Chapter 1

INTRODUCTION

1. INTRODUCTION AND BACKGROUND OF THE WORK

Darjeeling hill is the part of Eastern Himalayan region of India, lying between 87º59′ to 88º53′ E and 28º31′ to 27º13′ N. It covers an area of about 2320 km^2 with versatile altitude ranging between 130 m to 3660 m (Saha *et al*., 2011). Due to its geographic uniqueness, variability in altitude and climatic condition, this region favours the luxuriant growth of diversified vegetation. Bryophytes, the second largest group of green land plants after angiosperms, are one of the major constituents of this ecologically rich biodiversity. Bryophytes, also known as the "amphibians" of the plant kingdom, are small plants characterized by their simple organization and little or no organized vascular tissue. Their non-vascular nature is due to poorly developed conducting tissue that lack xylem. The bryophytes consist of three subgroups: Bryophyta (mosses), Marchantiophyta (liverworts or hepatics) and Anthocerotophyta (hornworts) and occupy an intermediate position in between algae and pteridophytes. This primitive group of plants play significant role in soil formation, maintaining soil moisture, recycling nutrients, preventing soil erosion, bio-monitoring pollutants, bio-accumulation of toxic minerals, pH indication and also as medicine.

For thousands of years nature has been a source of many therapeutic drugs. Traditional practices had provided knowledge about the useful plants for the development of many important drugs (Rado *et al*., 1989). Since time immemorial, different group of plants are used in different culture for the treatment of different ailments, and many scientific reports are also available indicating the application of bryophytes, especially liverworts in traditional medicine. But, in comparison to vascular plants their utilization was rather rare in ancient traditional medicine. However, they have been utilized in ancient Chinese, European, North American and Indian medicine to treat illness of cardiovascular system, tonsillitis, bronchitis, tympanitis in skin diseases and burns, etc. (Khanam *et al*., 2011). About 40 bryophyte species in China are reported to be used for the treatment of ulcer, cardiovascular disorders, bronchitis, tympanitis, cystitis, skin diseases and burns as well as antifungal and antibacterial agents. Recent studies have established cytotoxic, anticancer, anti-tumour (Shi *et al*., 2008a; Shen *et al*., 2010), antifungal (Niu *et al*., 2006; Veljic *et al*., 2010; Ivanova *et al*., 2007), and anti-inflammatory responses (Ivanova *et al*., 2007) properties of liverworts and mosses.

Nowadays, the increasing activities of mankind are directly leading to the alteration of distribution patterns of bryophytes and subsequently influencing the functional and structural roles of species in the ecosystem. Bryophytes are not well known to common people, even among some conservationists. They are thought to be quite useless members of the plant kingdom by the people and are being disturbed without even being noticed. However, several ethno medicinal studies and recent pharmacological studies have confirmed the unique medicinal properties of bryophytes, but still much is left to unearth.

For a long time the phytochemistry of bryophytes has been neglected and the information on biological activities of this second biggest group of land plants remained neglected and unknown (Sabovljevic and Sabovljevic, 2008). However, over the past two decades, biologists, chemists and pharmacologist have shown interest in this group of plants, and recent studies on liverworts and mosses have revealed that they produce numerous distinct active substances (Dulger *et al.*, 2005; Ilhan *et al.*, 2006; Ojo *et al.*, 2007). Nowadays, bryophytes are being considered as a remarkable reservoir of new and distinct natural secondary compounds. Studies have revealed the presence of wide array of secondary metabolites in bryophytes (Manoj *et al.*, 2012a). Many new compounds were described from this comparatively unexplored group of plants, mainly from liverworts (Asakawa, 2008; Sabovljevic and Sabovljevic, 2008). In spite of being treasure house for diverse, naturally occurring phytochemicals, the investigations on its phytochemicals are still in nascent stage only. In this context, assessment of bioactive components present in this group of plant is highly essential considering their therapeutic and clinical utility.

Secondary metabolites discovered from bryophytes have shown various important biological activities (Asakawa, 2008). Studies have revealed that very few reports on antioxidative activity of bryophytes have been detailed till now in comparison to other biological activities. In this aspect generation of appropriate knowledge base about antioxidant phytochemistry are essential. Antioxidative activity shown by plants is considered to be due to the phenolic compounds present in them. Antioxidants are substance that detoxifies reactive oxygen species (ROS) generated usually from exogenous chemicals and other common endogenous metabolic processes in human body (Nandy *et al.*, 2012). Reactive oxygen species, or free radicals, are molecules having one or more unpaired electrons in the outer shell which makes them extremely reactive. The production of reactive oxygen species like hydrogen peroxide (H_2O_2), superoxide ($O^{\cdot}{}_2^{-}$) and hydroxyl radicals is an

usual physiological phenomenon in the human body (Nordberg and Arner, 2001). However excess production of free radicals is considered harmful to health. These ROS are short lived but have the potential to initiate a chain reaction that results in creation of more free radical by destabilizing other molecules (Vats and Alam, 2013). Excessive production of free radicals imposes oxidative stress that causes hypertension, atherosclerosis, diabetes, emphysema, inflammation, cancer and many degenerative diseases including premature aging, neurodegenerative diseases like Parkinson's and Alzheimer's and cardiovascular diseases (Halliwell and Gutteridge, 1990; Kris-Etherton *et al.*, 2002; Pejin and Bogdanovic-Pristov, 2012). In addition to this, free radicals (ROS) also attack unsaturated fatty acids of cell membranes, resulting in lipid peroxidation, reduction in membrane fluidity, membrane protein damage and failure of enzyme and receptor activities (Dean and Davies, 1993). Thus, for the survival all living system is equipped with antioxidant molecules that detoxify the harmful effect of free radicals. However, under prolonged stress, tiredness, or diminished immunity inner antioxidant system may be insufficient. Thus, there is a need of exogenous supplimentation of antioxidants. Antioxidant can reduce the oxidative damage by chelating catalytic metals, neutralizing ROS and by acting as oxygen scavengers (Gulcin *et al.*, 2003). Antioxidant can be either natural or synthetic. The most commonly used synthetic antioxidants are butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propylgalate and *tert*-butylhydroquinone (Sherwin, 1990). However, synthetic antioxidants are considered harmful for health due to its carcinogenicity (Jayaprakasha *et al*., 2003). To meet the growing demand of natural antioxidants in pharmaceutical, cosmetic and food industries, the discovery of new natural sources with potential pharmaceutical and antioxidant capabilities are of great interest (Cowan, 1999). Several reports highlight scientific investigations regarding different antioxidant rich sources like vegetables, fruits, and other angiospermic, gymnospermic plant parts, but only little work has been done on other group of plants, especially bryophytes (Chobot *et al*., 2008). Recent pharmacological investigations have proved that active principles present in bryophytes are quite unique having potential therapeutic activity (Gokbulut *et al*., 2012). As this group can cope up with extreme climates and stresses, they are considered to possess strong antioxidative enzymatic machinery (Dey and De, 2012). Thus, investigation of antioxidative activity of this group of plants can prove to be beneficial in future for pharmaceutical and cosmetic industries.

Although liverworts have shown different biological activities, literature reviews have revealed that reports dealing with the antioxidant activity of bryophytes are rather few. In the

same way very little is known about the role of Eastern Himalayan bryophytes as natural sources of antioxidant. They are not as well explored as angiosperms in terms of antioxidant potential. Till now mosses like *Octoblepharum albidum, Hyophila involuta, Hyophila perannulata* (*Trichostomum criotum*), *Syrrhopodon subconfertus* (*Syrrhopodon confertus*), *Erythrodontium julaceum and Sematophyllum subhumile* (Mukhopadhyay *et al*., 2013) and the liverwort *Pellia endivifolia* (Dey *et al*., 2013) from Darjeeling Himalaya were studied to analyze their antioxidant activity, but there are still many more to unearth.

Liverworts have also shown some cytotoxic effect against cancer cells. Terpenoids and other aromatic compounds present in liverworts are reported to be responsible for the cytotoxicity shown by the plants. Liverworts like *Pellia endiviifolia, P. perrotetiana, Lophocolea heterophylla* and *Radula perrotetiana* showed cytotoxicity against P-388 cells (Toyota *et al*., 1990). Terpenoids from *Plagiochila pulcherrima* are cytotoxic against Hela cells (Wang *et al*., 2013). Metabolites, such as germacronolids, eudesmanolides, guaianolids, lunularin, plagiochiline A, (-)-ent-Arbusculin B (94b) and (-)-ent-costunolide, costunolide are reported to be responsible for cytotoxic activity of liverworts (Asakawa, 1995; Asakawa, 1990). However, studies have shown that no work has been done till date to explore the cytotoxic activity of liverworts of Darjeeling hills. Inhibitory activity against α-glucosidase was also displayed by marchantin C isolated from liverworts (Harinantenaina and Asakawa, 2007). But, only few reports are available on the anti-diabetic activity of liverworts worldwide. Thus, studies of α-glucosidase and α-amylase inhibitory activities can be of importance from pharmaceutical point of view.

Recently, there are increasing public demand for herbal medicines due to their low or no side-effects on human health. To meet this requirement there is a need to search for more natural resources with potential pharmacological activity. Till date, angiosperms have been mostly investigated for the drug development and no doubt secondary metabolites from these plants have lead to the development of a wide variety of therapeutic drugs. But, as plants belonging to same group are known to have similar phytochemical constituents, researchers are looking for novel resources of new therapeutic substances in unexplored group of plants. In comparison to other plant groups, bryophytes remain comparatively untouched in the course of drug discovery. At present, only a small percentage of liverworts and mosses are chemically studied worldwide. Similarly, in India only recently studies have been initiated to screen the phytochemicals and also to determine antioxidant activities of bryophytes (Alam,

2012). Eastern Himalayan Biodiversity hotspot is a huge repository of bryophytes with rich generic and species diversity, but they have not been explored chemically till date (Singh, 1997, 2001; Mukhopadhyay *et al.*, 2013). Further, pharmacological and chemical analysis on the secondary metabolites of bryophytes may give us a number of different new compounds which could be useful for pharmaceutical, cosmetic or agricultural fields. Therefore, more research on these aspects is required.

Liverworts despite being the storehouse of many phytochemicals unique to plant kingdom, very little is known about the phytochemistry of these plants. Various factors restricting the chemical analysis and isolation of bioactive compounds of bryophytes are (i) their seasonal availability, (ii) niche specificity restricting them to grow only in selected geographic area, and (iii) difficulty in collection of pure sample in sufficient quantity. These impediments, however, can be successfully addressed by *in-vitro* propagation of desired species (Vujcic *et al.*, 2017). The standardization of appropriate growth medium for liverwort culture will, therefore, be of great help to overcome the difficulties in the study of chemical constituents of liverworts.

Liverworts are significant component of biodiversity; still these plants are neglected by the conservationists as useless weeds. Nowadays increasing human population leading to various anthropogenic activities, like deforestation, urbanization, tourism, etc. are posing serious threat to natural habitats of fauna and flora including these plants. In India nearly 10% of the liverworts and hornworts are considered as rare, endangered and threatened due to various biotic factors (Singh, 2008). India has developed an elaborate Protected Area (PA) network comprising Wildlife Sanctuaries, National Parks, Community Reserves and Conservation Reserves for *in situ* conservation of its biodiversity. While, liverworts occurring in these PAs are conserved *in situ*, there is a need to conserve populations / habitats outside these areas by establishing 'Species Specific Sites' (Singh, 1999). Besides, to conserve small isolated populations of bryophytes *ex situ*, there is a need to establish 'moss houses' in different eco-climatic zones of the country. Lack of both proper knowledge on this group and appreciation by the masses, including general botanists, may cause serious threats leading to extinction of these plants. Through facilities like 'Bryophyte Gardens', 'Moss Houses' and 'Species Specific Sites', liverworts can be familiarized to students, researchers, scientists and the common people, which may inculcate interest among them and help to reduce threats to this unique group of plants. Thus more effort in establishment of suitable *ex situ* conservation

facilities is essential to save this fascinating yet less understood wealth of nature. Apart from this, study of habitat requirements of liverworts are also important to consider for their conservation. Factors such as light, moisture, temperature, substrate, etc. highly effect the distribution and density of liverworts. Several works focusing on the factors affecting species diversity and abundance have been carried out worldwide; however no such studies have been done in Darjeeling area. So, the record of habitat requirement can be of immense significance for proper conservation of this unique plant group.

Following are the objectives of this work:

1. Characterization of free radical scavenging properties of liverworts

2. To determine the anti-diabetic activity of bio-active molecules *in-vitro*

3. Quantitative evaluation of primary phytochemicals present in the plant sample

4. Detection and profiling of different phytochemicals by using TLC analysis

5. Bioassay guided isolation and purification of bioactive components

6. Spectral characterization and detection of bioactive components isolated through purification

7. To establish the influence of seasonal variation on the antioxidant property

8. To establish suitable conservation strategies for selected liverwort species