lagging far behind in these institutes. The present study approached to study in not only classical concepts of lichen biology but also focused on themodern scientific applications such as biotechnology, nanotechnology, etc for the better study of the conservative bioprospective study of the lichens.

The work on lichens in India is of ancient origin. Charilla, a mixture of lichens, mainly Parmotrema spp., Everniastrumcirrhatum; is in use from the ancient times. Dr. Dharani Dhar Awasthi, (Fellow of Indian National Science Academy and Fellow of Indian Academy of Sciences) who is also considered as Father of Indian Lichenology mainly
documents the work on Indian lichens; who’s monumental work (Awasthi, 1965, 1977 and 2000) have made immortal contribution to the Indian lichen flora. He was also a recipient of the prestigious award “Acharius Medal” (stuck by Swedish Royal Academy of Sciences in 1846 and restarted awarding from 1992 every fourth year) for his contribution to Lichenology, in 1992 by International Association of Lichenology. National Botanical Research Institute, Lucknow; Botanical Survey of India, Allahabad; MS Swaminathan Research Foundation, Chennai; Agharkar Research Institute, Pune have been the prime centre of lichenological study; focusing on the taxonomical identification, documentation of the flora, revisionary studies and modern aspects of bioprospection. The Indian lichen flora is represented by approximately 2350 species included under 305 genera, listed in 74 families. Indian lichen vegetation has high degree of endemism; represented by almost 520 species (22.5%).

The lichens can be broadly classified into two types: Macrolichens (relatively larger forms and easily visible morphologically) consisting of the foliose, fruticose and squamulose forms; and Microlichens (crust like, attached to substratum, with microscopic morphology) consists of crustose disco-lichens, lirrilate, leprose forms, terricolous and folicolous types of lichens come under this category (Figure 1.4). The thallloid form of compact light-exposed vegetative bodies of lichens, constitute the most complex and aesthetically pleasing morphologies evolved by fungi during the past 600 million years (Yuan et al., 2005).

Being the pioneering species in any ecological system, lichen help to create a suitable habitat and niche for other plants (such as bryophytes, pteridophytes etc.) in early successional stages to render growth in that proximity. From microscopic observations, lichens differentiate varieties of tissues including cortex (a tissue covering and protecting thallus and consisting of gathered and adhered hyphae), algal layer (a tissue in which algae in thallus is surrounded and supported by hyphae), medulla (a basic tissue of thallus consisting of loosely entangled hyphae) and rhizine (a tissue projecting on the under-side surface and sticking the thallus on a carrier), which are the structural characteristics of lichens (Yamamato et al., 1990). Many lichens are highly extreme-tolerant which allows
them to live as pioneers in the alpine zone and other cold environments. Life under these conditions correlates with the production of a variety of compound classes.

**Figure 1.4:** Lichens in their diverse habitat: (A): Terricolous; (B): Saxicolous; (C): Corticolous and (D): Ramicolous

Several of these metabolites are already found in plants or in other fungi, but reaches to highest diversity in lichens (Huneck and Yoshimura, 1996). The distribution patterns of secondary metabolites are usually taxon-specific and, therefore, have been widely used in lichen taxonomy and systematics (Carlin, 1987; W. L. Culberson, 1969b; Fehrer et al., 2008; Hawksworth, 1976; Nelsen and Gargas, 2008; Nordin et al., 2007; Nylander, 1866; Piercey-Normore, 2007; Schmitt and Lumbsch, 2004). Some of these
metabolites form an available biomass for phytochemical investigations, including the assessment of biological activities of the isolated compounds (Boustie et al., 2010).

1.2 Lichens- A rich source of active secondary metabolites and their biosynthesis

There are two main groups of lichen compounds; primary metabolites (intracellular) and secondary metabolites (extracellular). The intracellular primary metabolites are those involved in the primary metabolism of the organism including polysaccharides, vitamins, amino acids, and lipids etc. which are bound within the cell wall in the protoplast. The lichen is since a composite organism hence it is rather more difficult to decide whether a given class of compound will be regarded as a primary or secondary metabolite.

The intracellular compounds are generally water soluble and consequently during extraction processes easily extracted through aqueous or polar solvents and are present in the lichen thallus as a result of primary metabolism including the photosynthetic (exclusive for photobionts) and respiratory metabolism.

Lichens synthesize numerous metabolites, the “lichen substances,” which comprise amino acid derivatives, sugar alcohols, aliphatic acids, macrocyclic lactones, mono-cyclic aromatic compounds, quinones, chromones, xanthhones, dibenzofuranes, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl ethers (Clix et al., 1984; Fiedler et al., 1986). The more complex secondary compounds mainly which are of diverse class of terpenoids, alkaloids, depsides, depsidones, anthraquinones, etc are produced by a different mechanism and are produced as a result of the various biochemical changes in the intermediates of the metabolic cycles of the lichen which serve as a starting fuel. There are three major cycles (Figure 1.5) which are followed for the production of these secondary metabolites:

a. Sikhimic acid pathway
b. Mevalonic acid pathway

c. Acetate- polymalonate pathway.

The acetate- polymalonate pathway is the major pathway, through which majority of the secondary metabolites are produced (Culberson and Elix, 1989; Huneck, 2001).
Figure 1. 5: Classified Secondary metabolite pathway and the produced lichen compounds

(Based on Nash, 2008; Stöcker- Wörgötter, 2008)
These include primarily the lichen acids, mainly the primary β- orcinol derivatives, depsides, tridepsides, tetraepdsides, deipsoides, benzofurans, aryls, usnic acid and derivatives, polyphenolics, xanthones, anthraquinones etc. The malonyl CoA and acetyl CoA serve as the precursor compounds. The Sikhimic acid is responsible for the production of pulvinic acid derivatives, variety of disaccharides, polysaccharides, etc from the precursors such as amino acids specially phenylalanine, polyols, which are produced as an intermediates in the primary metabolism. The mevalonic acid pathway gives rise to the production of terpenoids including diterpenoids, triterpenoids, sesquiterpenoids, carotenoids, sterols, etc from the precursor compounds including mainly acetyl CoA. These secondary compounds give these lichens a very characteristic smell, taste and colour; which strengthen the base of chemotaxonomy. These compounds in lichen serve many purposes including self-protection through anti-herbivory (Lawry, 1989), anti-larval action (Emmerich et al., 1993) or nematicidal action (Ahad et al., 1991) and insecticidal action (Hesbacher et al., 1995).

1.3 Bioprospection of lichens with its relevance in medicinal world

In the recent past, much attention has been paid on the biological roles of lichen secondary substances; many lichen substances have been found to have a lot of bioactivities, such as anti-tumor, antibacterial, antifungal, antiviral, anti-inflammatory and also antioxidant activities (Oksanen, 2006). In the folklore of many European countries, lichens were used as a remedy for pulmonary tuberculosis and in the treatment of wounds and skin disorders. With a few exceptions, however, activity of lichen metabolites against gram-negative bacteria and fungi has not been reported.

Burkholder reported for the first time the presence of antibiotic substances in lichens (Burkholder et al., 1944). Several lichen metabolites were found to be active against Gram-positive organisms (Lauterwein et al., 1995). The anti-mycobacterial activity of lichen compounds was reported against non-tubercular species of Mycobacterium (Ingolfsdottir et al., 1998). The well known antibacterial topical drug in the market sold under the names of “USNO” and “EVOSIN” throughout the European
countries has usnic acid as one of the major constituent (Dayan and Romagni, 2002). Usnic acid, a very active lichen compound is used in pharmaceutical preparations. Usnic acid and vulpinic acid (produced by mycobiont) are cell division regulators of autotrophic partner of lichen symbiosis- the photobiont (Backor et al., 1998). Lichen extracts have cytotoxic activity in different degrees. The aqueous extract of *P. polydactyla* and the ethanol extract of the *R. farinacea* exhibited potent antibacterial activities (Karagöz et al., 2009).

Anthraquinones also constitute a major part of the secondary metabolites in lichens (Cohen and Towers, 1995; 1996 and Cohen et al., 1996); exhibiting antiviral activity against HIV. Hypericin, in particular is of great pharmaceutical importance because of its dramatic antiretroviral activity (Lavie et al., 1995).

![Figure 1. 3: Medicinal uses of lichens](image-url)

The polyketides are produced in the lichen through polyketide pathway, which is although not exclusive to lichens but the mononuclear polyketides produced by the mycobionts of the lichens such as methyl orcellinate, phloroacetophenone and phthalide derivatives are unique to lichens only. The probability of finding good compounds to be
used as drug in polyketides is extremely high, about 1 in 100 compounds, which in about 1 in 5000 in case of normal compounds (Borchardt, 1999). Many famous polyketide drugs are already present in the market such as Erythromycin A (antibiotic), rapamycin (immunosuppressant), lovastatin (cholesterol-lowering agents) etc. (Weismann, 2004).

The lichens in the tropical and the sub-tropical regions of the country are exposed to some of the very challenging climatic conditions along with the various biotic and abiotic factors, which determine the growth of the thallus. These mainly include the rainfall, the bark chemistry, the temperature and the presence of the various other competing microorganisms in their niche; which may be sometimes pathogenic. Hence, it is much healthier chance that a unique array of secondary metabolites, which may play a role in their own defense mechanism, may be present in these lichens (Galloway, 1992). But still, the novel search for the wonder drugs against some of the fatal global diseases, which claim for more than millions of lives every year, has not taken that pace and momentum from the herbals and that too specifically from the lichens, because of the following constraints:

- The composite nature of the lichen, which restricts them to a very slow growth; about 1-2 mm/year in nature.
- Lack of biomass availability for long term R and D restricts the scientist to explore the group.
- The culture protocols demand high sterility of the starting material, which brings about a major problem in the tissue culture studies and biomass production.
- Even in the cultured lichens, the induction for production of lichen substances is very tedious, thus limits the research in the labs and the transfer of the technology into the industries is curtailed.
- The exploration of the lichen flora for the purpose of pharmaceutical bioprospection is very poor, thus a little about their medicinal value is known and that too of well known lichen species.
• The flora of the extreme Himalayas is not well explored and still needs documentation by the taxonomists for the purpose to be resolved.

• Standardized protocols for the mass production of the secondary metabolites from cultured mycobiont and whole thallus culture and inflection studies in the pathways for these metabolite productions are still lagging behind.

Against all the odds, the demand is to not only explore the lichens for the search of the novel compounds of medicinal and pharmaceutical relevance, but to develop standard protocols and standardized optimum conditions, so as to get success in the industrial production of these compounds from the cultured tissues and thus limiting the use of the natural thallus; which will ultimately sustain the research for a longer time period and also serve in the process of biodiversity conservation, which is the ultimate need for the Mother Earth and necessary for the survival of mankind.

1.4 Dermatophytoses: A major problem for the tropical countries

Various dermatophytoses causing fungal pathogens are a class of anamorphic fungus which are widely increasing globally and are primary cause of diseases such as tinea capitis, tinea corporis, tinea inguinalis, tinea manuum, tinea unguium, tinea faciei and tinea pedis (Wang et al., 2006). The formation and morphology of conidia (for asexual reproduction) by fungi called dermatophytes forms the basis of their distinction into three genera class viz., *Trichophyton*, *Microsporum* and *Epidermophyton*.

Several factors involving physico-chemical structure of skin, UV light exposure, temperature, lack of humidity and normal microbiota occurrence restricts the fungus to grow. Moreover, in the process of keratinization, the stratum corneum is renewed by keratinocytes leads to epithelial shedding which acts as internal defense. They are keratinolytic in nature but can survive without keratin also; are responsible for superficial mycoses common in human and animals. Keratin is the human protein which forms the main building element of skin, nails and shells, protective in nature. It is cysteine rich with many disulfide bridges and acetamide bonds, which gives this high molecular weight protein a fibrous nature and assures higher stability. Dermatophytes produce specific
enzymes which catalyze the fungal penetration into the host keratin rich tissues (O'Sullivan et al., 1971; Takiuchi et al., 1982; Apodaca et al., 1989; Mignon et al., 1998; Padhye et al., 1999).

Many other important proteins and enzymes are identified which have been found to play prominent role in pathogenesis or in some way, either affect the immune system or the hypersensitivity response of affected individual (Peres et al., 2010). Appearance of the pruritic and typical circular erythematous lesions are the result of hypersensitive response of the host to the direct action of fungus and its active metabolites. Onychomycosis is characterized by thickening of the nails, white spots, often distrophic and even separation of nail from its bed occurs. At least 60% of all superficial fungal infection is caused by Trichophyton rubrum whose transmission is occurs mainly due to direct person to person contact or shedding of infected skin cells (Kwon-Chung et al., 1992; Kane et al., 1997).

**Table 1.1.:** Proteins identified in Dermatophytes probably involved in virulence

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>Function in the fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipase B</td>
<td>Gene inactivation attenuates the virulence of Cryptococcus neoformans and C. albicans</td>
</tr>
<tr>
<td>Subtilisin protease (Sub3)</td>
<td>Sub3 is the main protease secreted by T. rubrum during host infection</td>
</tr>
<tr>
<td>Subtilisin protease (Sub 5)</td>
<td>Secreted protease is an important virulence factor</td>
</tr>
<tr>
<td>Metalloprotease (Mep3)</td>
<td>MEP3 is produced by M. canis during infection of Guinea pigs</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>Glyoxylate cycle enzyme</td>
</tr>
<tr>
<td>Metalloprotease (Mep4)</td>
<td>MeP4 is the main metalloproteases secreted by T. rubrum during host infection</td>
</tr>
<tr>
<td>Dipeptidyl-peptidase V</td>
<td>Potential virulence factors of M. canis, helping to degrade substrates</td>
</tr>
<tr>
<td>P-type ATPase associated with copper resistance</td>
<td>Gene inactivation attenuates virulence of Listeria monocytogenes and C. neoformans</td>
</tr>
<tr>
<td>Urease</td>
<td>Gene inactivation reduces ammonia secretion in vitro and attenuates the virulence of Coccidioides posadasii</td>
</tr>
<tr>
<td>Glucosamine-6-phosphate deaminase</td>
<td>Gene inactivation attenuates the virulence of C. albicans in murine model</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>GAPDH contributes to the adhesion of Paracoccidioides brasiliensis to the host tissue and dissemination</td>
</tr>
<tr>
<td>ABC Transporter TruMDR2</td>
<td>Gene inactivation attenuates the virulence of T. rubrum in vitro</td>
</tr>
<tr>
<td>PacC</td>
<td>Transcription factor Gene inactivation attenuates the virulence of T. rubrum in vitro</td>
</tr>
</tbody>
</table>

Source: Peres et al., (2010).
Infection caused by dermatophytes

A- Tinea faciei  B- Tinea unguium (Nail Infection)
C, D & E- Tinea corporis, Tinea manus
F & G- Tinea pedis (Athlete’s foot infection)
The dermatophytes infectious fungi showed production of metalloproteases and subtilisin proteases during *in vivo* experiments which verified its significance in playing a vital role in dermatophytic infection (Brouta *et al*., 2002; Descamps *et al*., 2002). Studies done by Lemsaddek (2010) on various isolated strains of dermatophytes viz., *Epidermophyton floccosum, Microsporum audouinii, M. canis, T. mentagrophytes sensu lato* and *T. tonsurans* along with the reference strains of *A. benhamiae, A. vanbreuseghemii* and *T. rubrum* have shown positive results for the presence of MEP genes. Concurrently, some other isolates of *M. gypseum, T. rubrum*, and various isolates of *T. mentagarophytes, T.soudanense* and *A. vanbreuseghemii* lacked the MEP gene. A gene family of three genes namely MEP1, MEP2 and MEP3 coding for metalloproteases in *M. canis* has been described by Brouta and co-workers (2001) and also reported the *in vivo* production of Mep2 and Mep3 proteases in experiments with guinea pigs. Mep3 was characterized and showed collagenolytic, elastinolytic and keratinolytic activities (Brouta *et al*., 2001). Some authors also documented about two new genes MEP4 and MEP5 also in *M. canis* in five member gene family named MEP 1-5 encoding for fungalysins (endometalloproteases), also identified in *Trichophytonrubrum* and *T. mentagrophytes* (Jousson *et al*., 2004).

1.4.1. Growth of Dermatophytes in laboratory

The fungus isolated from the human as well as animal samples not only contains the dermatophytic fungi but some other common inhabiting fungi such as *Aspergillus, Candida* etc. After the isolation of the pure culture, the primary isolate can be cultured and further maintained at favourable conditions *in vitro* in Labs. The medium used is either PDA (Potato Dextrose Agar) or SDA (Sabouraud Dextrose Agar) and their broth media at pH 5.6 ± 0.1 with temperature ranging from 27- 32°C. Following these growth parameters, significant growth of the fungus can be obtained in a week time for further bioprospection related experiments.

1.4.2. Management by synthetic drugs available in the market and their side effects

Management of dermatophytic fungal infections more or less depends on the area affected and the type of infection detected. The simple inconspicuous single skin lesion
can be effectively cured by any antifungal topical lotion. But the complicated scalp, nail infection, groin injuries with tinea lesions and wound lesions are required with systemic therapy. Itraconazole, fluconazole, griseofulvin, terbinafin, clotrimazole, ketoconazole and seratoconazole some of the most commonly used antifungal agents found effective against various types of tinea such as tinea unguium (Onychomycosis), tinea capitis, tinea corporis, tinea cruris, tinea pedis and chronic and/or non-responsive tinea. These antifungals are though fast in action and have high responsiveness, but still they are unsafe for treatment of long chronic cases. Antifungals such Amphotericin B have nephrotoxic effects, Ketoconazole are associated with hepatocellular toxicity, anorexia and nausea. Skin rashes, itching, nausea, edema, headache, chilling, fever, etc are some common side effects of widely used antifungal agents. Moreover, the incidence of reoccurrence of the fungal infections is also high; when treated with these conventional antifungal agents. This has led to the emergence to a class of cosmeceuticals; the natural antifungals for the management of the cosmetic embarrassment.

1.5. Water borne diseases- A common health and hygiene related problem in developing countries

The presence of sunlight, plants and water has given life on the Earth. The locked source of pure drinking water in the glaciers, ice caps and glacial lakes and rivers count for 2.2% of the total water present on the Earth; thus limiting the useful source of drinking water to only 0.5% present in the rivers, lakes and the ground water. The rapidly increasing population has also increased the pollution at an alarming rate; thus several fatal bacterial, viral, protozoal, nematodal and fungal diseases have emerged which are somehow related to water, either for their dispersal or their growth. Water borne diseases, in particular, diarrhoeal diseases alone cause 2.2 millions death per year of the total water related deaths counting for 3.4 millions (WHO, 2002). This death toll has only increased in the coming years and will move on to increase least effective measures for its prevention are taken on a global scale. Even our Indian Constitution gives us the Right to clean drinking water under the Article 21 and Article 39 (a) and 39 (b). The tropics and
subtropical regions have the most favourable climatic conditions which suit for the spread of epidemics caused due to such diseases.

The main physico-chemical properties of water which determine its purity are turbidity (clarity), taste, odour, colour and pH. Biologically the water quality is determined by its BOD, COD, coliform count etc. WHO has provided a mandate which has documented the essential qualitative parameters, to be followed for determination of quality of water. Presence of the large amount of organic pollutant in the local vicinity has led to the emergence of water-borne bacterial diseases. These include some of the most fatal diseases such as fecal dysentery, diarrhoea, cholera, typhoid fever etc. caused by mainly *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Streptococcus aureus*. As stated earlier, synthetic drugs are though fast in action and provide speedy recovery, but come with lots of side-effects and high reoccurrence rate of the disease. Hence, prevention is always better than cure, i.e. rather than taking medication, one should focus on increasing the potability of water with the application of herbal antimicrobials. Hence, the present exploration of the lichen bioprospection will also be focused on the search of novel antibacterial compounds, with the application of modern biotechnological tools and techniques for the enhancement of quality of water.

1.6. Modern tools and techniques for better of compound extraction and its examination

The introduction of new analytical methods (thin layer chromatography, high-performance liquid chromatography, ultraviolet, infra red, magnetic resonance spectroscopy, mass spectrometry, X-ray crystallography) has led to the isolation of many new lichen substances, which by today number over 1500. These considerations explain the increasing interest of the pharmaceutical industries in these remarkable natural products (Huneck, 1999). TLC/ HPLC are known as standard lichenological methods for the identification, isolation and purification of the lichen substances; which always possesses a challenge in the path of bioprospective studies of novel active metabolites (Orange *et al.*, 2001; White and James, 1985 and Lisickov *et al.*, 2002). Bioinformatics,
Introduction

specifically dealing with the mechanism and functionality of the biological compound is also of great importance in present scenario of research. A study by Neamati et al., 1997; for identification of a pharmacophore among the total screened 17 compounds represented by depsides, depsidones and their synthetic derivatives came with 2 pharmacophore compounds having enzyme inhibitory activity against HIV-1 integrase enzyme.

In silico approach for the prediction and validation of the active secondary compounds for a particular parameter with a set of variable can be also obtained. In a study on the antifungal activity of Cladia aggregata extract Malassezia, commensal yeast; successful prediction and validation of the activity of the extract against the fungus was done using bioinformatical tools (Pandey et al., 2013). The sequencing and preparation of the cDNA library of the gene of interest and further its vector mediated transformation into suitable hosts may also prove to be an alternative for the rapid production of the secondary compound of interest. Similarly, the chemo-taxonomical approach towards the searching of the active compounds form the lichens with the similar evolutionary history can also serve the purpose and help in the meaningful exploration of lichen flora. But the above said techniques are limited to some big research units, and are not in availability to all the researchers. Hence, it is needed to collaborate and work efficiently.

1.7. Lichen culture- leading light to its conservation and bioprospection

The nature, the habitat and ecological niche which the lichens got hold of on earth consists of extremities of the various physical and biological environmental conditions such as temperature, altitude, humidity, suitable substratum for growth etc. These all factors contribute to the slow growing nature of this plant type. Artificial conditions for growth such as sulfurous acid gas or smoke and natural cultivation of lichens have never been successful (Yamamoto et al., 1990). Although all over the world as well as in some European countries there is interest for in vitro culture and artificial biosynthesis of lichens thalli, with the aim to produce high quantities of biomass for biotechnological applications (Armitage and Howe, 2006, 2007, Howe and Armitage, 2002, Ahmadjian V., 1990a) (Ahmadjian, V., Russell, L.A. and Hildreth, K.C. 1980). Ordinary culture processes have been attempted in order to increase the lichens artificially as mentioned above. However,
in conventional processes, importance has been given to fungal cells and continuous efforts have been put into increasing them (Yamamoto et al., 1990). Many authors are in a position to possess a good culture collection with more than 100’s of lichen derived cultures of lichenicolous fungi; many more have succeeded in obtaining culture-derived secondary substances (Yamamoto et al., 1993; Hamada and Miyagawa, 1995 and Behera et al., 2000). Main focus drawn in the culture studies is the establishment of the sterile cultures of the lichenized fungi with metabolite production, which is also the main objective of the present study.

1.8. Culture media modification and procedural inflections helping out to enhanced metabolite production

The sophisticated procedural requirements for the culture of lichens gave a challenge to the scientists for inflecting the media compositions and introduction of new additives in it. Specifically speaking, many studies focused on the alteration of the carbohydrate source, its concentration and addition of novel metabolites such as mineral salts and amino acids in it gave promising results for enhanced biomass production and metabolite synthesis. The addition of excess sucrose creates an osmotic pressure on the mycobiont thereby inducing the production of the metabolites (Hamada, 1993 and Hamada et al., 1996). The photobiont might also have an influence on the secondary metabolism of the mycobiont (Brunauer et al., 2007; Yamamoto et al., 1993; Yoshimura et al., 1994). In similar fashion, physico-ecological factors also play important role in the induction of the metabolite production. Many factors such as UV-light exposure, chilling, etc are known to induce the secondary metabolite pathways (Stöcker Wörgötter, 2001a; 2005 and Hager et al., 2008). Ecological conditions (including the circadian rhythm, total moisture content, pH, etc) and rate of thallus differentiation are regarded as factors responsible for activation of secondary metabolite pathways (Stöcker Wörgötter, 2008).

Henceforth, regardless of all the backdrops, the study focuses on three broad objectives:

- The collection, identification and preservation of the lichen species.
- Bioprospective studies of the medicinally important lichens.
- Culture study of screened out lichens found with active secondary substances.